COVER SHEET

Real-time monitoring of viable bioaerosols: capability of the UVAPS to predict the amount of individual microorganisms in aerosol particles

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ABSTRACT

The Ultraviolet Aerodynamic Particle Sizer (UVAPS) is a novel aerosol monitor for enumerating and sizing microbial aerosols. To explore the capability of the method to estimate the number of microorganisms (bacteria) in aerosol particles, and thus to provide information on the concentration of airborne microorganisms, in addition to the total number of microbe carrying particles, a linearity of the UVAPS fluorescent signals with respect to the concentration of the fluorophores was investigated. As the amount of intrinsic fluorophores in bacteria may vary depending on viability status of the cells, the linearity was initially investigated for the non-microbial aerosols (NADH, NADPH, or riboflavin), with preset concentrations of fluorescent material in aerosol particles. The succeeding tests were performed with bacterial aerosols containing carefully washed *Bacillus subtilis* or *Micrococcus luteus* vegetative cells. To correlate the fluorescence intensity with particle size, which determines the amount of fluorophores (or cells) in the aerosol particles, the UVAPS data were analysed for each of 64 size-channels individually. The fluorescence intensity was linear with respect to the particle volume at the fluorophore concentrations characteristic to bacterial cells (correlation factors were typically greater than 0.9) and became curvilinear at higher concentrations. As the linearity of the UVAPS signals was confirmed for bacterial aerosols, it was concluded that the UVAPS can be used to estimate the concentration of airborne viable bacterial cells in artificially generated bioaerosols. The predicted concentrations of viable cells in the *M. luteus* aerosols compared favourably with the results of the AGI-30 sampling for culturable cells.

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1. Introduction

The Ultraviolet Aerodynamic Particle Sizer (UVAPS, Model 3312, TSI Inc., St. Paul, MN) is a novel aerosol counter for the real-time detection of airborne microorganisms. The operating principles of this method to measure bioaerosols are based on the properties of microorganisms to contain numerous naturally occurring (or intrinsic) fluorescent compounds, which emit photons following excitation in the ultraviolet region (Cantor et al., 1980).

The UVAPS is a modified Aerodynamic Particle Sizer (APS) manufactured by the TSI Inc. It consists of a UV laser that provides for the particle fluorescence, in addition to the information on the size distribution and concentration of airborne particles within a size range of 0.5-15 μm. The fluorescence is produced by exciting particles with a pulsed ultraviolet laser beam at an excitation wavelength of 355 nm, followed by detection in the range from 420 nm to 575 nm. Under such conditions, the measured microbial fluorescence is largely determined by the reduced pyridine nucleotides (namely, nicotinamide adenine dinucleotide, NADH, and nicotinamide adenine dinucleotide phosphate, NADPH) and flavins (e.g., riboflavin, flavin mononucleotide, FMN, and flavin adenine dinucleotide, FAD). A detailed description of the UVAPS has been published previously (Hairston et al, 1997; Brosseau et al, 2000; Agranovski et al., 2003a).

The UVAPS (also known as FLAPS) was originally developed as part of the Canadian Integrated Biochemical Agent Detection System (CIBADS) for the real-time detection of aerosol particles that contain microorganisms (Ho et al. 1995; Boulet et al., 1996). As part of the CIBADS, the instrument was intended to be used as an alarm system and thus designed to detect sudden increases (i.e., relative changes) in the concentration of the fluorescent aerosol particles above the typical background levels, indicating dissemination of microbial aerosols upwind of the spectrometer. Accordingly, in addition to the laboratory-based feasibility studies (Hairston et al, 1997; Brosseau et al, 2000), the FLAPS/UVAPS was mainly evaluated for the capability to follow changes in the total concentration of fluorescent particles present in ambient air (Ho et al., 1995; Boulet et al., 1996; Ho et al., 1999).

In order to understand the full capability of the UVAPS in characterising bioaerosols in various research settings, an experimental program aimed towards the systematic evaluation of the instrument performance has been initiated in our laboratory. Previous studies were concerned with the primary parameters of the spectrometer to measure microbial aerosols such as counting efficiency, selectivity, sensitivity, and detection limits (Agranovski et al., 2003a; Agranovski et al., 2004) as well as the correlation of the UVAPS signals with the viability status (namely, the metabolic state) of airborne bacteria (Agranovski et al., 2003b). The research presented in this paper was focussed on the linearity of the fluorescent signals of the UVAPS, the effect of particle size on its performance, and exploring the capability of the spectrometer to estimate the amount of individual organisms (bacterial cells) in aerosol particles. The linearity of the fluorescent signals was initially investigated for non-biological aerosols containing one of the three major fluorophores (NADH, NADPH, or riboflavin) responsible for the UVAPS fluorescence signals while measuring microbial aerosols. The following tests were conducted with bacterial aerosols containing *Bacillus subtilis* or *Micrococcus luteus* vegetative cells. To investigate the UVAPS response for the cells in various metabolic states, the bacteria

were harvested at both exponential and stationary growth phase. In addition, the *M. luteus* samples were subjected to heat-stress.

