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Effect of sampling time and air humidity on the bioefficiency of filter samplers for bioaerosol collection

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Abstract

The effect of sampling time on the biological collection efficiency of viable airborne microorganisms was studied with two personal filter samplers (the Button Inhalable Aerosol Sampler and the 37-mm cassette) under relative humidities (RH) of 30 and 85%. Polycarbonate filters mounted in the samplers were challenged with five aerosolized microorganisms (fungal spores, endospores, and bacterial vegetative cells) and air was drawn through the samplers for a period of time ranging from 2 min to 8 h. The microorganisms were extracted from the filters at extraction efficiency of 96–98% by vortexing and ultrasonic agitation. The suspension of extracted microorganisms was analyzed by cultivation and by epifluorescence microscopic counting. Most of the tests did not indicate statistically significant differences in the bioefficiency of the two samplers. The culturability of *Penicillium melinii* and *Aspergillus versicolor* fungal spores at low and high RH values of 30 and 85% decreased during the first few minutes, but remained approximately the same for sampling times ranging from 30 min to 8 h. The relative culturability of *Bacillus subtilis* endospores decreased with sampling time, and was $\leqslant 17\%$ at RH = 30% and $\leqslant 32\%$ at RH = 85%. *Pseudomonas fluorescens* and *Serratia marcescens* vegetative cells were culturable only if sampled for 10 min or less. This concluded that the bioefficiency of filter samplers not only depends on the microbial species, but also on the sampling time and relative humidity.

Keywords: Bioaerosols; Filtration; Microorganisms; Spores

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1. Introduction

The effect of airborne microorganisms on human health has recently increased in importance. Human exposure to bioaerosols may result in diseases such as allergic alveolitis, organic dust toxic syndrome, asthma and allergy (Lacey, Pepys, & Cross, 1972; Donham, 1990; Malmberg, 1990; Lacey & Dutkiewicz, 1994; Heldal, Skogstad, & Eduard, 1996). Increased awareness of these problems has raised an interest in reliable methods for the collection and determination of airborne microorganisms.

Many commercially available sampling devices have been adapted for use in environments contaminated by bioaerosols. Several studies have determined the bioefficiency of samplers for viable microorganisms (Eduard & Heederik, 1998; Thorne, Kiekhaefer, Whitten, & Donham, 1992; Lin & Li, 1998; Li & Lin, 1999). Filter samplers have been found to meet several of the criteria for an "ideal bioaerosol sampler" (Macher, 1997). The collection efficiencies of commercially available filter samplers such as the IOM Sampler and the Button Inhalable Aerosol Sampler (both SKC Inc., Eighty Four, Pa) have been found to follow the inhalability curve of the American Conference of Governmental Industrial Hygienists (ACGIH) reasonably well (Kenny et al., 1998; Aizenberg, Grinshpun, Willeke, Smith, & Baron, 2000a). In contrast to impactors, the filter samplers — when used with an appropriate filter — can achieve high physical collection efficiency over a wide range of particle sizes (Aizenberg, Reponen, Grinshpun, & Willeke, 2000b). Some of the filter-sampling devices such as the two mentioned above, can also be used for personal sampling, and the microorganisms collected on the filter may be assessed in several different and complementary ways.

Blomquist, Palmgren, and Ström (1984a) and Palmgren, Ström, Blomquist, and Malmberg (1986) have introduced a filter sampling and elution method, referred to as the CAMNEA method (Collection of Airborne Microorganisms on Nuclepore Filters, Estimation and Analysis). In this method the microorganisms are sampled onto a Nuclepore polycarbonate filter, from which they are later extracted. One part is cultivated and the other is stained with acridine orange, followed by counting under an epifluorescence microscope. Thus, both the viable and total bioaerosol concentrations are determined. The extraction procedure in this method includes adding extraction fluid to the sampling cassette, plugging the cassette, and then vigorously agitating the cassette on a shaking table for 15 min. The extraction efficiency of this method has been reported to be approximately 65%. It has been improved to approximately 80% when further agitation was applied with an ultrasonic bath (Heldal et al., 1996). However, agitation in an ultrasonic bath may damage the microorganisms. We found vortexing to be a more efficient method than mechanical shaking when extracting bacteria from filter materials (Wang, Reponen, Willeke, & Grinshpun, 1999).

