



Field Testing of a Personal Size-selective Bioaerosol Sampler

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Existing samplers for the collection of bioaerosols have been designed with the aim of maintaining biological stability of the collected material, and in general do not select particles in accordance with international conventions for aerosol sampling. Many have uncharacterised sampling efficiencies and few are designed as personal samplers. If standard personal dust samplers are used for bioaerosols the viability of collected microorganisms may be compromised by dehydration. The objective of this study was to evaluate a novel personal bioaerosol sampler designed to collect the inhalable dust fraction and further subdivide the sample into thoracic and respirable fractions. The new sampler was tested to see whether it enhanced the survival of the collected microorganisms, and was assessed for ease of use in the field and in subsequent laboratory analyses. A number of occupation-related field sites were selected where large concentrations of bioaerosols were to be expected. The prototype sampler was found to be simple to use. Analysis could be carried out with similar efficiency either with all three fractions together for a total count, or separately for size selective data. The sampler performed at least as well as the standard IOM filter method but with the added advantage of size fractionation. The field trials showed that for sampling periods lasting several hours, microorganism survival within the sampler was adequate for culture and identification of the organisms present. This new sampler is now commercially available. In addition to bioaerosol sampling, the principle of size selective sampling using porous foams can be applied to other occupational hygiene problems, and also to indoor air monitoring of PM10 and PM2.5 concentrations.. Crown Copyright © 1999 Published by Elsevier Science Ltd..

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INTRODUCTION

Exposure to airborne microorganisms or other bioaerosols can result in respiratory sensitisation (asthma and alveolitis) or toxicological effects in the lung (inhalation fever or organic dust toxic syndrome). This can contribute to progressively debilitating ill health, causing personal hardship and economic loss. Investigations into the extent of exposure to bioaerosols, the constituents of the bioaerosol, the sources of exposure and the effectiveness of control measures can be important in reducing the incidence of ill health. Methods are needed therefore to sample bioaerosols in the workplace, in order to both quantify the concentration, and to maintain the collected organisms in an intact state for subsequent characterisation. The numbers of cells pre-

sent and the identities of the prevalent species may be important in assessing potential health effects.

Sampling devices designed specifically to collect airborne microorganisms (bioaerosol samplers) have limitations. Most existing instruments are mains operated, static samplers (Henningson and Ahlberg, 1994). Their design is often driven by the need to culture collected microbial cells, with little attention paid to inlet or sampling efficiency. Consequently, most samplers can at best be regarded as 'semi-quantitative' for the purposes of exposure assessment (Griffiths *et al.*, 1997). Viable cascade impactors (for example, Andersen Microbial Sampler, Andersen, 1958) are capable of separating the aspirated aerosol into size fractions, but not in accordance with the current ISO/CEN conventions for inhalable, thoracic and respirable fractions (ISO, 1995; CEN 1993). Personal sampling for bioaerosols has mainly been carried out using the same personal filtration devices used for gravimetric dust monitoring. This

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has the disadvantage that the dehydration effect of collection onto a filter surface may compromise the viability of the collected microbes, and give poor detection rates for sensitive microorganisms (Mullenberg and Burge, 1994).

Against this background, we sought to develop an efficient, personal, size-selective sampler designed to collect ISO/CEN health-related fractions of bioaerosols and to maintain their viability and culturability. A new sampler was developed that incorporates two size-selective polyurethane foams (PUF) placed in series, in front of a polycarbonate filter. The aerosol collection substrates—thoracic selector foam, respirable selector foam and filter—are contained within an adapted cassette for use with a standard IOM gravimetric dust sampler (Mark and Vincent, 1986). The thoracic selector foam has 50% penetration for particles with aerodynamic diameters equal to 10 µm, and the respirable selector foam has 50% penetration for particles with aerodynamic diameters equal to 4.5 µm. These values were chosen to give the optimum (that is, lowest) sampling bias with respect to the CEN thoracic and respirable conventions, taking into account discrepancies between the foam size-selection characteristics and the target sampling conventions.

The development and laboratory evaluation of the adapted sampler has been fully described in earlier publications (Crook *et al.*, 1997a,b; Kenny *et al.*, 1998). The latter reference gives the design specifications for the polyurethane foams, filter and adapted IOM cassette, and these parts are now available commercially from the manufacturer of the IOM sampler [SKC (UK) Ltd]. With this new sampler (referred to as IOM-f) the inhalable fraction of the aerosol is subdivided into three parts. The respirable fraction of the aerosol is collected on the filter only; the thoracic fraction is the sum of material collected on the filter and that on the respirable selector foam; the inhalable fraction is the sum of material collected on the filter, the respirable selector foam and the thoracic selector foam (that is, the entire contents of the cassette).

This paper describes field trials carried out using the prototype personal bioaerosol sampler, to examine the practicalities of using it for on-site sampling work, and to compare its performance with existing bioaerosol sampling methods.

MATERIALS AND METHODS

The new sampler was tested at five field sites with the following objectives:

- to identify any operational problems that might arise during normal usage;
- to compare microorganism survival in the IOM-f with existing bioaerosol samplers, that is, the Andersen viable impactor (a mains operated static

impaction sampler which collects directly on to agar plates), and the standard personal IOM sampler with filter only;

- to assess the performance of each of the three sampling stages of the IOM-f;
- to develop suitable post-sampling handling procedures that ensure adequate microbial survival rates in the IOM-f;
- to assess the impact of sampling time on survival rates.