2. Materials and methods

2.1. Research approach

The main objective of the tests was exploring the capability of the UVAPS to estimate the number of individual bacterial cells in an aerosol particle. To achieve this, it was necessary to derive a relationship between the intensity of fluorescence measured by the spectrometer and the amount of fluorophores in the aerosol particles.

In accordance with the Beer's law, in the absence of quenching, fluorescence intensity should be directly proportional (linear) to the concentration of fluorophores. The linearity of a sample may, however, be related to many factors, including the chemical composition of the aerosol particles and the path-length traversed by the light (e. g, the particle diameter). Once the UVAPS linearity is established, it would be possible to estimate from the particle size and fluorescence intensity the amount of individual organisms within a single fluorescent particle.

2.2. Tested aerosols

Some preliminary experiments were conducted with non-bacterial aerosol particles containing predefined amounts of NADH, NADPH, or riboflavin. These aerosols were selected for the study as, under operating conditions of the UVAPS, these three compounds are the primary microbial intrinsic fluorophores responsible for the measured fluorescence. The procedure for preparing these aerosols has been described in detail previously (Agranovski et al., 2004). Briefly, the non-bacterial aerosols were generated with a 6-jet Collison nebuliser from solutions having fluorophore concentrations from 0.005 to 0.1 mg/ml.

The bacterial aerosols were generated using washed *B. subtilis* or *M. luteus* vegetative cells, following the procedure described by Agranovski et al. (2003b). To investigate the UVAPS response for the cells in various metabolic states, the bacteria were harvested at the exponential (or log) and stationary growth phases. In addition, the *M. luteus* samples were subjected to heat-stress as described previously (Agranovski et al., 2003b).

2.3. Experimental set-up and procedures

A detailed description of the experimental set-up and procedures has been reported previously (Agranovski et al., 2003a-b; Agranovski et al., 2004). In brief, the aerosols were continuously generated with the Collison nebulizer (BGI Inc., Waltham, MA, USA), neutralized (10-mCi ^{85}Kr, model 3012, TSI Inc., St. Paul, MN), and then mixed with the drying air stream at the flow rates providing the total particle concentration at an arbitrary level of approximately 20 cm^{-3} (typically, 30-40 L/min). Adjusting the particle concentration at a certain level was important as accuracy of the UVAPS in measuring the fluorescent particles varies with concentration (Agranovski et al., 2003a). Thus, to eliminate the effect of particle concentration on the results, while investigating the UVAPS responses on other aerosol parameters (such as the particle size and nature) maintaining the total concentration of the aerosols relatively constant was critical. To restrain possible fluctuations in aerosol concentration, the

aerosols were introduced into a mixing chamber, which was also used as a manifold for simultaneous sampling by the UVAPS and the AGI-30 impingers.

By employing the Collison nebulizer it was possible to generate aerosols with aerodynamic diameters up to 2.5 μm. In order to investigate larger particles a second series of the experiments was conducted with aerosols generated with commercially available nasal sprayers (PS1823; Douglas Pharmaceuticals Ltd, Auckland, New Zealand). The glass bottles of the sprayers were carefully washed with distilled water, to remove the organic residues which could affect the results, and then sterilised by autoclaving. The plastic tops were thoroughly washed with sterilised distilled water (SDW) and then sanitised with alcohol. Before filling the sprayers with the suspensions used to generate test aerosols, the controlled tests (for contamination) were performed with the SDW. The washing was considered satisfactory when the UVAPS fluorescent signals were detected in up to the third channel (background fluorescence).

Unlike the Collison nebuliser, the sprayers generated aerosols in pulses, which made it difficult to maintain the particle concentration constant. To avoid this problem, the aerosols generated with the nasal sprayers were introduced into the chamber directly, without mixing with the dilution air. The chamber used in this series of experiments was a stirred-settling type of volume 160 L. The pulses were initiated every 60 seconds over the duration of the experiment, with the UVAPS measuring the aerosols continuously (with a sampling time of 3 s). In this manner, it was possible to collect a statistically sufficient number of samples with a total particle concentration of approximately 20 cm^{-3}. More frequent pulses (< 60 s) produced aerosols with greater particle concentrations than was required. The experiments for a given aerosol were repeated at least three times.