The growth and survival of airborne microorganisms depend on many factors including the type of culture medium, method of aerosolization, method of collection and analysis, and environmental factors such as the presence of sufficient moisture at a favorable temperature. It is known that a microorganism stops growing when its growth medium dries out (Blomquist, Ström, & Strömquist, 1984b; Heldal et al., 1996). As a consequence, microorganisms may survive better in an environment with high relative humidity. It is well known that the dehydration effect during filter sampling may cause injury and even death of collected microorganisms, especially of the sensitive ones (Muilenberg & Burge, 1994; Willeke & Macher, 1999). However, little information is available

on how much dehydration affects the survival of various microbial species ranging from hardy fungal spores to sensitive vegetative cells. Even less data are available on the recovery and survival rates of collected microorganisms after different sampling times. Lin and Li (1998) observed that the length of sampling time did not significantly influence the recovery of fungal spores of *Penicillium citrinum* collected by filtration. However, their study was limited to a sampling time of 1 h and did not include any bacterial species.

In the present study, we investigated the effect of sampling time and air humidity on the culturability of microorganisms collected by two commercially available filter samplers. The microorganisms were extracted from the polycarbonate filters by a method that combined vortexing with ultrasonic agitation. The extraction efficiency of this method and its effect on the relative culturability of sensitive microorganisms were evaluated for five microorganisms, including fungal spores, endospores and vegetative cells. The results may be used to estimate the composition and concentration of airborne microorganisms collected in the field by personal filter samplers.

2. Material and methods

2.1. Filter samplers

Two different personal filter samplers were used in this study: the standard 37-mm closed-face cassette and the Button Inhalable Aerosol Sampler. Both are commercially available devices used for personal and stationary aerosol sampling. The 37-mm cassette is made of conductive plastic, and the recommended flow rate is $2\,l\,min^{-1}$. The Button Sampler operates at a flow rate of $4\,l\,min^{-1}$, and collects particles on a 25-mm membrane filter, which is located behind its porous-metal inlet screen. The inlet screen consists of a spherical surface with a subtended angle of 160° and a porosity of 21%. Orifices of 381 μm diameter are evenly spaced throughout the curved surface which results in uniform particle deposition on the entire exposed area of the filter. In addition, the Button Sampler was found to be quite wind-insensitive (Aizenberg et al., 2000a). Black polycarbonate filters with a pore size of 0.2 μm (Millipore Co., MA) were employed for both samplers.

2.2. Microorganisms and their preparation

The testing was performed with five species of microorganisms: *Penicillium melinii* and *Aspergillus versicolor* fungal spores, *Pseudomonas fluorescens* and *Serratia marcescens* vegetative cells, and *Bacillus subtilis* var. *niger* endospores. These microorganisms are common in indoor and outdoor environments. The aerodynamic diameters of *P. melinii* and *A. versicolor* spores are approximately 3.1 and 2.4 μm, respectively. *P. fluorescens* and *S. marcescens* are gram-negative bacteria with an aerodynamic diameter of 0.7 and 0.8 μm, respectively. *B. subtilis* is a gram-positive bacterium with endospores, which have an aerodynamic diameter of ca. 0.9 μm. The aerodynamic diameters of these microorganisms were determined by use of an aerosol size spectrometer (Aerosizer; Amherst Process Instruments, Inc., Hadley, MA, now a division of TSI Inc.).

P. melinii and *A. versicolor* strains had previously been isolated from a moldy building. Prior to their use in the experiments, they were cultured in dispersion tubes containing malt extract agar,

and were then incubated at 25°C for 7 days. The dispersion tubes were inserted into an agar-tube disperser for dry spore generation, as previously described by Reponen et al. (1997). *B. subtilis* endospores were received from the US Army Edgewood Laboratories (courtesy of Agnes Akiyemi and Dr. Edward Stuebing, Edgewood Research, Development, and Engineering Center, Aberdeen Proving Ground, MD). The spores were activated at 60°C for 25 min and washed twice with sterile deionized water by centrifugation at 7000 rpm for 10 min. *P. fluorescens* (ATCC 13525) and *S. marcescens* (ATCC 13880) cultures were obtained from the American Type Culture Collection (Rockville, MD). They were subcultured by incubating in a trypticase soy broth at 28°C for 18 h. The cells were then washed twice, similar to the procedure used for *B. subtilis* spores. The bacterial suspensions of *B. subtilis*, *P. fluorescens* and *S. marcescens* were diluted with deionized water until a concentration of 10^7–10^8 ml^{-1} was achieved which resulted in a suitable aerosol concentration upon dispersion by a Collison nebulizer (see below).