Preparation of samplers

Before use in the field both the (0.8 µm pore size, 25 mm diameter) polycarbonate filters and the PUF plugs must be sterilised. Prior to sterilisation the PUF plugs were washed in order to remove any manufacturing contaminants that might interfere with their size selective properties and, although less likely, microbial contaminants. The unwashed foams were taken directly from their factory delivery bags. Twenty-five foam plugs and 250 ml of distilled water were placed in a sonic bath for 20 min, after which the plugs were thoroughly rinsed with distilled water before being dried overnight at 70°C to drive off any remaining volatile residue. The same procedure was used for both types of foam plugs utilised within the IOM-f.

Polycarbonate filters are suitable for sterilisation by autoclaving, whereas the PUF structure is damaged by the high temperature. Alternatives are to soak overnight in 70% v/v ethanol or to use a standard UV light exposure method. Soaking in ethanol can result in the PUF plugs becoming rigid and this therefore increases the risk of the foam plug falling out of the cassette during sampling. This problem was not encountered with the UV exposure and so this method was adopted as the preferred method for PUF sterilisation. The adapted IOM-f cassettes, which were made from aluminium, were sterilised by overnight soaking in ethanol and subsequent flaming. Using sterile plastic gloves the PUF plugs and the filters were inserted into the cassettes, which were then placed in sterile plastic bags for transportation to the sampling site. Here, the loaded cassettes were installed into the sampler heads, again while wearing sterile gloves.

Post sample handling

Following sampling, and using sterile tweezers and plastic gloves, the foams and filter were removed from each cassette and placed in labelled sterile 30 ml vials containing 10 ml Peptone Inositol Tween (PIT—a biological buffer, comprising 0.1% peptone, 2.0% inositol and 0.05% Tween) solution for transportation back to the analytical laboratory. If the units were being analysed as a whole the filter and both foams and were placed together in the

same 10 ml of PIT. If the units were being analysed separately then each individual component was placed in a separate 10 ml of PIT. It is important when removing the foams that they are pushed down through the flanged end of the cassette and not up through the contaminated inlet. As an alternative procedure the intact exposed cassettes were placed in sterile plastic bags for transportation to the laboratory. They were dismantled into PIT as above immediately before analysis. Sites chosen were within 2 h travel of the laboratory. On reaching the laboratory the samples were stored overnight at 4°C (in vials or bags) and then analysed the next day.

CHOICE OF FIELD SITES

Candidate sites were identified where there was expected to be a significant number and variable species of airborne microorganisms present. Volunteer workers were selected at each site and equipped with a torso harness, two battery-powered personal sampling pumps and two personal samplers (IOM and IOM-f). The samplers were mounted on the same side of the harness within the breathing zone. Sampler flow rates were set at 2.0 l min⁻¹ and checked before, during and after the sampling period using a calibrated bubble flow meter. Sampling periods typically ranged from 3 to 6 h.

Engineering works No. 1

Workers using lathes cooled by oil in water emulsion metalworking fluids may be exposed to large concentrations of bacteria that colonise the fluids. The volunteer workers were engaged in the following activities: grinding, turning, drilling, milling and lathe operation. In this visit, microorganism survival in the IOM-f was compared to a standard IOM personal inhalable sampler used with a polycarbonate filter. Each worker was fitted with one standard IOM and one IOM-f sampler. At the end of sampling, the cassettes from each IOM-f (containing two PUF plugs and a polycarbonate filter) were placed intact into the same PIT solution for subsequent analysis as if they were one substrate. The cassettes from the standard IOM sampler (containing filter only) were treated in the same way.

Waste water treatment

Sewage and waste waters are heavily contaminated by bacteria and maintenance workers may be exposed to aerosols created during aeration and filtration processes. In this visit the viable microorganisms sampled using the IOM-f were compared to those detected using the Andersen viable impactor. Additionally, two post-sampling handling procedures for the IOM-f were compared. One volun-

teer worker was engaged in blower house maintenance and the other in aeration tank probe cleaning. Due to the small number of workers at the site (only two) some additional sampling was also carried out using personal samplers mounted on two life-size manikins. These manikins wore identical equipment to the workers and were placed in fixed positions above the aeration tanks. Two fixed sites were chosen for the Andersen sampling. The first was directly above the sewerage aeration tanks, close to one of the fixed manikins, and the second was inside the blower house. All personal samples were taken using the IOM-f, two of which were mounted on each worker or manikin. Following sampling, one of the IOM-f cassettes from each worker or manikin was unloaded directly into PIT solution (all three stages together) and the other paired cassette was placed, intact, into a sterile plastic bag.

Compost preparation

Large concentrations of microorganisms are fundamental to the preparation of compost from vegetation waste and workers handling the compost, for example, spawning mushroom compost, bagging prepared compost, may be exposed to bioaerosols. The factory visited was mixing and packaging compost prepared from wood bark, and this was chosen to compare results from the three stages (two foams and one filter) of the IOM-f when analysed together to the three stages of the IOM-f when analysed separately. The volunteer workers were a front-end loader driver and two packers. Manikins were again used owing to the small number of workers at the site. The manikins wore identical equipment to the workers and were placed close to the compost mixing hoppers. All personal samples were taken using the IOM-f, two of which were mounted on each worker or manikin. After sampling, one cassette from each worker or manikin was unloaded into a single PIT solution (all three stages together) and the other cassette was unloaded into three separate PIT solutions (that is, one for each stage).