2.4. The UVAPS data analysis

The fluorescence emitted by an individual particle is registered by the UVAPS in one of 64 channels, depending on the intensity of the signal. The increasing channel numbers represent gradually increased fluorescence intensity. The UVAPS does not provide the nominal values for the fluorescence intensity of the particles in arbitrary units such as relative fluorescence units (rfu), as is typically reported by the spectrophotometers. Instead, the intensity of the signals is reported on a scale from 1 to 64, corresponding to the channel numbers. Specifically, non-fluorescing particles are registered in the first channel and the closer the particle signal is to the 64^{th} channel the higher its fluorescence intensity. To investigate a correlation between the intensity of the signals and the amount of the fluorophores within the aerosol particles, the UVAPS data were analysed for each fluorescence channel individually. The information on particle size distribution was used to determine the count median diameter (CMD) of particles detected in a particular fluorescence channel, for each of the 64 channels. The particle CMD values were subsequently used to calculate the particle volumes and the amount of the fluorophores within the particles, taking into account the concentration of the solutions which were used to generate the aerosols. A detailed procedure for calculating the amount of fluorophores in the aerosol particles has been reported previously (Agranovski et al., 2004). The calculated volumes and the amount of fluorophores were then plotted against the number of the fluorescent channels.

2.5. The number of individual bacterial cells in aerosol particles

The number of individual cells which may occur within the generated droplets depends on several factors, including the droplet size, the size of bacterial cells, the concentration of bacterial cells in the nebulised suspension, and the distribution (mixing) of cells in the suspension. The number of cells inside the droplets may therefore vary considerably within the two extreme values, namely from a single bacterial cell to the maximum possible number. Apparently, a probability of that that the number of cells within the droplets approaches the maximum increases with the increase of cell concentrations in the nebulised suspension. The maximum number of cells which may occur within the droplets depends on droplet size and cell size as well as the type of cell packing (packing degree or density) in the droplets.

Similar to the droplets, the packing of aerosol particles from evaporating droplets can vary considerably affecting the number of cells in aggregates forming the particles. The ability to objectively examine aggregation of cells requires a quantitative measure of the closeness (density) of cells. Nevertheless, it was possible to estimate the density of aggregated cells, and thus the number of cells, in the particles measured by the UVAPS based on the geometrical principals of sphere packing theory.

Under assumption that the volume of a particle is completely filled with cells and contains no intercellular space, the number of cells in a particle would be given by the ratio (R) of the total volume of the particle ($V_{particle}$) to the volume of a single bacterial cell (V_{cell}):

$$R = V_{particle} / V_{cell} \quad (1)$$

To account for intercellular space, this ratio (R) should be multiplied by the fraction of the total volume occupied by cells, or the packing density (P). The number of cells in a particle is therefore given by

$$N = RP \quad (2)$$

It is known that for spheres P may vary between 0.5235 and 0.7405, depending on a particular type of packaging (Conway and Sloane, 1993; Martin et al., 1997; Zong and Talbot, 1999). For the densest possible packing of spherical cells, such as face-centered cubic packing or hexagonal packing, P = 0.7405 (Arnold and Lacy, 1977; Conway and Sloane, 1993; Zong and Talbot, 1999; Arnold, 2000). On the other hand, in case of simple cubic packing, known to have the lowest packing degree between the individual spheres, P = 0.5235 (Conway and Sloane, 1993; Martin et al., 1997).

The upper and lower levels for the number of spherical cells (i.e., cocci such as *M. luteus*) in an aggregate/particle can therefore be estimated by using a method proposed by Martin et al. (1997). Namely, the number of cocci in the aerosol particles can be estimated as

$$0.5235R < \text{cell number} < 0.7405R \quad (3)$$

For the purposes of this study, however, the calculations of the number of cells in the particles were performed under assumption of randomly packed cells as the most likely scenario for the formation of cell aggregates in aerosol particles.

The packing density of the random densely packed spheres is known to be 0.64 (Seife, 2000; Berzukov et al., 2001; Donev et al., 2004). Accordingly, the

number of individual *M. luteus* in the agglomerates forming the aerosol particles was calculated as

$$N_{cocci} = 0.64R = 0.64\ D^3_{particle} / D^3_{cell} \quad (4)$$

where $D_{particle}$ is the count median diameter of the fluorescent particles detected in a particular channel and D_{cell} is the aerodynamic diameter of the bacterial cell.

In regard to bacilli, such as *B. subtilis* cells, the number of individual cells in the particles can be calculated in accordance with the theory of dense packing of ellipsoids (Coelho et al., 1997; Schurmann, 2002; Donev et al., 2004). Taking into account that ellipsoids can randomly pack with P = 0.68-0.71 (Donev et al., 2004), the number of *B. subtilis* cells in the aerosol particles measured by the UVAPS was estimated assuming the average number of cells in each aggregate to be

$$N_{bacilli} = 0.695R = 0.695\ D^3_{particle} / D^3_{cell} \quad (5)$$

2.6. Microbiological analysis

The Spread-Plate Count technique (Greenberg et al., 1992) was used to enumerate viable bacteria in the samples collected with the AGI-30 impingers. The concentration of airborne viable bacteria was determined by converting number of colony forming units (CFU) into an aerosol concentration in accordance with the procedure described previously (Agranovski et al., 2003b). The proportion of viable bacteria (further referred to as culturable fraction) in the total population of aerosol particles was determined by dividing the total aerosol count measured by the UVAPS to viable aerosol count measured by plate technique. The culturable fractions were subsequently compared with the fluorescent aerosol fractions, which were determined by dividing the total aerosol count to the fluorescent count measured by the UVAPS.