2.3. Test system

The experimental set-up is shown in Fig. 1. The "dispersion unit" represents one of two aerosol generators used in this study. The first unit was an agar-tube disperser (Reponen et al., 1997) designed to release dry fungal spores. It was used for the aerosolization of *P. melinii* and *A. versicolor* spores at a flow rate of 15 $l\,min^{-1}$. The second generator was a three-nozzle Collison nebulizer (BGI Inc., Waltham, MA). This liquid generator was used to aerosolize *B. subtilis* spores as well as *P. fluorescens* and *S. marcescens* cells at a flow rate of 6 $l\,min^{-1}$. The bioaerosol flow was diluted with HEPA-filtered clean air at a flow rate of 30 $l\,min^{-1}$. The desired relative humidity of the air was achieved by passing dilution air through a silica gel drier or a humidifier. The conditioned air was HEPA-filtered before being mixed with bioaerosol flow. Finally, the diluted aerosol flow entered a test chamber where the two filter samplers were placed side by side. The temperature and relative humidity in the chamber were monitored by a sensor (TRH-100-20FT) and recorded by a data logger (Pocket Logger, model XR440) (both Pace Scientific, Inc., Charlotte, NC). In addition, an optical particle counter (Model 1.105; Grimm Technologies Inc., Douglasville,

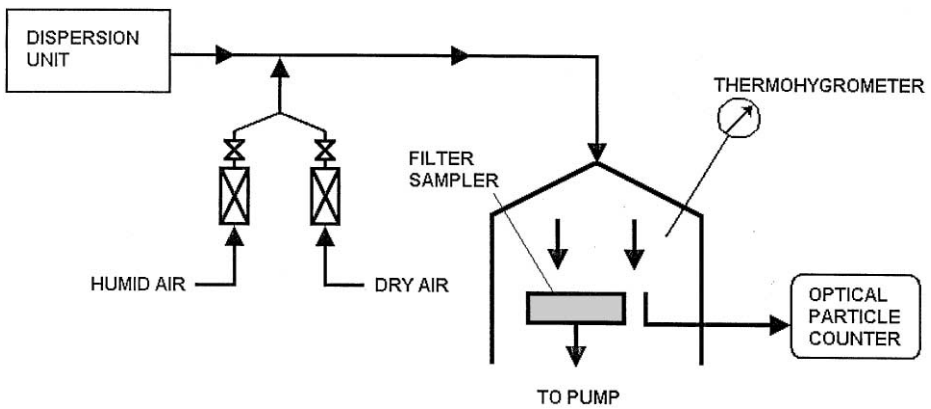

Fig. 1. Experimental set-up for the generation and collection of bioaerosols.

GA) was used to monitor the stability of the aerosol concentration in the chamber. The entire set-up was located in a biological safety cabinet (Model 6TX; Baker Co., Sanford, MA).

The tests were performed at two relative humidity (RH) levels: 30 and 85%. The temperature in the chamber ranged from 22 to 24°C during the tests. The sampling times ranged from 2 min to 8 h. In order to evaluate the influence of sampling time on the recorded bioaerosol concentrations, the aerosolization of microorganisms was discontinued after sampling for 10 min; after that only clean, HEPA-filtered air passed through the samplers. This limited the bioaerosol generation time to a maximum of 10 min and prevented bias from fresh microorganisms collected after $t = 10$ min. Furthermore, this resulted in similar filter loads for tests with different sampling times. Three variables were tested with each microorganism: sampler type, relative humidity and sampling time. Each experiment was repeated three times, and the results were reported as means and standard deviations.

2.4. Extraction of microorganisms from filters

The extraction method was modified from the CAMNEA method developed by Palmgren et al. (1986). Due to the open pores of the inlet screen, it was not practical to perform the extraction procedure while the filter was still held in the Button Sampler. Therefore, each filter was removed from the sampler immediately after completion of sampling, and was then subjected to extraction and analysis of the collected microorganisms. The extraction fluid consisted of 0.1% (w/v) sterile peptone water containing 0.01% Tween 80. The loaded filters were soaked with 20 ml of the extraction fluid in a centrifugal tube for 10 min. The extraction efficiency was compared for two methods: (1) vortexing only, and (2) vortexing followed by ultrasonic agitation. Vortexing was performed for 2 min with a vortex touch mixer (Model 231; Fisher Scientific, Pittsburgh, PA). Ultrasonic agitation was achieved by inserting the centrifugal tube containing the extraction liquid into an ultrasonic bath for 15 min (Branson Cleaning Equipment Company, Shelton, CT). Because of concerns that the extraction of microorganisms by ultrasonic agitation or vortexing may cause cell injury (especially for sensitive microorganisms such as *P. fluorescens* or *S. marcescens*), the relative culturability of *P. fluorescens* was determined after extracting them by the two extraction methods.

