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Aerosol Science 34 (2003) 301–317

Journal of
Aerosol Science

www.elsevier.com/locate/jaerosci

Real-time measurement of bacterial aerosols with the UVAPS: performance evaluation

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Received 12 August 2002; accepted 6 November 2002

Abstract

This paper presents the results of the laboratory evaluation of the Ultraviolet Aerodynamic Particle Size Spectrometer, the novel instrument for real-time continuous monitoring of bioaerosols. The main focus of this study was on evaluating selectivity, sensitivity, counting efficiency, and the detection limits of the UVAPS. The tests were performed with two types of aerosols, bacterial (e.g., *Bacillus subtilis* spores or vegetative cells, and *Pseudomonas fluorescens*) and non-bacterial (e.g., NaCl, latex, peptone water, and nutrient agar/broth). To control viability of airborne bacteria, the bioaerosols were simultaneously sampled with the AGI-30 impingers. The study has demonstrated the UVAPS cross-sensitivity to the non-bacterial organic materials and the need for careful preparation (washing) of test bacteria, in order to avoid the interference with the fluorescence signals of nutrient media used to grow bacteria. The results were indicative of strong sensitivity of the UVAPS to the type of airborne bacteria. The limitations in the capability of the UVAPS to measure bacterial spores were also found. Counting efficiency of the fluorescent particles was shown to depend on particle concentration with the upper limit of detection of the UVAPS approximately 6×10^7 particles/m³.

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Keywords: Bacterial aerosols; Real-time monitoring; Intrinsic fluorescence; Performance evaluation

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1. Introduction

Real-time continuous monitoring of airborne microorganisms is becoming increasingly important. Conventional methods used for detecting bioaerosols involve two stages, field sample collection and off-line laboratory analysis, that make bioaerosol studies labour intensive and time consuming. In situations when a risk from an exposure to bioaerosols has to be identified urgently to allow the appropriate control measures, any delays with identification and enumeration of airborne microorganisms may be vital. Real-time monitors would eliminate a time-consuming analytical stage and allow microbial detection in seconds rather than in hours. In addition, continuous bioaerosol monitoring may facilitate increased accuracy of the results compared to conventional sampling methods, the majority of which are capable of only short-term bioaerosol collection. The drawback of such practice is that some sources may release bioaerosols as “concentration bursts” that may not be detected by short-term grab sampling, whilst such episodic releases may produce significant health effects (Burge, 1995; Macher, Chatigny, & Burge, 1995). Although variations in biological contaminant concentrations over space and time are common, specifying environmental variability for a particular sampling location is not possible. The representative data should, therefore, be collected over the long-term sampling periods. If maintaining viability of the collected microorganisms over the entire sampling time is critical, sequential short-time sampling is essential, as is the case with the majority of commercially available bioaerosol samplers. This exercise involves collection of a great number of samples. Considering the potential cost of such a sampling approach, the number of samples is usually limited in practice that may consequently affect the accuracy of the results and may not reflect real bioaerosol exposure. In studies aimed towards establishing correlation between concentration of biological agents and health effects, an insufficient number of samples may be a reason for the failure to find significant links (Harrison et al., 1992; Nelson, Kaufman, Burt, & Karr, 1995).

There has been considerable interest in recent years in the development of methods for nearly real-time continuous detection and identification of biological airborne particles. These methods can be broadly subdivided into two types: those that are based on mass spectrometry of ions (Snyder, Smith, Dworzanski, & Meuzelaar, 1994; Beverly, Basile, Voorhees, & Hadfield, 1996; Kishnamurthy, Ross, & Rajamani, 1996; Snyder, Thornton, Dworzanski, & Meuzelaar, 1996; Dworzanski et al., 1997; Gieray, Reilly, Yang, Whitten, & Ramsey, 1997; Arnold & Reilly, 1998) and those that employ the intrinsic fluorescence of biological material (Pinnick et al., 1995; Hill et al., 1995; Chen, Nachman, Pinnick, Hill, & Chang, 1996; Nachman et al., 1996; Hairston, Ho, & Quant, 1997; Pinnick et al., 1998; Seaver, Eversole, Hardgrove, Cary, & Roselle, 1999; Pan et al., 1999; Cheng et al., 1999).

Despite numerous attempts to develop an aerosol sampler for the real-time monitoring of viable bioaerosols, the Ultraviolet Aerodynamic Particle Sizer (UVAPS, Model 3312, TSI, St. Paul., MN) is the only commercially available aerosol counter of this nature. The monitor measures aerodynamic size, scattered light intensity, and fluorescence of airborne particles within a size range of 0.5–15 μm . Fluorescence is produced by exciting particles with an ultraviolet laser beam at an excitation wavelength of 355 nm and detected at emission region between 420 and 575 nm.¹ At such conditions, the

¹ These operational conditions correspond to the commercial model but vary to some extent in the prototype models described previously in literature that is discussed further in Section 2.

measured fluorescence is considered to be specific to living microorganisms (Hairston et al., 1997), namely to NAD(P)H molecules (excitation peak at 340 nm and emission peak at 470 nm (Li, Asali, & Humphrey, 1991)) present in all living cells and to riboflavin (excitation peak at 385 nm and emission peak at 525 nm (Li et al., 1991)). Though the UVAPS cannot be used to identify airborne microorganisms, it can be very beneficial in studying dispersion of bioaerosols in various environments (e.g., agricultural facilities, the workplace, the natural environment), controlling the spread of bacterial diseases, source identification and for characterisation of viable bioaerosol particles.

Although the feasibility of this instrument for the detection of bioaerosols has been previously demonstrated by the designers of the monitor (Hairston et al., 1997; Ho, Spence, & Hairston, 1999; Brosseau, Vesley, Rice, Goodell, & Hairston, 2000), the collection of meaningful data and their adequate interpretation are still not possible without a full understanding of its capabilities and limitations. An efficient application of the UVAPS to various bioaerosol studies requires further comprehensive systematic evaluation of its performance.