Wool sorting

Raw cotton or wool are contaminated by microorganisms, to which workers may be exposed during scouring and blending processes. The wool sorting house presents an environment with high concentrations of dust from unscoured fleeces, providing a rich bioaerosol microflora. Four of the volunteer workers were engaged in manual sorting of the raw wool and another in packing the sorted wool. The aim of the field visit was to compare the viable performance of the IOM-f to the IOM personal inhalable sampler used with a polycarbonate filter (that is, similar to the first visit but in a different environment). Each volunteer was fitted with one IOM and

one IOM-f personal sampler for comparison. The three stages from each IOM-f (two PUF plugs and a polycarbonate filter) were placed together in the same PIT solution and were analysed as if they were one substrate. The filter-only cassettes from the standard IOM samplers were treated in the same way.

Engineering works No. 2

This visit sought to assess the effects of sampling time on the survival of microorganisms in the IOM-f. The engineering works used high-speed grinding processes with a mineral-oil-based metal working fluid. An array of IOM-f samplers was co-located in the breathing zone of a life-sized manikin and exposed to a metal working fluid bioaerosol. The manikin was equipped with a torso harness, five personal pumps and five IOM-f samplers. The samplers were mounted across the upper torso of the manikin within the breathing zone. Sampler flow rates were set at 2.0 l. min⁻¹ and checked hourly using a calibrated bubble flow meter. The manikin was positioned approximately 1 m downwind of the gentle air stream created by the grinding machine and was orientated to maximise exposure to the metal working fluid mist.

Sample time duration ranged from 1 to 6 h. Six consecutive 1 h samples provided a base line of measurements of airborne bioaerosol concentration. The concentrations measured over 2, 4 and 6 h periods were compared with the sums of the 1 h measurements taken over the same periods of time.

After sampling all IOM-f cassettes were removed from the IOM sampling heads using sterile tweezers and gloves. The substrates were then immediately placed in 10 ml of PIT. Some of the IOM-f samples were divided into their three component parts (foams 1 and 2 and the polycarbonate filter) following sampling. These were placed in separate vials of PIT for three-stage analysis to provide data on the inhalable, thoracic and respirable bioaerosol fractions. All other IOM-f samples had the three substrates placed together in one vial of PIT and were analysed together to provide data on only the inhalable fraction of the bioaerosol.

LABORATORY ANALYSIS

Extraction

Filters in PIT were whirlmixed for 1 min to resuspend the collected particles. Foams (either separately or together with the filter according to the different analyses) were whirlmixed for 1 min and then plunged rapidly for 1 min *in situ* using a sterile 20 ml hypodermic syringe plunger. This procedure had been found in earlier experimentation by the authors to maximise the extraction of microbial cells from the foams (Kenny *et al.*, 1998). Using this

method in laboratory tests, average yields of the bacteria *Bacillus subtilis*, *Pseudomonas alcaligenes* and *Escherichia coli* "spiked" into foams were 78%, 72% and 82% of the inoculum respectively.

Dilutions and plating out

Five ten-fold serial dilutions (to 10⁻⁵ of original suspension) were prepared from each suspension, and 0.1 ml of each dilution (plus 0.2 ml of undiluted sample if a low bioaerosol count was anticipated) was spread on to the surface of duplicate agar plates. A range of media relevant to the expected microbial species (depending on where the samples originated) was used as follows:

- Nutrient agar at 25°C to isolate mesophilic (MP) bacteria;
- Fastidious anaerobe agar (incubated aerobically) at 30°C to isolate mesophilic bacteria. This medium has been found to assist recovery of stressed bacteria, especially from metalworking fluids;
- Nutrient agar at 37°C to isolate bacteria capable of growth at human body temperature;
- MacConkey agar at 37°C to isolate coliform bacteria;
- R8 agar at 55°C to isolate thermotolerant (TT) and thermophilic bacteria and actinomycetes;
- Malt extract agar at 25°C to isolate mesophilic (MP) fungi and yeasts;
- DG18 agar at 25°C to isolate xerotolerant (XT) fungi, which has been found to perform well in isolating aerosolised fungi (Verhoeff *et al.*, 1990).

All plates were incubated at the appropriate temperatures and emerging colonies were counted after 4 and 7 days' incubation. The total number of colony forming units (CFU) per plate was used to calculate airborne viable cell concentrations. Depending on the volume of air sampled, this gave a lower limit of detection of 70 CFU per m³ for the longest sampling period of 6 h. All samples were analysed in duplicate.

Staining and epifluorescent microscopy cell counts

Subsamples of the suspensions prepared as above were prepared for direct epifluorescent microscopy counting (DEFT) using the CAMNEA method (Plamgren *et al.*, 1986, modified by Heldal *et al.*, 1996). One millilitre of each neat sample was drawn under vacuum through a black, 0.2 µm pore size, 25 mm polycarbonate membrane filter. The filter was overlaid with Acridine Orange stain and then rinsed with buffer and isopropyl alcohol. The filter was air-dried and placed on a microscope slide in a small drop of non-fluorescent immersion oil, another drop was placed on the top of the filter and sandwiched between a 25 mm diameter coverslip.

Slides were examined under a Zeiss epifluorescent microscope with UV light at an excitation wavelength of 490 nm, and the cells counted at ×1000

magnification. A total count (orange + green fluorescing cells) and a viable count (orange fluorescing cells only) was made. These data were used to calculate total airborne cell concentrations and viable cell concentrations. Using this method the lower detection limit for the number of cells per cubic metre of air was 8.82×10^3 .