2.5. Microbial count

2.5.1. Culturable count in the extraction fluid

Part of the extraction fluid (5 ml) was diluted and inoculated on malt extract agar (DIFCO Laboratories, Detroit, MI) for the culturable count of *P. melinii* and *A. versicolor*, and on standard methods agar (Becton Dickinson and Company, Cockeysville, MD) for the culturable count of *B. subtilis*, *P. fluorescens* and *S. marcescens*. The culture plates with *P. melinii* and *A. versicolor* were incubated at 25°C for 4–7 days, the plates with *B. subtilis* at 37°C for 18 h, and the plates with *P. fluorescens* and *S. marcescens* at 28°C for 40 h. The culturable microbial count in the extraction fluid, C_{cfu}, was calculated as follows:

$$C_{\mathrm{cfu}} = (\mathrm{cfu}/10^{-n})(V_1/V_2), \tag{1}$$

where cfu is the average number of colony-forming units on the plates, n is the dilution factor, V_1 is the volume of extraction fluid applied to each filter sample (20 ml), and V_2 is the volume of diluted suspension spread on each agar plate (0.1 ml).

2.5.2. Total microbial count in the extraction fluid

For determining the total count, 1 ml of formaldehyde (37%) was added to the 15 ml of remaining extraction fluid. The resulting fluid was stained with 30 ml of acridine orange solution (0.1 mg ml^{-1}) for 5–10 min and filtered through a black polycarbonate filter. The filter was mounted on a microscope slide with immersion oil and a cover slip. An epifluorescence microscope was used to count the microorganisms on the filter at a magnification of 1000 × (Model Laborlux S; E. Leitz, Inc., available from W. Nuhsbaum Inc., McHenry, IL). Forty randomly chosen microscopic fields were counted.

The total microbial count in the resulting extraction fluid, C_{total}, was determined as follows:

$$C_{\text{total}} = N(\pi R^2/A)(V_1/V_3), \tag{2}$$

where N is the average microbial count on each microscope field, R is the effective radius of the filter (8.5 mm), A is the area of the microscope field (0.02404 mm^2), V_1 is the same as defined above for Eq. (1), and V_3 is the volume of the resulting extraction fluid that was stained and filtered (15 ml).

The culturable fraction of organisms in the extraction suspension, CF_{extr}, was defined as the culturable count (C_{cfu}) divided by the total count (C_{total}):

$$\text{CF}_{\text{extr}} = C_{\text{cfu}}/C_{\text{total}}. \tag{3}$$

In this study, we compared CF_{extr} with the original culturable fraction in the generation suspension CF_{orig}, by defining the relative culturability as follows:

$$\text{Relative culturability (\%)} = \text{CF}_{\text{extr}}/\text{CF}_{\text{orig}} \times 100\%. \tag{4}$$

CF_{orig} was calculated by Eqs. (1)–(3) for $V_1 = 1$ ml, $V_2 = 0.1$ ml, and $V_3 = 0.05$ ml. CF_{orig} was determined separately for each batch of microorganisms which were freshly prepared for each day of experiments. The value for CF_{orig} was found to be 1.0 for *P. melinii* and *A. versicolor*, from 0.3 to 0.7 for *B. subtilis*, from 0.2 to 0.5 for *P. fluorescens*, and from 0.3 to 0.9 for *S. marcescens*. The detection limit of the relative culturability ranged from 0.1 to 0.95%.

2.5.3. Remaining total count on the sampler filter

To calculate the extraction efficiency, it is necessary to enumerate the total microbial count remaining on the sampler filter after performing extractions. Since black polycarbonate filters can be used directly for epifluorescence microscopic counting, they were utilized as collection filters. After extraction, each filter was removed from the extraction fluid and stained with 5 ml of acridine orange solution for 5 min. The remaining microorganisms on the filter were counted under the epifluorescence microscope as described in the previous section.

The extraction efficiency (EE, %) was calculated as follows:

$$\text{EE}(\%) = C_{\text{total}}/(C_{\text{total.rem}} + C_{\text{total}}), \tag{5}$$

where the total microbial count in the extraction fluid (C_{total}) was calculated using Eq. (2), and the remaining total count on the filter after extraction ($C_{\text{total.rem}}$) was calculated by modifying Eq. (2) to the following:

$$C_{\text{total.rem}} = N(\pi R^2/A). \tag{6}$$

2.6. Bacterial recovery from filtration stress

The effect of filtration stress on the bacterial recovery was studied through direct inoculation of a suspension with a known concentration of bacteria on 25-mm polycarbonate filters, which were mounted on a filtration funnel. Then air was drawn through the filters for 5 min with *B. subtilis* and for 2 min with *P. fluorescens* at a flow rate of 4 $\mathrm{l\,min^{-1}}$ to simulate the dehydration stress of filter sampling with the Button Sampler. These time intervals correspond to the shortest sampling times which were used for these bacteria in the tests. The resulting filter samples were extracted and analyzed for bacterial culturability by the same method as the filters loaded with bioaerosols. This testing did not include aerosolization of the microorganisms, thus eliminating viability loss due to bioaerosol generation and impaction onto the filter.