The experimental study has been undertaken in order to evaluate the UVAPS performance as a preparation for the use of the instrument to study bioaerosols in diverse atmospheric environments. This paper reports on the laboratory tests aimed at evaluating the selectivity, sensitivity, and counting efficiency of the UVAPS.

2. The UVAPS description

The detailed descriptions of the UVAPS can be found in Hairston et al. (1997) and Brosseau et al. (2000). It has to be noted, however, that a prototype monitor described by Hairston et al. (1997) differs from the commercial model used in this study in some respects. In particular, the UVAPS is equipped with a pulsed laser as opposed to a continuous laser in the prototype model used by Hairston et al. (1997). In addition, some operational parameters (e.g., excitation and emission wavelengths) are somewhat different as well as the optics, with lower background noise and better signal-to-noise characteristics in the commercially available model.

The UVAPS (Model 3312A)² uses a double crest optical system, as in the Aerodynamic Particle Sizer (APS, Model 3320, TSI, St. Paul, MN), capable of providing both aerodynamic and scattered light measurements for particles from 0.5 to 15 μm . A separate, ultraviolet laser beam produces fluorescence by exciting particles at a specific wavelength of 355 nm (325 nm at the prototype model used by Hairston et al. (1997) and 349 nm used by Ho et al. (1999)). The resulting fluorescence between 420 and 575 nm (420 and 580 nm in the models used by Hairston et al. (1997) and Ho et al. (1999)) is detected with the photomultiplier tube (PMT).

The monitor performance may be affected by the control settings in various ways. The particle size and scattered light measurements are influenced by the red diode laser power setting, the avalanche photodiode gain setting, and the particle detection threshold setting. The fluorescent measurements (the fluorescence sensitivity) are influenced by the UV laser power settings and the PMT gain settings. Unless the fluorescent material content is near saturation, changes in both parameters should produce a linear effect, e.g., doubling either the PMT gain or UV laser pulse energy should produce

²Model 3312A has been currently changed to Model 3314 due to a change in the TSI numbering system and doesn't affect the specified descriptions for the Model 3312A.

a doubling of the measured fluorescence intensity. At saturation levels, the increasing of either parameter will not result in an increase of the measured fluorescence intensity. The intensity of the fluorescence is read by a high-speed analog-to-digital converter.

The UVAPS operates with an external computer and is supplied with custom software that offers advanced instrument control and data management capabilities. The aerosol is sampled at a flow rate of 1 l/min with a sampling time programmable from 1 s to 18 h. The instrument software allows the measurements to be saved and then analysed in three modes: time-of-flight, fluorescence, and light scattering. The collected information may be afterward assessed as paired data in three two-dimensional arrays. The instrument may be therefore used in the same way as the APS (further referred to as the APS mode) or, in addition to other two parameters, to measure fluorescence of the individual particles (further referred to as the UVAPS mode). This study was only concerned with the measurements of time-of-flight and fluorescence of the individual particles. The data were subsequently taken and analysed for both the APS mode (for analysing concentration and size distribution of the particles regardless of their nature, i.e., total particles) and the UVAPS mode (for analysing concentration and size distribution of both, fluorescent and non-fluorescent particles).

3. Methods

3.1. Experimental setup

The experimental setup developed for this study is schematically shown in Fig. 1. The aerosols were generated by a 6-jet Collison nebulizer (BGI Inc., Waltham, MA), which was operated at a flow rate of 6 l/min. After generation, the aerosol stream was passed through an electrostatic charge neutraliser (10-mCi ^{85}Kr , model 3012, TSI Inc., St. Paul, MN) in order to minimise electrostatic

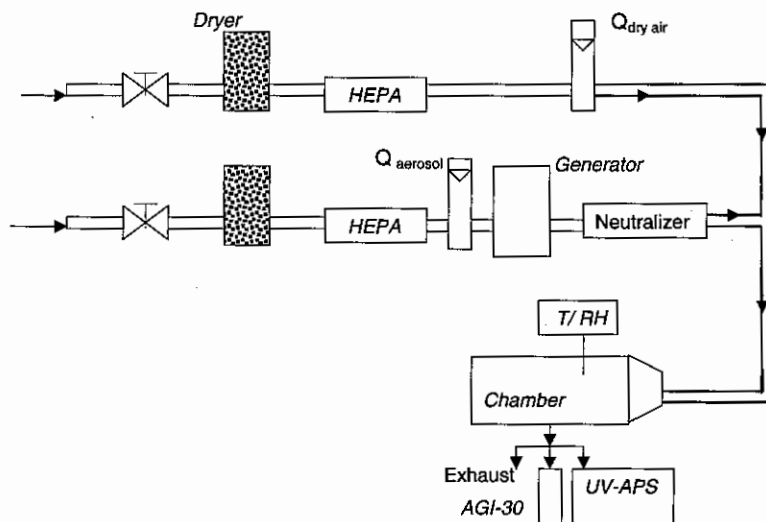


Fig. 1. Schematic diagram of the experimental set-up.

removal of particles by the inner surfaces of the test system. The aerosolised droplets containing test particles or microorganisms were then rapidly dried and diluted by filtered compressed air before introducing into a mixing chamber to restrain possible fluctuations in aerosol concentration. Considering the droplets' lifetime to be proportional to diameter squared at a given temperature and relative humidity (May, 1973), the aerosolised aqueous droplets evaporated very rapidly as a result of mixing with dry air. Specifically, for a droplet size range generated by the Collision nebulizer (Gussman, 1984), the droplet wet lifetime would be in order of 0.01–0.1 s. The mixing chamber was also used as a manifold for simultaneous sampling by the various samplers employed in this study. Thus, all monitors sampled aerosol from the same location of the chamber. To equalise and to minimise the effect of particle transport loss (and bias) during sampling, short tubes of the same length and diameter were used for connecting the monitors with the chamber. The experiments were performed at the air temperature in the chamber between 20°C and 23°C and the relative humidity (RH) within 45–55%. The entire setup was placed inside a Class II, Type A, Biological Safety Cabinet (SG-400 SterilGARD, E-mail Westinghouse Pty Ltd., Australia).