Acridine orange fluorescent staining and microscopic counting allows a very high proportion of microbial cells present to be counted. Recent evidence of the ability of this technique to detect Gram-negative bacteria has been presented by Heidelberg *et al.*, (1997). The proportion of viable to non-viable acridine orange stained cells for three bacterial species was observed to remain fairly constant over 4 h of sampling by liquid impinger, while numbers of culturable bacteria declined considerably. This demonstrates the tendency for bacteria to enter a non-culturable but viable state under aerosolisation stress and adverse sampling conditions. Possible limitations to detection of microbial cells may be found however with some fungal spores, such as *Rhizopus* spp., which are reported to turn translucent under fluorescent staining conditions rendering them difficult to see (Blomquist, G; Eduard W., personal communication).

Treatment of data

Only the field visits to the composting factory and woollen mill provided results in sufficient numbers to allow statistical analysis. Simple unpaired *t*-tests were applied to the complete sets of results for total viable counts using the statistical graphs package Sigmaplot. For the compost field visit, unpaired *t*-tests were performed to identify any significant difference between the "all-in-one" and separate three-stage methods of analysis previously described. For the wool field visit, unpaired *t*-tests were performed to identify any significant difference between the IOM-f and the conventional IOM results.

RESULTS

Engineering works No. 1 and wastewater treatment

Bioaerosol yields from Field Visits 1 (engineering works) and 2 (wastewater treatment plant) are summarised in Tables 1 and 2 respectively. Although the visits provided useful opportunities to test the sampler in the field, and general observations could be made about the performance of the IOM-f sampler, the total cell counts were too low to allow statistical comparisons of the data.

Bioaerosols at the engineering works No. 1 were almost exclusively Gram-negative bacteria. This finding is consistent with previous studies of bacterial growth within contaminated metalworking emulsion (Thorne *et al.*, 1996). Yields with both types of sampler were low, ranging from below the threshold for detection, up to concentrations of 6×10^3 CFU per m^3 .

Bioaerosol yields at the wastewater treatment plant were also low, with some samples below the level of detection, up to concentrations of 3×10^3 CFU per m^3 . Site observations suggested that this was a result of the general conditions (outdoor environment, steady dispersing breeze and little aerosolisation) rather than poor performance of the samplers, as there was a low yield both with the IOM-f sampler and with the Andersen impactor, which inoculates directly onto agar plates. Further evidence of low bioaerosol concentrations was the failure to isolate coliforms on MacConkey agar with either sampler. The bacteria isolated were predominantly general environmental species and the fungi were mainly *Cladosporium* spp.

Compost plant

At the compost handling plant each worker wore two IOM-f samplers, one of which was analysed by extracting all three substrate components (thoracic selector foam + respirable selector foam + filter) together and the other was analysed by extracting the three components separately. As may be expected where compost was being handled (Crook *et al.*, 1994), predominant isolates included the fungi

Table 1. Bioaerosol sampling at engineering works using prototype porous foam bioaerosol sampler (IOM-f) and conventional IOM sampler (IOM filter)*

Work task	Foams and filters	CFU per m^3 nutrient at 25°C	CFU per m^3 nutrient at 37°C	CFU per m^3 malt at 25°C
Grinding	IOM-f	9.55×10^2	6.37×10^2	4.77×10^2
	IOM filter	3.19×10^2	6.37×10^2	4.77×10^2
Turning	IOM-f	2.52×10^3	3.46×10^3	7.86×10^2
	IOM filter	7.86×10^2	2.36×10^3	1.57×10^2
Drilling	IOM-f	6.21×10^2	ND	6.21×10^2
	IOM filter	4.66×10^2	3.10×10^2	6.21×10^2
Milling	IOM-f	6.17×10^3	4.32×10^3	4.63×10^2
	IOM filter	ND	4.63×10^2	ND
Lathing	IOM-f	6.1×10^2	7.62×10^2	3.05×10^2
	IOM filter	ND	4.57×10^2	4.57×10^2

* ND = none detected.

Table 2. Analysis of bioaerosols at waste water treatment plant*

(a) Comparison of yields from placing samples into diluent (PIT) on site or immediately prior to analysis in the laboratory next day						
Work task	Sample process	CFU per m ³ nutrient at 25°C	CFU per m ³ nutrient at 37°C	CFU per m ³ MacConkey 37°C	CFU per m ³ malt at 25°C	
Blower house maintenance	PIT on site	ND	1.97×10 ²	ND	ND	
	PIT in lab.	9.84×10 ²	3.94×10 ²	ND	3.94×10 ³	
	PIT on site	ND	ND	ND	1.10×10 ³	
Probe cleaning	PIT in lab.	5.43×10 ²	2.72×10 ²	ND	2.72×10 ²	
	PIT on site	1.41×10 ³	1.01×10 ²	ND	5.04×10 ²	
Manikin 1 aeration	PIT in lab.	5.04×10 ²	ND	ND	1.71×10 ³	
	PIT on site	ND	8.9×10 ¹	ND	3.19×10 ³	
Manikin 2 aeration	PIT in lab.	6.21×10 ²	8.9×10 ¹	ND	1.51×10 ³	
	PIT on site	ND	3.29×10 ²	ND	1.54×10 ³	
Sampling	PIT in lab.	4.39×10 ²	1.09×10 ²	ND	1.43×10 ³	

(b) Area samples taken using Andersen microbial impactors collecting directly onto agar plates						
Site	CFU per m ³ nutrient at 25°C	CFU per m ³ nutrient at 37°C	CFU per m ³ MacConkey 37°C	CFU per m ³ malt at 25°C		
1: Aeration tank	2.13×10 ³	3.09×10 ²	ND	1.27×10 ³		
2: Blower house	2.30×10 ³	3.99×10 ²	ND	1.25×10 ³		

* ND=none detected.