2.7. Statistical analysis

Statistical analysis was conducted on the complete data set using the Student's *t*-test. Statistical significance was accepted at the $P<0.1$ level of probability.

3. Results and Discussion

3.1. Non-bacterial aerosols

It has been shown previously that the amount of intrinsic fluorophores in bacteria may vary depending on the viability status of the cells (Agranovski et al., 2003b). In order to control the concentration of fluorescent material in the aerosol particles, the initial tests were performed with non-bacterial fluorophores. To correlate the fluorescence intensity with the particle size, which determines the amount of fluorophores within the aerosol particles, the CMD's (and then the volumes) of particles detected in each of 64 fluorescent channels were calculated. The CMD of each fluorescent channel was then plotted against the channel number. Thus, the results are presented in terms of

the average size of particles with the same fluorescence. To test for linearity, the measurements were obtained for the aerosols generated from the serially diluted solutions.

The fluorescence intensity was linearly proportional to particle volume (correlation factors were typically greater than 0.88) for all three non-microbial aerosols (NADH, NADPH, and riboflavin). The results for the NADH aerosols generated from the 0.1 mg/ml, 0.05 mg/ml, and 0.025 mg/ml solutions are presented in Fig.1, where each data point is the mean value of the five replicate measurements. The slope of the lines decreased by the same factor as the dilution (within the limits of the experimental error) demonstrating the linearity of the signals. Similar results, in terms of linearity, were obtained for the NADPH and riboflavin aerosols. To demonstrate variability of the data, the results (both mean values and standard distributions) for the NADPH aerosols generated from the 0.1 mg/ml solution are shown independently in Fig. 2.

Comparative analysis of the NADH and the NADPH results, for the aerosols generated from the solutions of the same concentration, showed that the NADH particles produced approximately four to six times higher fluorescence signals than the NADPH particles of the same volume (Figs. 1 and 2). These results are consistent with the results of the previous study (Agranovski et al., 2004), where NADH was found to be a stronger fluorophore than NADPH. Similarly, the fluorescence intensity of the riboflavin particles was found to be significantly higher than that for the particles having the same amount of NADH.

3.2. Bacterial aerosols

As with the non-biological aerosols, the fluorescence intensity was found to vary linearly with the particle volume of bacterial aerosols (Figs. 3-4). Comparative analysis of the data for the cells in various metabolic states showed, however, that fluorescence intensity of the particles containing cells harvested during the stationary growth phase (further referred as "stationary-phase particles") was higher than the fluorescence intensity of the log-phase cells. For example, inspection of the data for the *B. subtilis* aerosols (Fig. 3) showed that the stationary-phase particles (open squares and the linear fit presented with a solid line) of the same size as the log-phase particles (filled diamonds and a dashed line) typically produced stronger fluorescence, corresponding to higher channel numbers. In addition, while the fluorescent signals for the log-phase aerosols were recorded only in the first 15 channels, the stationary-phase aerosols were detected in up to the 33rd channel. Similar tendencies were also observed for the *M. luteus* aerosols, generated with the cells harvested at different growth phases. These results are consistent with the results of the previous study (Agranovski et al., 2003b) where, due to their greater susceptibility to the aerosolization stress, the log-phase particles were shown to contain less fluorescent material than the stationary-phase particles.

In relation to the heat-stressed *M. luteus* log-phase cells, the stressed cells produced less fluorescence than the cells not subjected to heat treatment, or the so-called "healthy" cells (Fig. 4). Although both aerosols produced signals in the first 34 channels, the comparison of the dependence of the fluorescence signals for the particles of the same size showed that the amount of fluorophores in the heat-stressed bacteria was smaller ($P< 0.1$) than in the healthy bacteria. Namely, intensity (which corresponds to the channel number) of aerosol particles of the same size containing healthy *M. luteus* cells that were untreated by heat was higher than for the particles

containing the heat-stressed cells (Fig.4). These results are consistent with the results by Agranovski et al (2003b), where the UVAPS data were compared with both the amount of injured and healthy culturable bacteria and where cell injury was first demonstrated to affect the amount of the intrinsic fluorophores compared with the intact bacterial cells.