3.2. Test aerosols

Two types of test aerosols, bacterial and non-bacterial, were generated to evaluate the UVAPS performance. The latter were used for the size calibration purposes, for adjusting the UVAPS sensitivity, and for the control blank (without bacteria) tests in order to evaluate the instrument selectivity.

3.2.1. Non-bacterial aerosols

Latex aerosols. The aerosols were generated using monodispersed Polystyrene Latex (PSL) Particles (Duke Scientific Corporation, Palo Alto, CA) with diameters of 0.7, 0.993, and 2 μm and blue fluorescent (BF) microspheres (Duke Scientific Corporation, Palo Alto, CA) with a diameter of 0.91 μm , all with a density of 1.05 g/cm^3 , that corresponded to aerodynamic equivalent diameters of 0.72, 1.02, 2.05, and 0.93 μm , respectively (Hinds, 1982). Aliquots of stock suspensions were diluted with sterile distilled deionised water (SDW) to provide suspensions with the concentrations in order of 10^7 particles/ml.

NaCl aerosols. Some preliminary experiments were performed with NaCl aerosols, which were generated by nebulising 10 wt% aqueous NaCl solutions.

Control "blank" aerosols. Depending on the method of preparation of the bacterial suspensions used for aerosolisation, the aerosolised particles may, in addition to bacteria, also contain some residue of non-bacterial organic materials such as peptone water (which is often used for bacterial washing and then as a liquid media, in attempt to minimise a risk of the osmotic shock of the bacteria), nutrient agar or broth. Though the UVAPS is designed to be selective for only microbial fluorophores (Ho et al., 1999), a possibility of interference of the instrument fluorescent signals that originated from the airborne microorganisms with the signals that originated from non-bacterial particle counterparts was investigated using the control "blank" aerosols. Those were generated from the 0.1 vol% aqueous solutions of the Trypticase Soy Broth (TSB) and peptone water (PW), and from the washings of the sterile, bacteria-free Trypticase Soy Agar (TSA) plates with sterile distilled water.

3.2.2. Bacterial aerosols

Bacterial test aerosols were generated using three types of microorganisms: *Bacillus subtilis* spores (ATCC 6633), vegetative cells of *Bacillus subtilis* (grown from spores ATCC 6633), and *Pseudomonas fluorescens* (QUT 0980; obtained from the QUT culture collection). These microorganisms are commonly found in the ambient air environment and were selected to represent bacteria that are robust (*B. subtilis*) or sensitive (*P. fluorescens*) to environmental stresses. An additional advantage of using these bacteria in this study is the similarity of the cell size. This fact is important for facilitating data comparison and interpretation, until the effect of bacterial particle size on the UVAPS fluorescent signals has been assessed.

B. subtilis is gram-positive, non-motile, facultatively anaerobic, endospore forming, rod-shaped bacterium, commonly found in soil, water sources and in association with plants. The vegetative cells have size of 0.7–0.8 μm in width and 2.0–3.0 μm in length and may occur singly or in chains (Buchanan & Gibbons, 1974). It is considered a model organism for gram-positive bacteria and has been extensively studied in the laboratory. Optimum growth temperature is about 37°C.

B. subtilis spores are rod-shaped, have a size of approximately 0.5 \times 1.0–1.8 μm and are often used in aerosol studies as a model for small and robust microorganisms.

P. fluorescens is gram-negative, motile, strictly aerobic, non-sporulating bacillus (straight or slightly curved rods) with the size of about 0.7–0.8 μm in width by 2.0–2.8 μm in length (Buchanan & Gibbons, 1974). It is common environmental microorganism, sensitive to environmental stresses (Buchanan & Gibbons, 1974; Stewart et al., 1995) with optimum growth temperature of 25–30°C.

All bacteria were maintained as part of the culture collection at QUT. Cultures were prepared by sub-culturing bacteria in Trypticase Soy Broth (TSB; Difco) at the relevant optimal temperature overnight.

3.3. Preparation of bacterial spray suspensions

The suspensions of the *B. subtilis* spores were prepared by suspending one vial of supplied material in 50 ml of sterile distilled water (SDW) to yield a concentration of culturable microorganisms in order of 10^9 colony forming units (CFU)/ml. The suspension was subsequently heated at 75°C for 10–15 min in a waterbath, to activate the spores and to kill any vegetative cells that may have possibly been present.

The suspensions for aerosolisation of bacterial cells were prepared in two ways: by transferring bacteria growing on agar plates into a suspending liquid or by harvesting bacteria from nutrient broth. The former method was initially used in the preliminary tests for comparative purposes with the literature data (Brosseau et al., 2000; other relevant publications on the UVAPS performance are not well documented in respect to the preparation of the bacterial suspensions). The procedure used was as follows: 200 μl of active bacterial culture was inoculated on TSA plates and incubated aerobically for 48 h at the relevant optimal temperature—*B. subtilis* at 37°C and *P. fluorescens* at 30°C. Bacterial growth was then aseptically harvested from the plates by a loop, placed onto the wall of the conical sterile centrifuge tubes, emulsified and then suspended in 50 ml of SDW followed by vortexing to assist breaking the bacterial clumps. For the control tests, a blank spraying liquid was prepared by pouring sterile distilled water over a sterile agar plates free of bacteria.