2.7. Statistical analysis

All statistical analyses were performed using SAS/STAT procedures. For each type of microorganism, a general linear model (GLM) procedure was applied. The factorial experimental design had three factors: relative humidity, sampler type and sampling time. Each cell had three observations. The analyses were initially run on the full model containing all the interactions. The statistically insignificant interactions were then pooled in the model. Final analyses were performed with the reduced model. Once the model was determined to be appropriate, multiple comparisons of the means were conducted using the Scheffe's test available in the GLM procedure to test the effect of relative humidity, sampler type and sampling time. A family-wise significance level of 0.05 was used.

Simple *t*-tests were performed to compare the extraction efficiency of the two methods for extracting microorganisms from polycarbonate membrane filters: vortexing only versus ultrasonic agitation combined with vortexing. The effect of ultrasonic agitation on the relative culturability of *P. fluorescens* was also tested by a simple *t*-test.

3. Results and discussion

3.1. Extraction methods

Table 1 presents the extraction efficiency achieved when the microorganisms were extracted from polycarbonate membrane filters by the two extraction methods. The extraction efficiency was found to range from 77 to 85% when vortexing was applied to the filter samples for 2 min. The efficiency improved significantly (*t*-test: $p < 0.05$) to approximately 96–98% when vortexing was followed by 15-min agitation in an ultrasonic bath.

Table 1
Extraction efficiency (arithmetic mean ± standard deviation of three repeats) of two methods of extracting microorganisms from polycarbonate membrane filters[a]

Microorganism	Extraction efficiency (%)	
	Vortexing only	Vortexing + ultrasonic agitation
P. melinii	Not tested	96 ± 2
B. subtilis	85 ± 3	98 ± 1
P. fluorescens	77 ± 4	96 ± 1

[a]Sampling time = 10 min.

When studying the effect of ultrasonic agitation on the culturability of *P. fluorescens* cells, the average relative culturability was found to be 37% (ranging from 31 to 43%) when extraction was performed by vortexing only. It was 43% (ranging from 37 to 49%) when ultrasonic agitation followed the vortexing. The difference was not statistically significant (t-test: $p > 0.05$).

The data at Table 1 show that the second extraction method (vortexing for 2 min followed by ultrasonic agitation for 15 min) is sufficiently effective for extracting microorganisms from the filter samples. We also found that ultrasonic agitation performed in addition to vortexing does not reduce the relative culturability of the microorganisms, even for the most sensitive microorganisms tested, such as *P. fluorescens*. Therefore, vortexing followed by ultrasonic agitation was used in all other experiments. The extraction efficiency of this method was 96–98%, exceeding 80% achieved by the conventional CAMNEA procedure (calculated from the data presented by Heldal et al., 1996). The difference may partially be attributed to the fact that the filter was removed from the sampler before the extraction procedure was applied. Our procedure probably improves the extraction efficiency because the frequency and amplitude of excitation are more directly applied to the microorganisms for detaching from the filter when the filter is floating in the extraction fluid instead of being mounted on the sampler during extraction.

3.2. Effect of sampling time and relative humidity

3.2.1. Fungal spores

Figs. 2 and 3 show the relative culturability of *P. melinii* and *A. versicolor* fungal spores as a function of sampling time at a relative humidity of 30%. The figures represent experimental data collected at sampling times ranging from 10 min to 8 h. The mean relative culturability of *P. melinii* had its highest value of 109% at $t = 10$ min and its lowest value of 40% at $t = 8$ h, see Fig. 2. For *A. versicolor*, it reaches a maximum of 126% at $t = 10$ min (Button Sampler) and a minimum of 51% at $t = 8$ h (37-mm cassette), see Fig. 3. The difference between the initial relative culturability and 100% may be within the measurement accuracy of the experiments; i.e. application of the agitation procedures may not increase the culturability of the microorganisms. For both fungal species, the mean relative culturabilities obtained with the Button Sampler and the 37-mm cassette overlap within one standard deviation at every sampling time. Statistical analyses proved that the sampler type had no significant effect on the relative culturability of the *P. melinii* and *A. versicolor* spores. Analyses using the GLM procedure resulted in statistical evidence that the relative