In terms of particle concentration, the percent fluorescence (e.g., the percentage of the aerosol particles that produced a fluorescent signal above a threshold) was found to be 34.5 ± 0.1 % and 23.6 ± 0.1% for the healthy and the heat-stressed *M. luteus* samples, respectively.

3.3. Effect of particle size on the UVAPS performance

Although the fluorescence intensity was generally directly proportional to the amount of the fluorophores in the aerosol particles, closer inspection of the graphs showed that the linearity was slightly affected by the particle size (Figs. 1-2). Specifically, the slope, although constant at smaller sizes, was observed to decrease at larger particle sizes. These results may be explained by the reduced capability of the UV light to penetrate larger or more concentrated (in terms of fluorescent material) particles. When the amount of the fluorophores inside the particle increases, the light either cannot penetrate the entire volume, to cause excitation, or the surface portion of the particle nearest to the beam absorbs the majority of the light, leaving little available for the rest of the particle. This will subsequently decrease the intensity of the bigger (and more concentrated) particles and affect the slope of the line. Nevertheless, at smaller fluorophore concentrations, particularly at the concentrations characteristic to the bacterial cells, the linearity of UVAPS fluorescent signals was apparent over the range of particle sizes (Figs. 3 - 4).

3.4. Number of bacterial cells in the particles

Due to the apparent linearity of the UVAPS signals for bacterial aerosols, it was possible to estimate the number of individual microorganisms within the aerosol particles. The relationships between the intensity of the fluorescent signals and the amount of individual microorganisms in aerosol particles are presented graphically in Fig. 5 and 6. The numbers of bacterial cells inside the particles were calculated in accordance with equations (4) and (5), as discussed earlier in the Methods. In this series of the experiments, in order to produce bacterial clumps, the *B. subtilis* aerosols were generated by means of the nasal sprayer. Unlike *B. subtilis* aerosols, it was possible to produce *M. luteus* clumps with the Collison nebulizer (Agranovski et al., 2003b).

Due to the specificity of the UVAPS results, which were analysed for each of the 64 channels, the calculated values cannot be directly verified experimentally with conventional bioaerosol methods. Comparison of the experimental data with the calculated values is, however, possible for the whole set of the UVAPS data, for a particular sample. In other words, rather than analysing the UVAPS data for each channel individually, the results should be analysed for the total concentration of fluorescent particles, i.e., for the sum of the fluorescent particles detected through channels 2 to 64. In this manner, the number of individual microorganisms may be related to the number of fluorescent particles measured for a specific bacterial aerosol. For example, in the case of the *M. luteus* aerosols comprised of heat-stressed cells (corresponding to the results presented in Figs. 4 and 6), the total concentration of the

fluorescent particles ($N_{particles}$) was approximately 4.7 particles/ cm^3 and the CMD of the fluorescent particles was 1.55 μm. Considering the aerodynamic diameter of *M. luteus* cells (D_{cells}), which was approximately 1.22 μm, the estimated concentration of individual microorganisms (N_{cells}) contained in the aerosol particles will be approximately 6.1 cells/cm^3 ($N_{cells} = N_{particles} \times 0.64 \times D^3_{particle}/ D^3_{cell}$ = 4.7 particles/$cm^3 \times 0.64 \times 1.55^3$ μm /1.22^3 μm). Whereas the results for *M. luteus* aerosols comprised of the healthy cells were as follows: $N_{particles}$ = 6.9 particles/ cm^3, CMD = 1.58 μm, and D_{cells} = 1.26 μm, that corresponded to the concentration of the individual organisms of approximately 8.7 cells/cm^3. These calculated values for the *M. luteus* aerosols compared favourably with the results of the AGI-30 sampling, which were reported previously (Agranovski et al., 2003b). Namely, the concentrations of culturable organisms were 5.1 CFU/cm^3 and 7.4 CFU/cm^3 for the stressed and healthy cells, respectively. The calculated values for the number of *M. luteus* cells in the aerosols measured by the UVAPS were therefore approximately 18 - 19 % higher than the number of culturable organisms detected with the AGI-30. As the AGI-30 impingers are known to break the microbial clumps and, therefore, to provide data for the amount of airborne microorganisms rather than airborne microbial particles, it was appropriate to compare the AGI-30 results with the calculated values based on the UVAPS data.

4. Conclusions

The results of the present study have demonstrated that the UVAPS data on the fluorescence intensity and the size distribution of fluorescent particles can be used to estimate the concentration of airborne microorganisms. However, it has to be noted that the capability of the UVAPS in this regard is limited to aerosols generated under controlled laboratory conditions. In the case of ambient aerosols, which may contain a number of different microorganisms, as well as other biological and non-biological fluorescent materials, the interpretation of the UVAPS data in terms of numbers of individual microorganisms will be extremely complicated.

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Figure Legends

Fig. 1. Relationship between particle volume and intensity of the fluorescent signals for the NADH aerosols.