Alternatively, spray suspensions were prepared by harvesting bacteria from broth as follows. One millilitre of the overnight starter culture was subcultured in 100 ml of Trypticase Soy Broth

(Difco) to the early stationary growth phase on a rotary shaker at the relevant optimal temperature. *B. subtilis* cultures were grown at 37°C for 8–14 h and *P. fluorescens* at 30°C for 14–16 h. Cells were harvested by centrifugation (1000g, 15 min, room temperature), washed three times with and then resuspended in 50 ml of SDW. Harvesting bacteria at the stationary growth phase is important in order to maximally preserve the viability during aerosolisation and sampling processes, as such bacteria are known to be more resistant to stresses than that at the exponential growth phase (Black, 1996). For *B. subtilis* cultures, harvesting at the early stationary phase is additionally important in order to avoid the sporulation process, which is known to begin at the end of exponential growth and requires approximately 8–10 h to complete under standard laboratory conditions (Sonenshein, Hoch, & Losick, 1993; Errington, 1993). Avoiding the sporulation was important for this study as it could possibly affect the viability of the *B. subtilis* cells and subsequently the UVAPS signals. To control the bacterial growth, the growth curves were constructed for the bacteria. The optical densities were measured hourly with a spectrophotometer at 540 nm. To monitor and control the *B. subtilis* sporulation process, the post-exponential cells were periodically examined under an optical microscope.

3.4. Experimental procedure

Prior to evaluating the UVAPS with the bacterial and control “blank” aerosols, the instrument was calibrated with PSL and BF microspheres, with the sizes reported earlier in the preceding sections. Then, a series of the experiments were performed on adjusting the UVAPS sensitivity.

3.4.1. Adjusting the UVAPS sensitivity

As was discussed earlier, the sensitivity can be adjusted by setting two parameters: the gain of the fluorescence sensing PMT and the UV laser pulse energy. In accordance with the instrument instruction manual, when the fluorescence sensitivity is set high, the UVAPS internal fluorescence baseline will result in measured fluorescence even from particles that do not contain fluorophores. Thus, the instrument fluorescent baseline should be occasionally checked using non-fluorescent test particles such as PSL or NaCl particles.

Following this recommendation, prior to monitoring bacterial aerosols, the UVAPS settings had to be checked and adjusted with non-bacterial particles. It was, however, unclear how the characteristics of such particles may possibly affect the UVAPS performance. Thus, it was an aim of this study to investigate the effect of the size, concentration, and composition of the aerosols used for adjusting the instrument settings (sensitivity) on the UVAPS fluorescent response. The corresponding experiments were performed with the PSL and NaCl aerosols.

In the tests evaluating the effect of PSL particle size, the aerosol concentration was supplied at arbitrary level of approximately 20 particles/cm³. The PSL particles of 0.7 µm were used to adjust the UVAPS optimal settings by a gradual slow increase of both UV laser pulse intensity and PMT gain, until some weak fluorescent signals started to appear in the first two channels. Holding these (optimal) settings, the comparative tests were performed with the PSL particles of 0.993 and 2 µm, after cleaning a chamber with HEPA-filtered air.

To investigate the effect of the particle concentration, the tests were performed for each particular PSL aerosol at the fixed concentrations of approximately 5, 10, 40, or 80 particles/cm³. The particle concentration was adjusted by changing the flow rate of the diluting air stream and, in some cases,

by bypassing the main aerosol stream. In this way, the UVAPS was tested within the aerosol concentration range of 0.01–1500 particles/cm³. The upper levels were adjusted in accordance with the UVAPS specification for the maximum particle concentration, which are 1500 and 600 particles/cm³ (for < 10% coincidence) for 0.5 and 10 µm particles, respectively (TSI Incorporated, 1998). The aerosol sampling time was set at 3–5 s.

To evaluate the effect of aerosol composition, some tests were conducted with the NaCl aerosols generated from the 10% aqueous solutions.

3.4.2. Measurement of the test (bacterial and control “blank”) aerosols

After adjusting the UVAPS sensitivity with the PSL (0.993 µm) particles, the UVAPS measurements were first taken for the PSL aerosol (“background” aerosol). Then, following cleaning the chamber with filtered air, the continuous aerosol measurements were taken using a sampling time of 3 s. The aerosol concentrations were adjusted in the same way as discussed above.

For comparative tests, the bacterial aerosols were simultaneously sampled with the UVAPS (for monitoring both total and fluorescent particles) and the AGI-30 impingers (for monitoring culturable microorganisms) for 10–15 min. Though the UVAPS measures bacterial particles rather than individual microorganisms and AGI-30 is known to break particles and, thus, measure single bacteria, it was appropriate to compare their data since the majority of the airborne bacteria generated by Collison nebuliser were single microorganisms, which was evident from the size distribution of the aerosols. Samples collected with the AGI-30 impingers were analysed by standard culture technique, on the TSA plates. The *B. subtilis* samples were incubated at 37°C and *P. fluorescens* at 30°C. The plates were inspected on daily bases and analysed after 20–24 h of incubation for *B. subtilis* colonies and after 40–48 h for *P. fluorescens* colonies.

After completing sampling of the test aerosols, the measurements were repeated for the PSL background aerosol. The average background concentrations of the fluorescent particles were subtracted from the measured values of the test aerosols. To obtain statistically significant results, all measurements were repeated at least three times and the results have been presented as the averages.

4. Results and discussion

4.1. Adjusting the UVAPS sensitivity

NaCl aerosols did not produce the fluorescent signals over a range of the UVAPS settings. In contrast, the PSL particles started to produce fluorescence at the settings close to the instrument default conditions (PMT gain at 500 V and UV laser pulse energy at 50%). The PSL particle size was found to have no effect on the instrument settings. Although the aerosol concentration did not affect the UVAPS settings, at which a weak fluorescent signals started to appear in the first two channels, it affected “percent fluorescence” that was calculated as a percent of the fluorescent particles to the total PSL particles. The “percent fluorescence” was found to gradually decrease with an increase of particle concentration. This fact should be taken into account when PSL spectra compared with the spectra of other tested aerosols. Accordingly, whenever “percent fluorescence” of two aerosols was further compared, it was done for the aerosols of the corresponding concentrations.