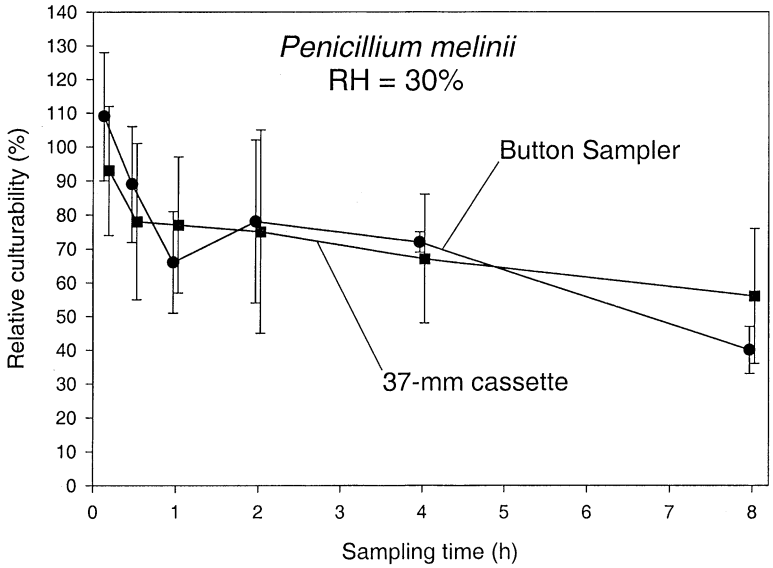

Fig. 2. Effect of sampling time on the relative culturability of *Penicillium melinii* spores at relative humidity (RH) of 30%. The error bars indicate the standard deviation of three repeats.

culturability of *P. melinii* and *A. versicolor* spores were affected by the sampling time during 8 h of sampling ($p < 0.05$). A multiple comparison was then performed through the Scheffe's test at a family-wise significance level of 0.05. The results indicate that the relative culturability of *P. melinii* was significantly higher at the sampling time of 10 min than after 8 h. For *A. versicolor*, the relative culturability at the sampling time of 10 min was higher than after 4 and 8 h. However, for the sampling time ranging from 30 min to 8 h — which is the usual sampling time when collecting airborne microorganisms with filter samplers — there was no statistically significant change in the relative culturability of *P. melinii* and *A. versicolor*.

The data for Figs. 2 and 3 were obtained at a relative humidity of 30%, because this RH level was assumed to be an unfavorable condition for microbial survival. Since the relative culturability at this low humidity did not decrease significantly with sampling time, as it increased from 30 min to 8 h, the tests at high humidity (RH = 85%) were performed only for two sampling times (10 and 30 min). Table 2 lists the relative culturability of *P. melinii* spores at RH = 30 and 85%. The mean relative culturability of *P. melinii* spores ranged from 77 to 109%. A paired *t*-test showed that there was no significant difference in the relative culturability of *P. melinii* spores at humidities of 30 and 85% ($p > 0.05$).

3.2.2. Endospores

The effect of sampling time and relative humidity on the relative culturability of collected *B. subtilis* spores is shown in Fig. 4. At RH = 30%, the relative culturability of *B. subtilis* spores decreased from 17 to 5% when the sampling time was increased from 5 min to 4 h for both the Button Sampler and the 37-mm cassette. At RH = 85%, the relative culturability decreased from 32 to 17% for the Button Sampler and from 32 to 5% for the 37-mm cassette. Since the relative

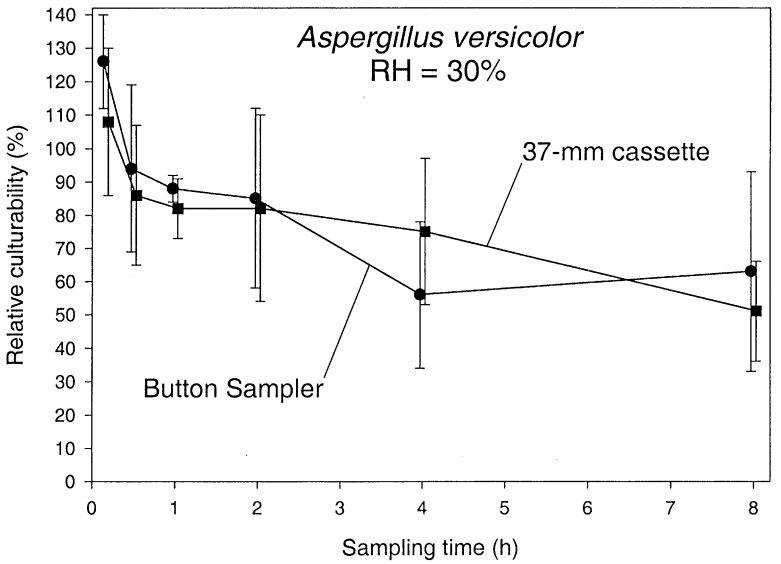

Fig. 3. Effect of sampling time on the relative culturability of *Aspergillus versicolor* spores at relative humidity (RH) of 30%. The error bars indicate the standard deviation of three repeats.

culturability had decreased to as low as 5% after sampling for 4 h, the 8-h sampling time was not analyzed for *B. subtilis*.