Table 3. Analysis of bioaerosols at compost handlers. Comparison of yields of culturable and total collected microorganisms between IOM-f and the total of its individual components analysed separately*

Task	Foam and filter	Total† CFU per m ³	DEFT viable cells per m ³	DEFT non-viable cells per m ³	DEFT total‡ cells per m ³
Driver front-end loader	IOM-f all-in-one	3.98×10 ⁵	1.27×10 ⁵	2.80×10 ⁵	4.07×10 ⁵
	Total	3.99×10 ⁵	1.04×10 ⁶	0	1.04×10 ⁶
Bagger	IOM-f all-in-one	8.24×10 ⁵	1.60×10 ⁶	1.20×10 ⁶	2.80×10 ⁶
	Total	7.65×10 ⁵	1.47×10 ⁶	8.48×10 ⁵	2.32×10 ⁶
Bagger	IOM-f all-in-one	4.50×10 ⁵	1.52×10 ⁶	4.42×10 ⁵	1.96×10 ⁶
	Total	6.35×10 ⁵	3.70×10 ⁵	2.82×10 ⁵	6.52×10 ⁵
Manikin 1 wood bark hopper	IOM-f all-in-one	1.09×10 ⁶	1.12×10 ⁷	8.78×10 ⁵	1.21×10 ⁷
	Total	1.11×10 ⁶	1.85×10 ⁶	7.17×10 ⁵	2.57×10 ⁶
Manikin 2 fine wood bark hopper	IOM-f all-in-one	7.14×10 ⁴	2.52×10 ⁷	6.50×10 ⁵	2.59×10 ⁷
	Total§	8.98×10 ⁵	1.79×10 ⁶	2.46×10 ⁵	2.04×10 ⁶

* IOM-f all-in-one = all components (foams + filter) extracted together.

† Total CFU = sum of maximum yields for each microbial group.

‡ Total cells = sum of "viable" and "non-viable" counts by direct epifluorescence microscopy (DEFT).

§ Total = sum of yields from each component (thoracic selector foam + respirable selector foam + filter) extracted separately.

Paecilomyces and *Aspergillus* spp. and the actinomycetes *Streptomyces*.

Table 3 shows the overall results for culturable and total counted cells. Fig. 1 shows the ratios of viable cell concentrations for the “all-in-one” analysis to the sum of the viable cell concentrations for three substrate components. Yields were also compared separately for different analytes; bacteria grown on nutrient agar at 25°C, bacteria grown on nutrient agar at 37°C, thermotolerant bacteria and actinomycetes grown on R8 agar at 55°C, fungi grown on malt agar at 25°C and xerotolerant fungi grown on DG18 agar at 25°C. Fig. 2 shows the proportions of each type of microorganism in different particle size fractions, calculated from the analyses of the individual IOM-f substrates. Results for the different work activities are pooled as the same trends are evident for each volunteer subject. The inhalable fraction is the sum of analyses from both selector foam substrates + filter; the thoracic fraction is found on the filter + the respirable selector foam, and the respirable fraction is on the filter only.

Wool sorting

Each worker wore one IOM-f sampler and one conventional IOM sampler. The IOM-f sampler was analysed by extracting all three components (thoracic selector foam + respirable selector foam + filter) together and compared to yields from the IOM sampler with filter only. The results for culturable and total direct counted cells are summarised in Table 4. A large proportion of the yields on fungal isolation plates (malt agar and DG18) were yeasts, so these were totalled separately to provide further data for comparison. Fig. 3 shows the ratios of viable cell concentrations for the “all-in-one” IOM-f analysis to the viable cell concentrations for the conventional IOM filter-only analysis.

Engineering works No. 2

In the second engineering works the numbers of viable microorganisms were low and statistical errors in the counts are therefore relatively large. Consequently the reported viability effects are not statistically significant. However, the results derived from the fastidious anaerobic agar growth conditions provided some useful information despite viable counts being low (of the order of 1×10^1 CFUs per undiluted sample). Fig. 4 shows the relative viable efficiency of the 2, 4 and 6 h samples compared to the sum of the 1 h samples covering the same period and terminating at the same time. Fig. 5 illustrates the subdivision of the viable inhalable bioaerosol into thoracic and respirable fractions for the 1, 2, 4, and 6 h samples, all of which terminated at the same time. No viable cells were detected on the filters from these samples, suggesting either that bacteria were associated with

Table 4. Analysis of bioaerosols at wool sorters. Comparison of yields of culturable and total collected microorganisms between IOM-f components analysed together and conventional IOM sampler*