In addition, during the preliminary tests of the UVAPS performance, it was found that UV laser pulse intensity value is not kept at the fixed setting value but rather fluctuates, sometimes quite considerably, during a measurement session. This inevitably affected the UVAPS fluorescent signals. Subsequently, the settings should be frequently adjusted to avoid measurement bias. This approach, however, seems impractical, especially for field applications. An alternative strategy is to use once defined (but periodically checked) optimal settings and measure background non-bacterial aerosol (e.g., latex), before and after monitoring of bioaerosols. The fluorescent background signals (measured for background aerosol) should then be subtracted from the bioaerosol readings, during data analysis. This approach was later applied in the instrument evaluating tests. The instrument default settings were confirmed to be optimal and were therefore used during the further tests.

4.2. Instrument software

The three-dimensional spectra of the aerosols are presented in Figs. 2–4. The particle aerodynamic diameters are presented in 52 size channels, with the respective concentrations recorded in number of particles per cubic centimeter. The fluorescence intensity is presented at 64 channels representing gradually increasing fluorescence intensity of the particles. Data on non-fluorescing particles are presented in the first channel and the data on fluorescing particles in channels from 2 to 64. Total particle concentration, therefore, corresponds to the sum of particle concentrations from channel 1–64 and should be equal to the total particle concentration obtained in the APS mode (spectra are not shown). Nevertheless, the APS data were always higher than the corresponding data recorded in the UVAPS mode. The correlation of the data obtained for PSL particles of 0.993 μm in these two modes are presented in Fig. 5, as well as the difference as a function of the total particle concentration. As can be seen, the difference between the results obtained in two modes increased with the concentration of the particles.

4.3. Selectivity

The characteristics of the test aerosols are summarised in Table 1. Count median diameters (CMD) and geometric standard deviations (GSD) are presented for both total (counted by the UVAPS through the channels 1–64) and fluorescent (counted through the channels 2–64) particles. The proportion of the fluorescent particles as a percentage of the total particle number is also presented. The last two columns in Table 1 are the concentration of culturable particles as was determined from the samples collected with the AGI-30 impingers by cultivation (see Methods for the details) and the percentage of culturable particles to the total particles measured by the UVAPS.

Although the UVAPS is designed to be selective to only specific microbial fluorescent molecules, strong fluorescent signals were observed for non-microbial organic aerosols, which were generated from the suspensions of 0.1% peptone water, broth, or washing from sterile agar plate (Figs. 2a–c, Table 1). Those can be compared with the spectra of the bacterial aerosols (Figs. 3a–c, Table 1).

These results indicate that fluorescent signals from the non-microbial components of test aerosols may seriously interfere with the bacterial signals and thus obscure interpretation of the results. To avoid this problem, the test aerosols should be prepared from well washed microorganisms suspended in distilled sterile water. However, a possibility and an extent to which such signal interference may occur in the field environments should be further investigated.