A GLM procedure was performed to the experimental data. The independent variables were sampling time, relative humidity and sampler type, and the dependent variable was microbial relative culturability. The analysis showed that all three independent variables were significant factors affecting the relative culturability of collected *B. subtilis* spores ($p < 0.05$). After removing insignificant interaction items from the structural model further analyses were performed for the data at each relative humidity level for each sampler type. Using Scheffe's test, a multiple comparison was also performed on the effect of sampling time. The results indicate that the relative culturability decreased significantly when the sampling time increased. This has been attributed to the desiccation stress on bacteria. The relative culturability was higher when airborne *B. subtilis* spores were collected by the Button Sampler than by the 37-mm cassette at a relative humidity of 85%. However, the analysis did not indicate a statistically significant difference when the two samplers were operated at RH = 30%. In addition, the analyses performed on the Button Sampler data at all tested sampling times indicate that the relative culturability was significantly higher at RH = 85% than at 30% ($p < 0.05$).

The significant effect of sampler type on the relative culturability of collected *B. subtilis* spores at a relative humidity of 85% can be explained by analyzing the differences between the two samplers. Under the experimental conditions, the effective face velocities for the filters of the Button Sampler and the 37-mm cassette were 26 and 4 $\mathrm{cm\,s^{-1}}$, respectively. This would implicate higher desiccation stress with the Button Sampler. However, the amount of water per unit filter area passing through the filters during 10 min, calculated using a psychrometric chart (ACGIH, 1995) at RH = 85% and a temperature of 23°C, is 0.3 $\mathrm{ml\,cm^{-2}}$ for the Button Sampler and 0.04 $\mathrm{ml\,cm^{-2}}$

Table 2
Effect of relative humidity (RH) on the relative culturability (arithmetic mean ± standard deviation of three repeats) of *P. melinii*, *P. fluorescens* and *S. marcescens* spores collected on filters with the Button Sampler and the 37-mm closed-face filter cassette

Microorganism	Sampling time (min)	Relative culturability (%)			
		Button Sampler		37-mm cassette	
		RH = 30%	RH = 85%	RH = 30%	RH = 85%
P. melinii	10	109 ± 19	77 ± 20	93 ± 19	99 ± 9
	30	89 ± 17	97 ± 28	78 ± 23	97 ± 9
P. fluorescens	2	0	2 ± 1	0	17 ± 5
	5	0	2 ± 1	0	6 ± 2
	10	0	0.8 ± 0.3	0	5 ± 1
	30	0	0	0	0
S. marcescens	2	0.2 ± 0.1	0.3 ± 0.2	0.1 ± 0.2	0.7 ± 0.8
	5	0.8 ± 0.4	0.1 ± 0.1	1.0 ± 1.2	4.3 ± 6.8
	10	0.2 ± 0.3	0.5 ± 0.8	0.1 ± 0.1	1.5 ± 2.4
	30	0	0	0	0

for the 37-mm cassette. The higher relative culturability of *B. subtilis* found with the Button Sampler as compared to the 37-mm cassette may thus be related to the higher amount of water per unit filter area passing through the filter at the higher humidity.

3.2.3. Bacterial vegetative cells

Taking into consideration the high sensitivity of *P. fluorescens* and *S. marcescens* cells, a shorter sampling time of 2 min was added to the test sequence when testing the effect of sampling time and relative humidity. At a relative humidity of 30% in the sampling chamber, the relative culturability was below the detection limit for *P. fluorescens* and below 1% for *S. marcescens* (see Table 2). At RH = 85%, the relative culturability was higher than at RH = 30%, but was still low with a maximum of 17 ± 5% for *P. fluorescens* and 4 ± 7% for *S. marcescens*. No colonies of either bacterial species were observed at RH = 85% when the sampling time was longer than 10 min.

The relative culturability of collected microorganisms is expected to be strongly dependent on the microbial species. After generation and collection of the three bacterial species, it was observed that the relative culturability did not exceed 17% at RH = 30%. Additional tests were performed to investigate the extent to which desiccation of bacteria on the Button Sampler's filter affects the recovery rate of *B. subtilis* and *P. fluorescens*. When aerosolization was excluded and the bacterial stress was thus associated only with exposure to air of RH = 30% passing through the filter, the resulting microbial relative culturability was 72 ± 20% for *B. subtilis* (5-min sampling) and 43 ± 6% for *P. fluorescens* (2-min sampling).