Task	Foam and filter	Total† CFU per m ³	DEFT viable cells per m ³	DEFT non-viable cells per m ³	DEFT Total‡ cells per m ³
Wool sorting	IOM filters	6.14 × 10 ⁶	5.95 × 10 ⁶	9.29 × 10 ⁵	6.88 × 10 ⁶
	IOM-f all-in-one	5.82 × 10 ⁶	3.01 × 10 ⁶	9.91 × 10 ⁵	4.00 × 10 ⁶
Wool sorting	IOM filters	3.68 × 10 ⁶	1.60 × 10 ⁶	9.85 × 10 ⁵	2.59 × 10 ⁶
	IOM-f all-in-one	3.21 × 10 ⁶	2.65 × 10 ⁶	2.11 × 10 ⁶	4.76 × 10 ⁶
Wool packing	IOM filters	2.93 × 10 ⁵	1.35 × 10 ⁵	1.35 × 10 ⁵	2.70 × 10 ⁵
	IOM-f all-in-one	3.60 × 10 ⁵	9.64 × 10 ⁵	4.39 × 10 ⁵	1.40 × 10 ⁶
Wool sorting	IOM filters	2.62 × 10 ⁶	1.31 × 10 ⁶	1.52 × 10 ⁵	2.83 × 10 ⁶
	IOM-f all-in-one	5.69 × 10 ⁶	1.05 × 10 ⁶	1.35 × 10 ⁶	2.40 × 10 ⁶
Wool sorting	IOM filters	2.77 × 10 ⁶	1.31 × 10 ⁶	9.43 × 10 ⁵	2.25 × 10 ⁶
	IOM-f all-in-one	7.45 × 10 ⁶	3.75 × 10 ⁶	6.94 × 10 ⁵	4.44 × 10 ⁶

* IOM filters = Standard IOM sampler with polycarbonate filter; IOM-f all-in-one = all components of IOM-f sampler extracted together.

† Total CFU = sum of maximum yields for each microbial group.

‡ Total counts = sum of “viable” and “non-viable” counts by direct epifluorescence microscopy (DEFT).

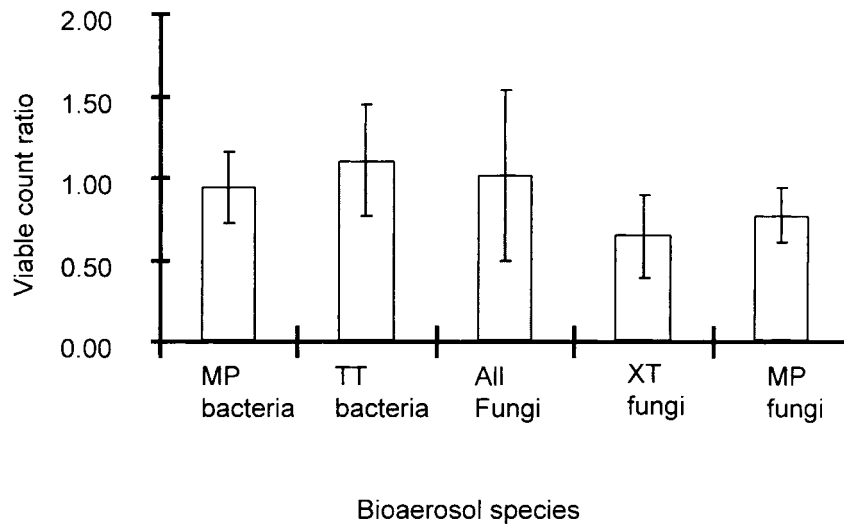


Fig. 1. Ratio of viable cell concentrations for "all-in-one" IOM-f analysis compared to the sum of analyses for individual IOM-f substrates, in the composting plant. Note MP = mesophilic; TT = thermotolerant; XT = xerotolerant.

non-respirable particles, or possibly that the survival rates on the polycarbonate filter were low in this case.

To demonstrate the bioaerosol source, two bulk samples of metal working fluid were taken from the sump of a milling machine at the field site and analysed, for culturable bacteria only, by diluting and plating out onto fastidious anaerobe agar incubated at 300°C. These gave an average count of $4.9 \times 10^7 + 0.5 \times 10^7$ CFU per litre, demonstrating considerable biological activity in the fluid.

DISCUSSION

Analytical methods

Although plate counts and enumeration expressed as "CFU per m³ air sampled" is the counting con-

vention generally used in bioaerosol sampling, it is acknowledged to have limitations in terms of the information it presents on the total number of intact microbial cells present. Spread plate and colony counting procedures measure only those microbial cells capable of growing on agar plates under the conditions provided. Therefore the total number of cells present may be greater, including those intact but no longer viable and those classed as "non-culturable but viable" (NCBV). Both may still retain allergenic and toxic properties, so it may be useful to record their presence. Alternatives to CFU counting include the measurement of a microbial product, such as endotoxin, where the important microflora are Gram-negative bacteria, or adenosine triphosphate (ATP). The usefulness of endotoxin assay in estimating total Gram-negative bacteria is the sub-

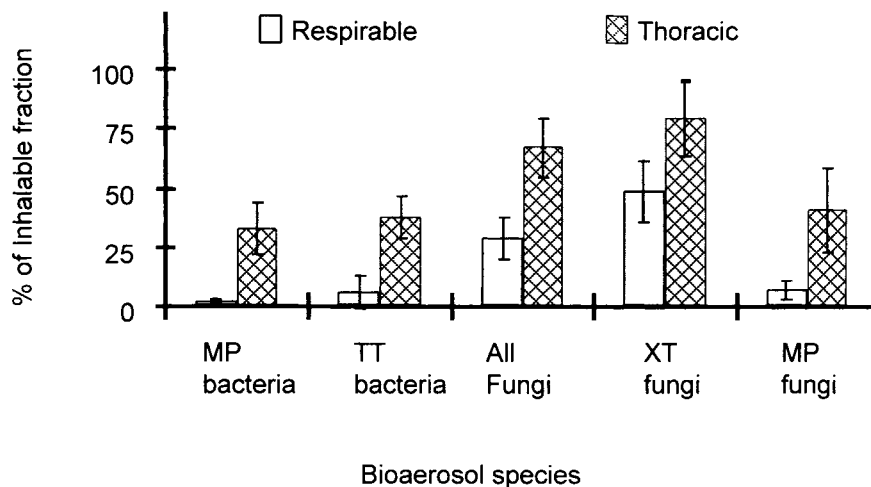


Fig. 2. Subdivision of the viable inhalable bioaerosol into thoracic and respirable subfractions, in the composting plant. Abbreviations as before.