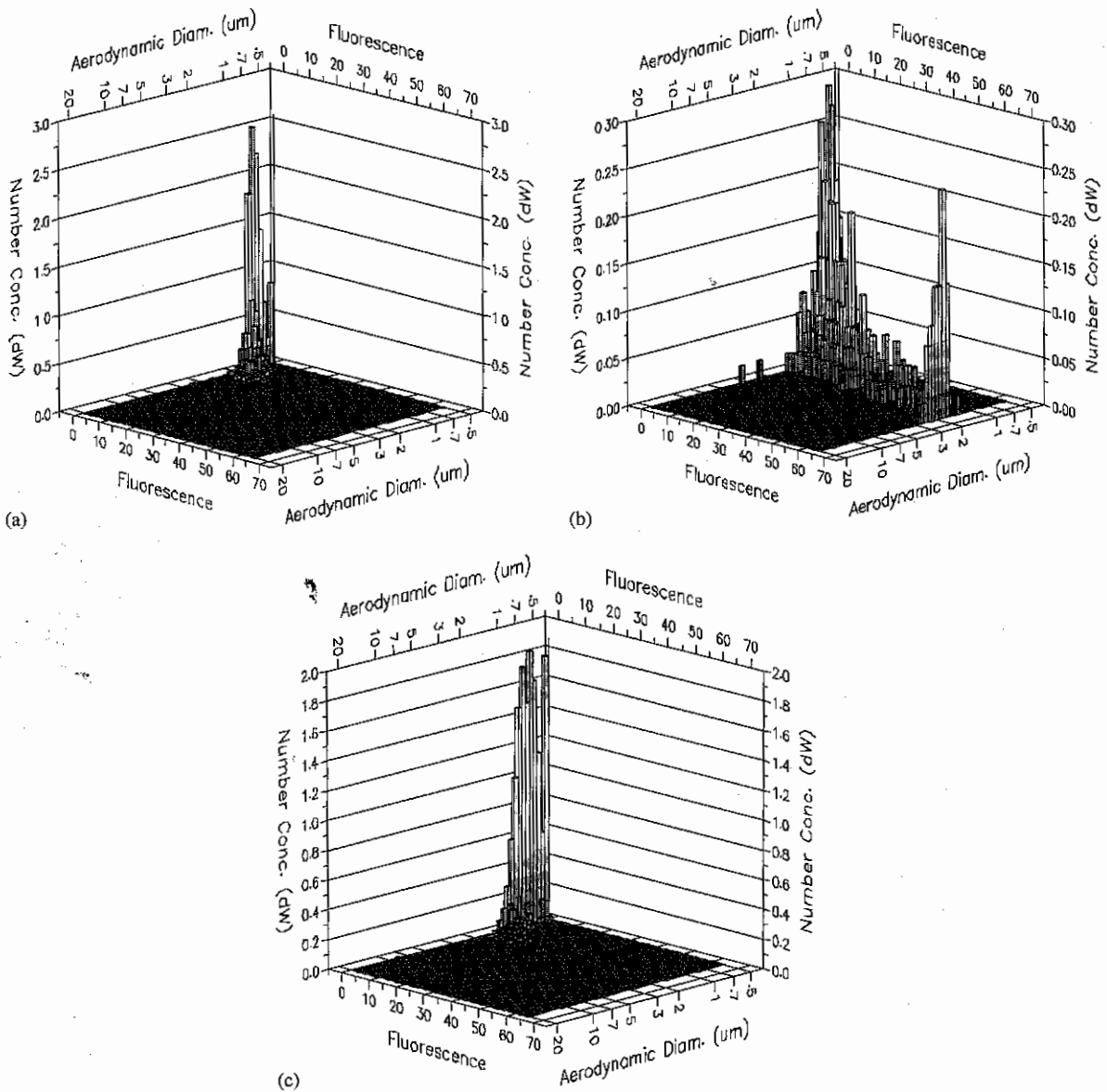


Fig. 2. The UVAPS spectra of the control blank aerosols: (a) TSA plate washing; (b) 0.1% solution of the TSB; (c) 0.1% of peptone water. The concentrations (dW) are given in particles/cm³ and display interval particle size distribution. The concentration in any channel represents the concentration within the particle size boundaries for that channel.

Since the agar washing solution was found to produce strong fluorescent signals (Fig. 2a) that noticeably affected the results of the bacterial aerosols (Table 1), the corresponding method of preparing the bacterial suspensions by transferring bacteria growing on agar plates into a suspending liquid was found inadequate for this study. Further tests were performed only with carefully washed bacteria.

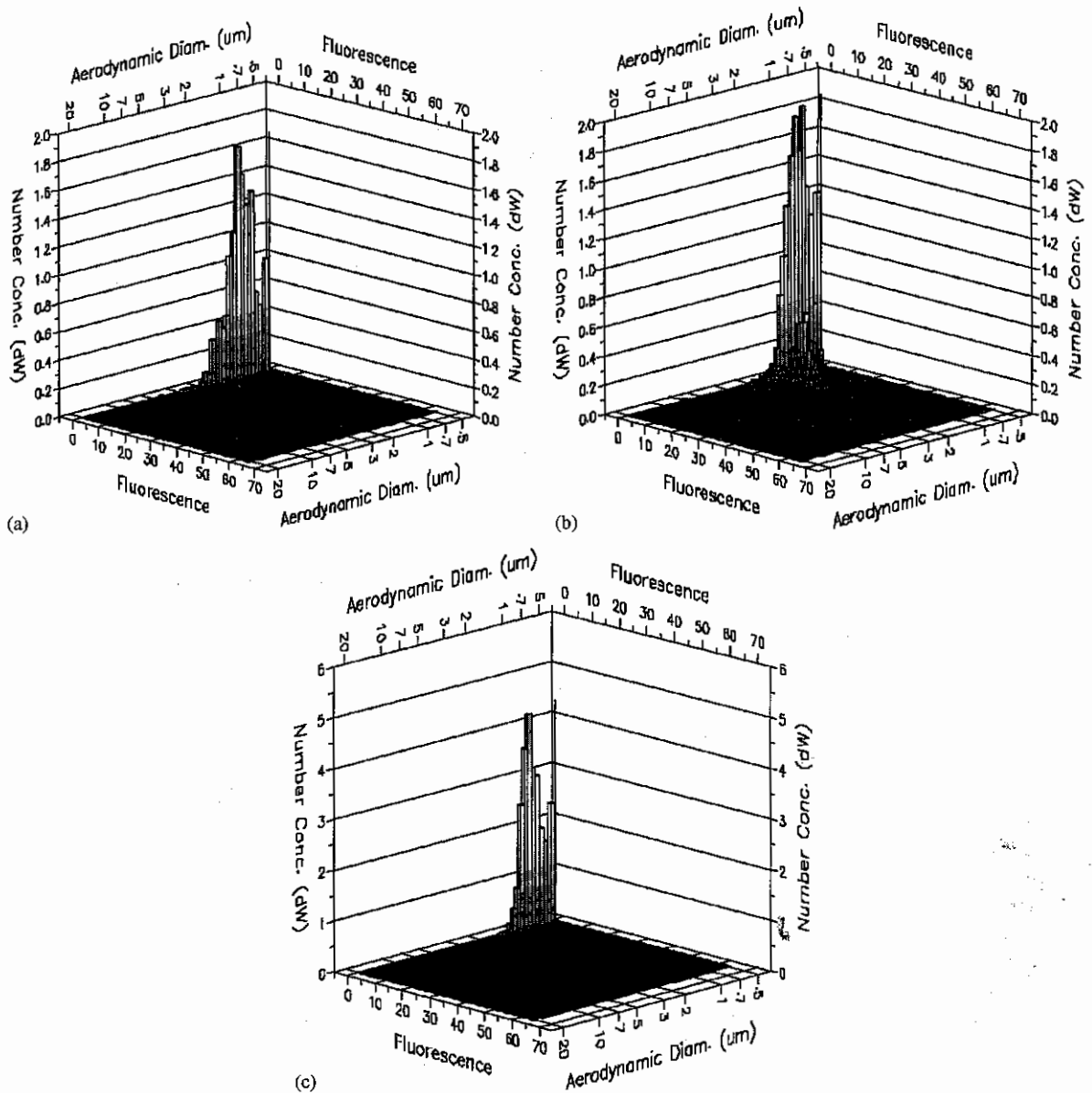


Fig. 3. The UVAPS spectra for the bacterial aerosols: (a) *B. subtilis* spores; (b) *B. subtilis* cells; (c) *P. fluorescens*. The concentrations are given in particles/cm³.