The relative culturability presented in Fig. 4 and Table 2 was affected by aerosolization, impaction and desiccation stress. At RH = 30%, the relative culturability was 17% for *B. subtilis* (5-min sampling) and 0% for *P. fluorescens* (2-min sampling) when using the Button Sampler. The

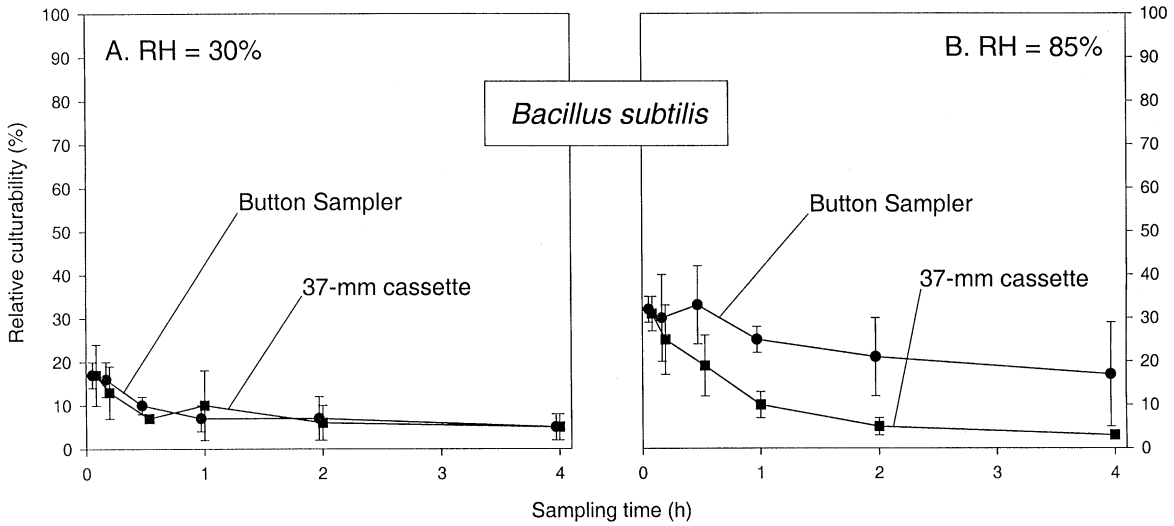

Fig. 4. Effect of sampling time on the relative culturability of *Bacillus subtilis* spores. The error bars indicate the standard deviation of three repeats. RH = relative humidity.

results of relative culturability caused simultaneously by the three factors of aerosolization, impaction and desiccation stress can now be compared with the results involving only desiccation stress. This comparison reveals that aerosolization during generation and microbial impaction on the filter accounts for an additional 55% loss in relative culturability for *B. subtilis* and a 43% loss for *P. fluorescens*.

4. Conclusions

Tests with five representative microbial species have shown that the personal filter sampling method maintains constant relative culturability when *Penicillium melinii* and *Aspergillus versicolor* fungal spores are collected during sampling periods ranging from 30 min to 8 h. The relative culturability of *Bacillus subtilis* spores did not exceed 17% at a relative humidity of 30% and 32% at a relative humidity of 85%. The relative culturability of sensitive *Pseudomonas fluorescens* and *Serratia marcescens* vegetative cells was near the detection limit when tested at a relative humidity of 30%; at RH = 85%, more colonies were observed if the sampling time did not exceed 10 min (the relative culturability was $\leqslant 17\%$). The relative culturability of collected bacteria generally increased when the relative humidity of the air was increased from 30 to 85%. The bioefficiencies determined for the Button Sampler and the 37-mm cassette did not show statistically significant differences in most of the tests. A significant difference in bioefficiency between the two samplers was observed only for *B. subtilis* at RH = 85%. This was attributed to the different amount of moisture collected on the filters of these samplers.

It should be noted that the above-discussed results of relative culturability include the combined effects of three factors: aerosolization, impaction and desiccation stress. A separate experiment

performed without aerosolization of microorganisms indicated that the combined effect of aerosolization and impaction on the filter accounts for 55% loss of culturability for *B. subtilis* and 43% loss for *P. fluorescens*.

The study revealed that a high extraction efficiency could be achieved (96–98% when the microorganisms were extracted from polycarbonate collection filters by a method combining vortexing with ultrasonic agitation. Ultrasonic agitation did not cause significant injury to *P. fluorescens* vegetative bacterial cells.

The results indicate that for sampling times typically used in filter sampling — 30 min or longer — only spores (fungal and bacterial) remain culturable, while bacterial vegetative cells quickly lose their viability, primarily due to desiccation stress. The culturability of bacterial spores is affected by sampling time and relative humidity more than that of fungal spores.

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