Fig. 2. Results for the NADPH aerosols generated from the 0.1mg/ml solutions.

Fig. 3. Relationship between particle volume and intensity of the fluorescent signals for the *B. subtilis* aerosols generated with the Collison nebuliser. The results correspond to the cells harvested at stationary (a solid line) and log (a dashed line) growth phase.

Fig. 4. Relationship between particle volume and intensity of the fluorescent signals for the *M. luteus* aerosols. The results correspond to the healthy (H) cells harvested at the exponential (or log) growth phase and for the heat-stressed (S) cells.

Fig. 5. Relationship between the maximal number of the *B. subtilis* individual cells within the aerosol particles and the intensity of the fluorescence registered by the UVAPS. These aerosols were generated with the nasal sprayer from washed cells harvested at the stationary growth phase.

Fig. 6. Relationship between the maximal number of the *M. luteus* individual cells within the aerosol particles and the intensity of the fluorescence registered by the UVAPS. These aerosols were generated from the washed cells harvested at the exponential (log) growth phase (labelled as "Healthy") and the heat-stressed cells from the same bacterial suspension.

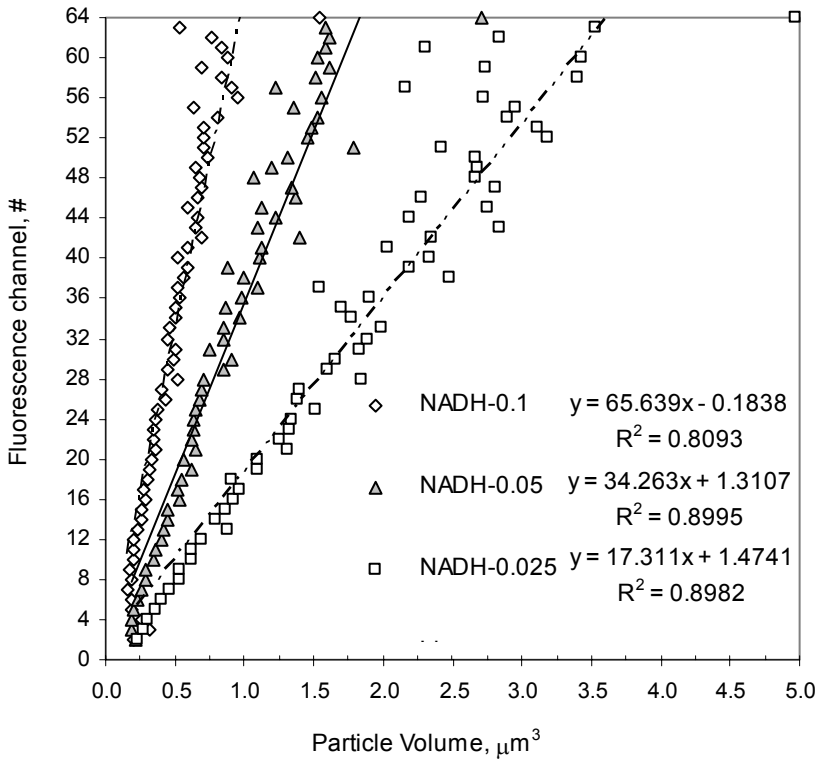


Fig. 1. Relationship between particle volume and intensity of the fluorescent signals for the NADH aerosols.