4.4. Counting efficiency and concentration range

Ideally, a proportion of fluorescent (viable) particles should be constant for the same aerosol, regardless of the concentration. However, counting efficiency of fluorescent particles was found to depend on total particle concentration. Fig. 6 demonstrates the result for *B. subtilis* aerosols (similar

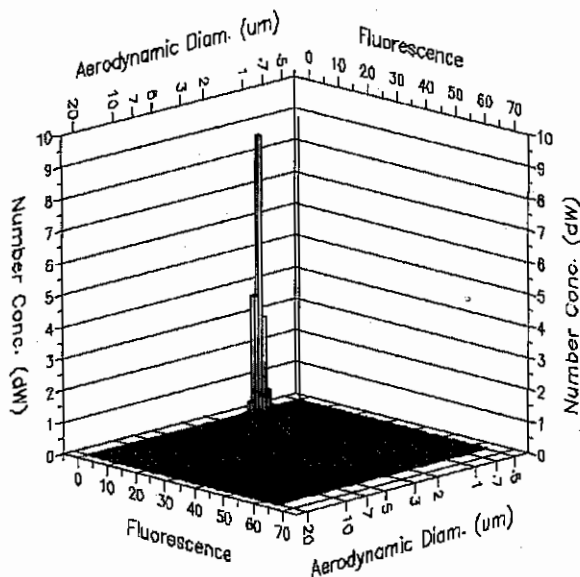


Fig. 4. The UVAPS spectra for PSL (0.993 μm) aerosol. The concentrations are given in particles/ cm^3 .

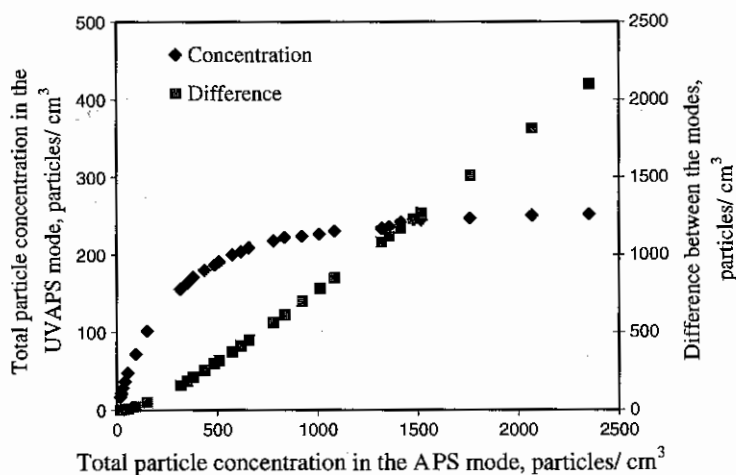


Fig. 5. Correlation of the results obtained for PSL particles of 0.993 μm in two (the APS and the UVAPS) measurement modes.

trends were observed for all aerosols). As can be seen, the counted proportion of fluorescent particles gradually decreased with an increase of the total particle concentration, reaching a “saturation” level at total concentration of about 100 particles/ cm^3 . These results can be explained by the operational principals of the UVAPS as follows. Individual particles can be resolved if the measurement time is less than the time between particle events. The UV laser has a repetition rate of 5 kHz, or a 200 μs “dead time”. This means that in the period shorter than 200 μs the UV laser cannot be consequently

Table 1
Characteristics of the tested aerosols

Aerosol	Total particles ^a			Fluorescent particles ^b				Culturable particles	
	Conc., #/m ³	CMD μm	GSD	Conc., #/m ³	CMD μm	GSD	%	Conc., CFU/m ³	%
PSL	1.9E+07	1.04	1.1	3.4E+05	1.03	1.6	1.8	—	—
Peptone	1.8E+07	0.81	1.3	3.2E+06	1.14	1.3	17.4	—	—
TSA	2.0E+07	0.79	1.2	6.9E+05	0.87	1.2	3.5	—	—
TSB	2.0E+07	0.97	1.3	1.5E+07	0.97	1.3	76.3	—	—
<i>B. s.</i> spores	2.0E+07	0.91	1.4	2.9E+05	1.09	1.0	1.5	6.6E+06	33
<i>B. s.</i> cells	1.9E+07	1.01	1.3	4.4E+06	1.17	1.2	23.4	8.6E+06	46
<i>B. s.</i> (plate)	2.0E+07	0.82	1.3	6.4E+06	0.91	1.3	32.0	8.0E+06	40
<i>P. f.</i> cells	2.1E+07	0.77	1.3	4.3E+05	1.09	0.8	2.0	6.9E+06	32
<i>P. f.</i> (plate)	2.0E+07	0.81	1.3	8.1E+05	0.92	1.2	4.0	7.1E+06	35

^aAs determined by counting aerosol particles through the channels 1 to 64.

^bAs determined by counting aerosol particles through the channels 2 to 64.

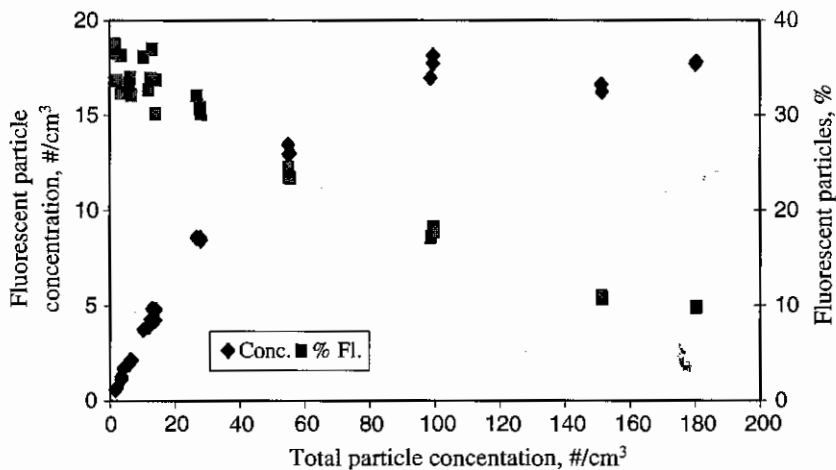


Fig. 6. Effect of total particle concentration on the fluorescent signals of the UVAPS. Though these results correspond to *B. subtilis* aerosols, similar and very consistent trends were observed for all tested aerosols.

triggered. If two particles follow each other into the detection area in a time interval shorter than 200 μs the first will be illuminated with the UV laser, and its fluorescent signal recorder, but the UV laser will not have time to recover before the second particle enters the detection area and it will not be illuminated with the UV light from the laser and therefore will have a zero fluorescence signal. The APS part, which is the part where the size of the particle is estimated has a much faster recovery time and will be able to detect the size of both particles. Therefore both particles will be size classified, counted, but only for the first particle will any fluorescence signal be detected. If

both particles are fluorescent (viable) then only the first one will be counted as viable while the second one will be counted as a non fluorescent (non-viable) particle.