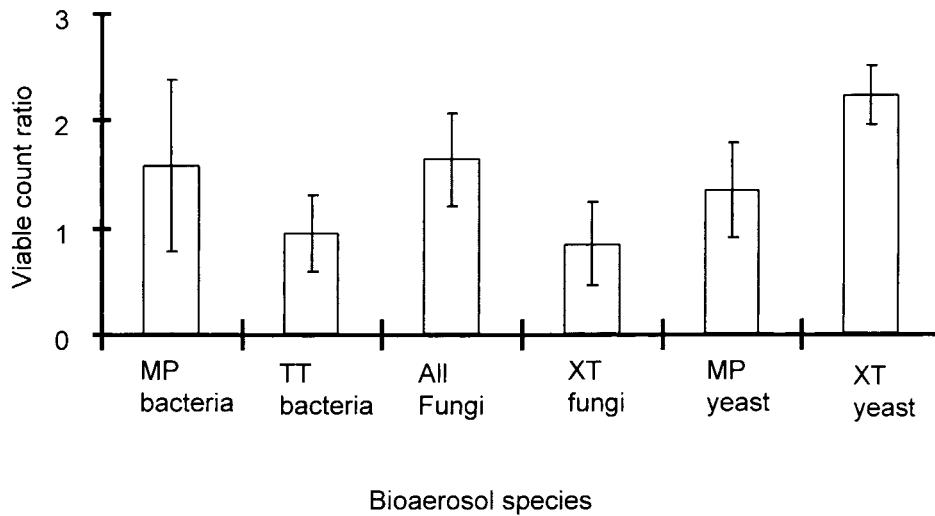


Fig. 3. Ratio of viable cell concentrations for "all-in-one" IOM-f analysis compared to conventional IOM sampler, in the wool sorting house. Abbreviations as before.

ject of an ongoing study, concerned particularly with endotoxins in metalworking fluids (more details are available from the authors). The potential for ATP analysis is discussed further by Crook and Sherwood-Higham (1997). Total counting by fluorescence microscopy, which combines direct microscopic techniques with fluorochrome staining, does not rely on cell culture. The method of collection is less critical therefore in terms of retaining culturability, although with some fluorochromes an estimate of viability can also be made from the fluorescent properties.

Direct microscopic measurement, using fluorescent staining techniques, is a less well established technique than culture based estimations of bioaerosol numbers, although microscopy is used frequently in other areas of environmental microbiology, such

as water bacteriology. Potential limitations associated with the CAMNEA or similar methods include unstable and non-specific fluorescence of non-microbial particles of similar size and shape to microorganisms, destaining of small microbial cells, losses during preparation or inability to count cells bound up in aggregates (Heldal *et al.*, 1996). Counting precision could be subject to operator error. Careful preparation of samples and microscopy to well validated protocols reduce these potential errors and increase the scope of the method. Newer alternatives to acridine orange as a fluorochrome, such as the nucleic acid binding dye SYTO13, offer further scope for fluorescent microscopic counting (Mason *et al.*, 1998).

In this project direct fluorescence microscopy counting generally yielded greater numbers than was

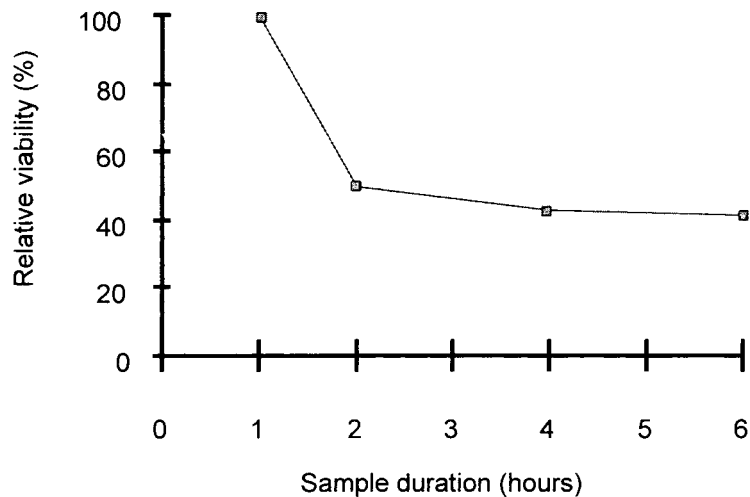


Fig. 4. Relative survival rates for microorganisms as a function of sampling time, referenced to 1 h samples, in the engineering works.