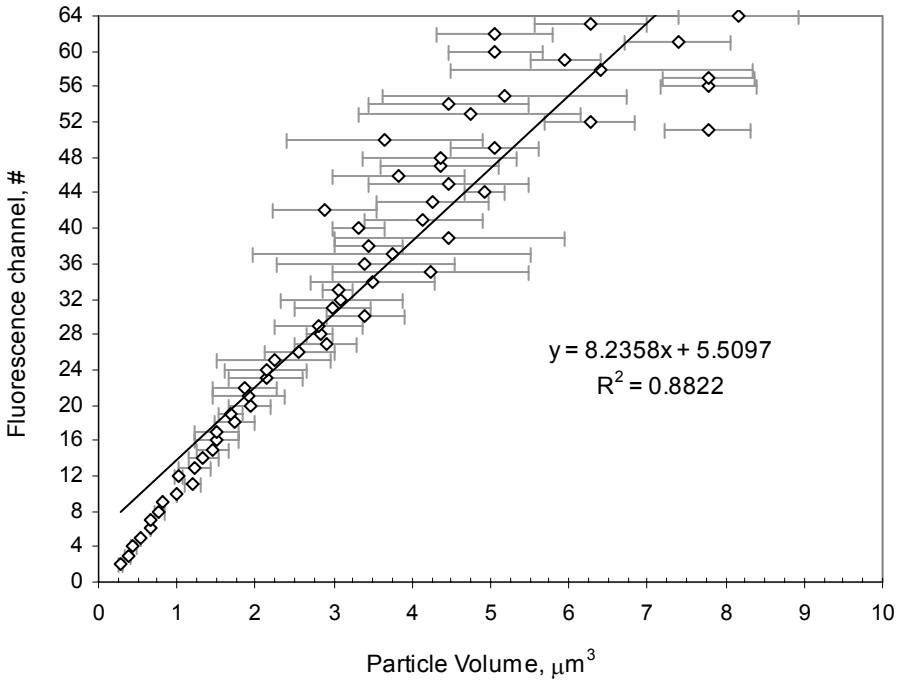


Fig. 2. Results for the NADPH aerosols generated from the 0.1mg/ml solutions.

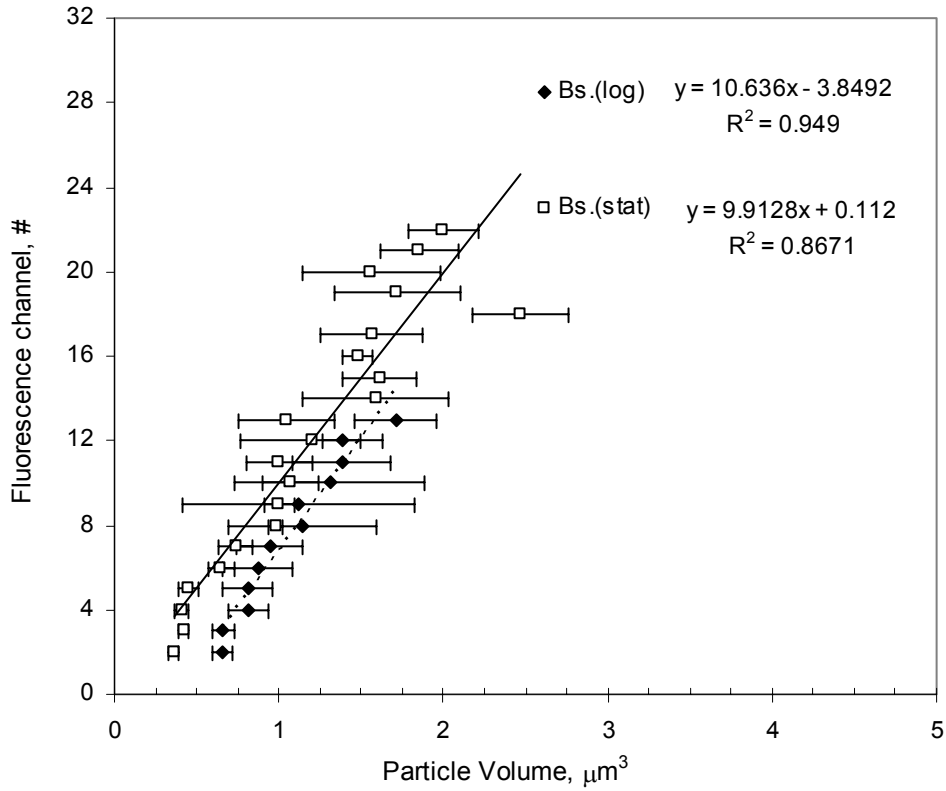
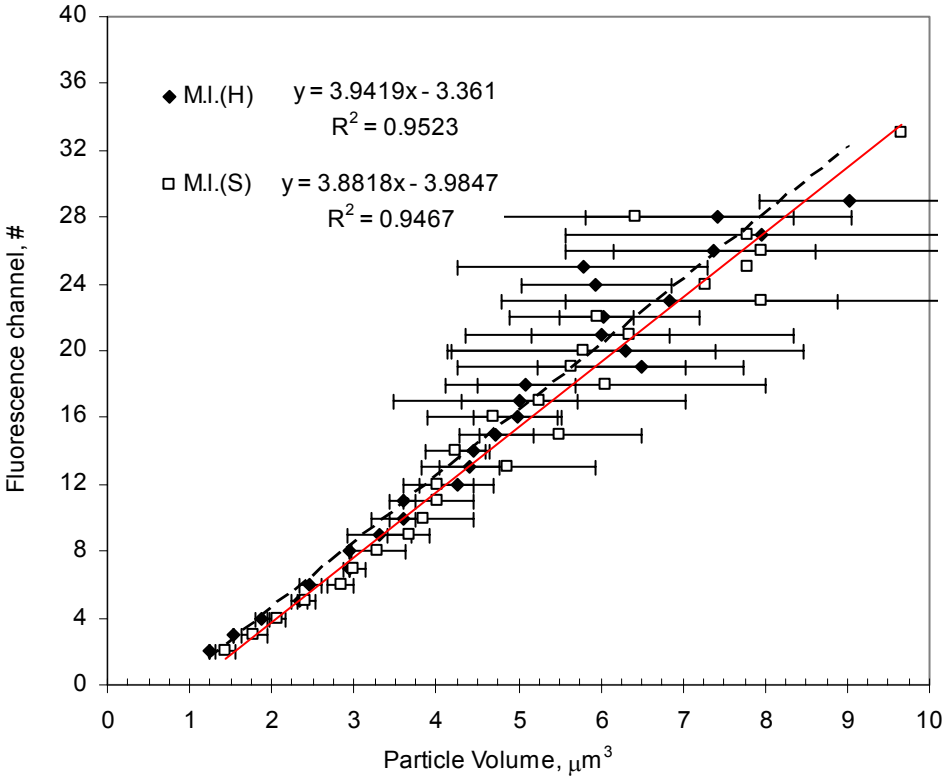