The maximum concentration can be estimated from the laser “dead time” of $\Delta t = 200 \mu\text{s}$ and the total (aerosol and sheath) flow rate of $q = 5 \text{ lpm}$. In the volume of $\Delta V = q\Delta t = 0.0167 \text{ cm}^3$ there should be only one particle. If more than one particle is present then the laser will not have time to recover. This provides us with an estimate of the highest concentration at which all fluorescent particles will be detected: $n = 1 \text{ particle}/\Delta V = 60 \text{ particles}/\text{cm}^3$. It can be seen (Fig. 6) that at around this concentration the number of counted fluorescent particles starts to deviate from the straight line indicating that some are not counted. Thus, the *upper limit of detection (ULD)* of the UVAPS appears to be about $60 \text{ particles}/\text{cm}^3$.

4.5. Sensitivity

The tests have revealed that the instrument response can be dramatically affected by the nature of bacterial aerosol.

In regards to endospores, the UVAPS has demonstrated very limited capability to detect individual *B. subtilis* spores (Fig. 3a). The proportion of the fluorescent particles in *B. subtilis* spores aerosols was similar to that of the background PSL aerosols (Table 1), though simultaneous sampling with the AGI-30 impingers resulted in high concentrations of viable (culturable) aerosol particles—in order of $10^6 \text{ CFU}/\text{m}^3$. These results contradict the previously published UVAPS data by Hairston et al. (1997) and Brosseau et al. (2000), where relatively strong fluorescence signals (with percent fluorescence of 17% and 44%, respectively) were measured for the *B. subtilis* spore aerosols. While initially unexpected, the results obtained in this study can be explained by the physiology of the spores. By their nature, the spores are dormant and lack metabolic activity and thus do not contain NAD(P)H molecules and only a minute amount of riboflavin that appeared to be below the instrument sensitivity level. The difference between our results and those reported previously may be explained by the differences in the methods of preparation of the bacterial suspensions for aerosolisation. In the previous studies, they were probably prepared from a not highly purified spore collection (which is also the most likely scenario for the routine preparation of bacterial spores in large quantities). In addition, preparing a spore suspension of a *Bacillus* species is known to be difficult and any “in house” suspension is likely to be contaminated with vegetative cells. Thus, the origin of the measured fluorescence signals reported by Hairston et al. (1997) and Brosseau et al. (2000) could be explained by traces of the nutrient media (used to produce the spores) and vegetative cells in the aerosolised particles.

In regards to the individual bacterial cells, the UVAPS seems to be highly sensitive to the nature of the bacteria forming the aerosol. For example, very weak or no fluorescent signals were observed for *P. fluorescence* aerosols (Fig. 3c and Table 1). In contrast, fluorescent signals from *B. subtilis* aerosols (Fig. 3b) were typically in good agreement with the AGI-30 results (Table 1). Since the total amount of NAD(P)H molecules, a specific fluorophore most responsible for emitting fluorescence under the UVAPS operating conditions, in all bacteria growing under similar conditions is almost indistinguishable (Wimpenny & Firth, 1972) these results suggest a strong correlation of the UVAPS fluorescent signals with physiological state of bacteria. The results of the corresponding study are presented independently and will be submitted shortly.

5. Conclusions and recommendations

Overall conclusions from the study can be summarised as follows:

5.1. Selectivity

Although the UVAPS is designed to be selective to only microbial fluorescence, strong fluorescent signals were observed for non-microbial organic aerosols (generated from the suspensions of 0.1% peptone water, nutrient broth, or washing from sterile agar plate). Consequently, the fluorescent signals from the non-microbial components of test aerosols may seriously interfere with the bacterial signals and thus obscure interpretation of the results. This fact is particularly important when the instrument response on different microbial aerosols is investigated. To avoid this problem during laboratory tests, the aerosols should be prepared from well washed microorganisms suspended in distilled sterile water. However, the possibility and an extent to which such signal interference may occur in the field environments needs further investigation.

5.2. Sensitivity and correlation of fluorescence signals with bacterial viability

- The study has demonstrated limitations in the ability of the instrument to measure *individual bacterial spores*. These results are well explained by biochemical composition of the spores, which do not contain NAD(P)H and only minute amount of riboflavin that appeared to be below the instrument sensitivity level.
- The results obtained in this study are indicative of strong sensitivity of the UVAPS to the physiological state of bacteria. The instrument seems incapable of detecting single injured bacteria, thus, suggesting that the amount of fluorophore(s) in injured bacteria is below the sensitivity level of the UVAPS. Further experimental work is needed to provide quantitative data to confirm this hypothesis.

5.3. Counting efficiency

Counting efficiency of the UVAPS has been shown to depend on particle concentration. The proportion of fluorescent particles was found to decrease with an increase of total particle concentration, with a “saturation” level of total concentration at about 10×10^6 particles/m³. This fact should be taken into account during comparative measurements. Whenever the data such as “percent fluorescence” or the signal patterns of two aerosols are to be compared, it should be done for the aerosols of the same concentrations.

5.4. Detection limits

The upper limit of detection (ULD) of the UVAPS was found to be around 6×10^7 particles/m³.

Acknowledgements

This work was supported by Australian Pork Limited (grant STU 136/1456).

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