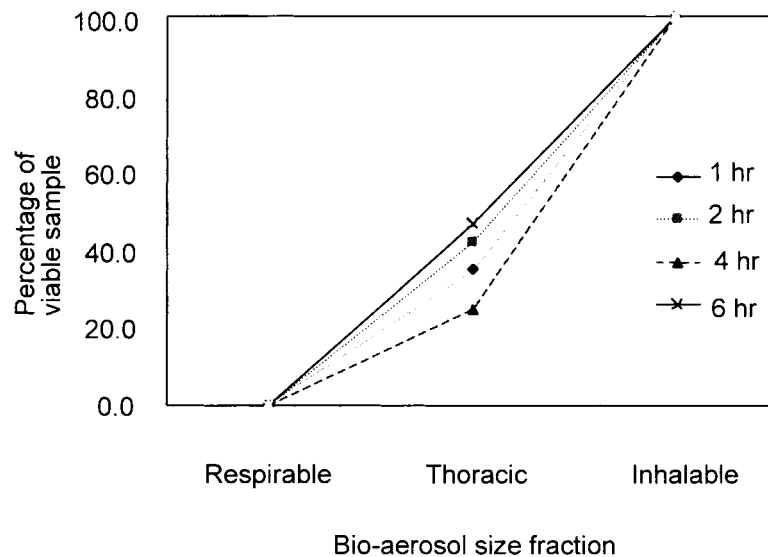


Fig. 5. Size fractionation of the bioaerosol as a function of sampling time, in the engineering works.

found with enumeration of colony forming units. This was as expected, because CFU counting will under-represent stressed cells unable to respond to growth conditions on agar plates. However, the relationship is not constant and obviously depends on the source of the bioaerosol. At the wool sorting factory the numbers obtained using both methods were similar. Here there were a large number of biologically robust yeasts, which may have increased the proportion of culturable cells in the bioaerosol. As direct microscopic measurement is a less well established technique, there is a need for further work to develop the method.

Observations on the usability of samplers in the field

The IOM-f sampler presented no differences in its use from the conventional IOM sampler. Special requirements applying to bioaerosol sampling in general include aseptic handling of samples, which is a fundamental requirement, and storage and transportation of bioaerosol samples. It was not possible to address all questions on this second point within the scope of this project; however, the procedures used presented few practical problems and yielded satisfactory results. One practical point was the possibility of foams becoming dislodged from cassettes during sampling. Two possible solutions are either that the inside of the cassette could be roughened to ensure better grip at the edges of the foam or that cross wires could be introduced at the inlet to hold the foams in place. The last solution would be valid because the best practice is to remove the foams from the back of the cassette.

Comparison of sampling methods and procedures

Statistically there was no significant differences between yields for the IOM-f analysed as all com-

ponents together (“all-in-one”) or for the three components analysed separately and the yields totalled, although indications are that a slightly greater yield was achieved from analysing the three components separately. Statistically there were no significant differences between yields for the IOM-f “all-in-one” and the conventional IOM sampler as used for bioaerosol sampling. General observation of the data however would indicate that a slightly greater yield was achieved with the IOM-f sampler, as shown in Fig. 3. Table 2 indicates higher CFU concentrations measured using the Andersen impactor when compared to the IOM-f sampler; however, it is not appropriate to make quantitative comparisons between these methods. The aspiration rates are greatly different (2 l. min^{-1} for IOM-f and 28 l. min^{-1} for the Andersen impactor), the Andersen collects directly on to agar medium, hence it is run for only short periods to avoid overloading. Consequently the two samplers cannot be run together over the same sampling period; the Andersen sampling time is much shorter. Qualitatively, the results from the two methods were comparable in that all microorganism species detected in the Andersen samples were also detected in the IOM-f samples.

The results for different sampling times, although again not statistically significant, indicate that the maintenance of the viability of the sample falls sharply during the first 2 h of sampling and thereafter falls much more slowly. While the decrease in viability over extended sampling periods may not compromise the qualitative analysis of organisms present it eliminates the possibility of quantitative analyses by culture-based methods. There was not any strong evidence to suggest that one particular bioaerosol fraction was more affected than another as sampling time was changed (see Fig. 5).

The particle size information provided by the IOM-f gives some useful insights into the nature of the inhaled bioaerosols. For example, it is possible to see in Fig. 2 that a greater proportion of the xero-tolerant fungal spores were respirable than was the case for bacteria, despite fungal spores being larger than individual bacterial cells. This suggested that bacteria were present in air as aggregates or associated with larger inert particles.

CONCLUSIONS AND FURTHER WORK

This field study has demonstrated the potential usefulness of the IOM-f personal bioaerosol sampler. In the comparisons that were possible (although not always statistically significant), the IOM-f performed at least as well and generally better than the conventional, filter-only, IOM sampler in terms of the ability to collect bioaerosols and maintain them in a viable and culturable state. An additional benefit is the availability of size fractionation with the new sampler, which will facilitate an understanding of the health effects associated with bioaerosol exposure. The new sampler is simple to use and, being an adaptation of an existing sampler, provides a low-cost method that is accessible to wider uses in addition to bioaerosol sampling. Further work to investigate the sampler's performance, limitations, and potential applications is currently underway. The principle of size selective collection using porous foams can be applied to other airborne particulate monitoring problems, including PM10 and PM2.5 sampling.

The results obtained in this work highlight the need to quantify exposures to bioaerosols in terms that do not have an excessive dependence on sampling and analytical conditions, such as numbers of colony forming units. Issues that could not be fully addressed included the identification of optimum conditions for sampling time, and for storage and transportation of bioaerosol samples after collection. Further work is needed to establish methods that preserve stable biological integrity for as long as possible.

Direct epifluorescence microscopy is a developing technique for bioaerosol analysis which still requires further work to establish clear analytical and data handling protocols. This is likely to be the subject of future work at European level through CEN Technical Committee 137 (Working Group 5, workplace bioaerosol sampling). The results from this project have established the potential value of the method, and shown that it is compatible with the prototype sampler evaluated.

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