Fig. 3. Relationship between particle volume and intensity of the fluorescent signals for the *B. subtilis* aerosols generated with the Collison nebuliser. The results correspond to the cells harvested at stationary (a solid line) and log (a dashed line) growth phase.



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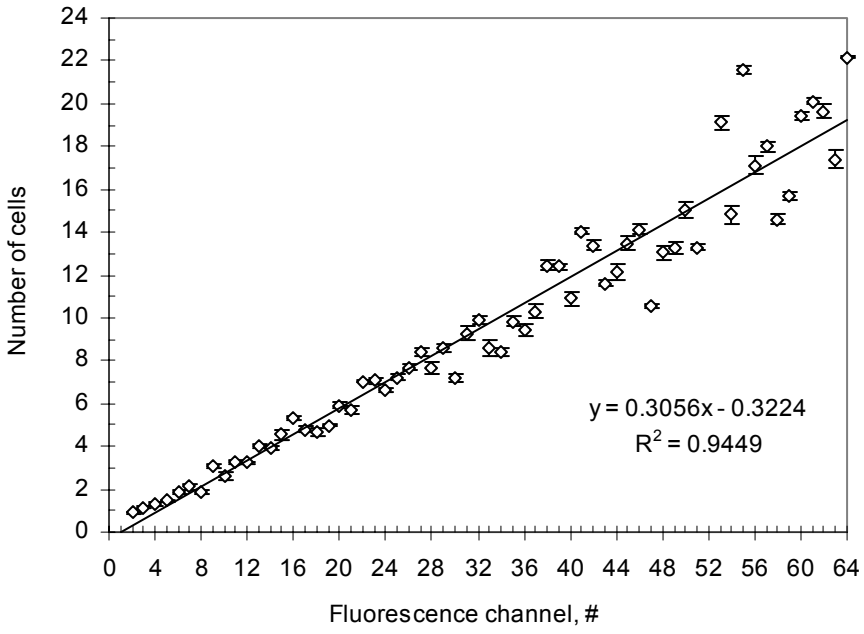

Fig. 5. [illegible] ividual cells within the aerosol particles and the intensity of the fluorescence registered by the UVAPS. These aerosols were generated with the nasal sprayer from washed cells harvested at the stationary growth phase.

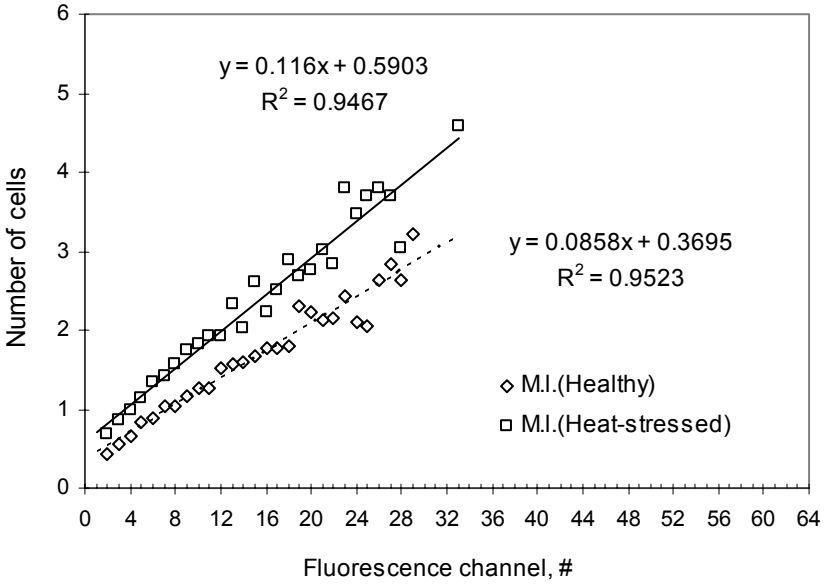

Fig. 6. Re ividual cells within the red by the UVAPS. sted at the exponentia essed cells from the s