COVER SHEET

Determination of the number of individual bacterial cells in aerosol particles with the UVAPS

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Intensity of the fluorescent signals detected by the UVAPS was found to be directly proportional to the amount of the fluorophores in the airborne particles (Agranovski et al., 2005), which indicates that the UVAPS data can be used to estimate the number of individual microorganisms in the particles. This study further explored a capability of the UVAPS to provide data on the concentration of airborne bacterial cells, in addition to the concentration of airborne bacterial particles.

Experiments were performed with *B. subtilis* and *M. luteus* vegetative cells. The cells were washed and suspended in 200 ml of sterile distilled water at the concentration of approximately 10^9 cells/ ml, as was determined by measuring the turbidity of the suspensions with a spectrophotometer at 540 nm. The suspensions were then further analysed using the haemocytometry method and the spread-plate-count technique, to enumerate the total and viable cells, respectively. The ratio of culturable to total counts provided data for the viable fraction (R_V) of cells in the spray suspensions. The aerosols were generated by a 6-jet Collison nebulizer. In addition to the UVAPS, the aerosols were collected with the AGI-30 impingers and then analysed by cultivation on the TSA agar plates.

Analysis of bacterial suspensions revealed that approximately 71 % of *B. subtilis* cells and 80 % of *M. luteus* cells were viable. Thus, the aerosol particles containing several bacterial cells could include both viable and non-viable organisms. While the number of total cells in each particle was proportional to the particle aerodynamic diameter, the number of viable cells was proportional to the amount of the fluorescent material and thus to the fluorescence intensity which was designated by the number of the fluorescent channel.

The number of individual cells (N) which may occur within the particles depends on particle size, cell size, and the packing density (P) and can be estimated as $N = P*V_{particle} / V_{cell}$, where $V_{particle}$ is the volume of the particle and V_{cell} is the volume of a single bacterial cell. The packing of aerosol particles from evaporating droplets can vary considerably. However, in theory, for spherical cells (e.g., cocci) P may vary between 0.5235 and 0.7405 (Martin et al., 1997), depending on type of packing. Specifically, in the case of randomly packed spheres, which is the most likely scenario for the formation of cell aggregates in aerosol particles, P = 0.64 (Donev et al., 2004). Accordingly, the number of individual *M. luteus* in the agglomerates forming the aerosol particles was calculated as $N_{cocci} = 0.64R = 0.64 D^3_{particle} / D^3_{cell}$, 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 was calculated in accordance with the theory of dense packing of ellipsoids 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 $N_{bacilli} = 0.695R = 0.695 D^3_{particle} / D^3_{cell}$.

To estimate the number of viable cells (N^V) in the particles, it was assumed that the fraction of viable cells in the aerosols was equal to the viable fraction of cells in the spray suspensions (R_V). Thus, the number of viable cells was calculated as $N^V_{cocci} = R_V*N_{cocci} = 0.8\ N_{cocci}$ for *M. luteus* aerosols and as $N^V_{bacilli} = R_V*N_{bacilli} = 0.71\ N_{bacilli}$ for *B. subtilis* aerosols.

The calculated values were then compared with the concentration of culturable organisms (N^C) collected with the AGI-30 impingers, 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.

On average, the calculated values for the number of *M. luteus* cells in the aerosols measured by the UVAPS were approximately 0.94 ± 0.03 times of the values for the number of culturable organisms detected with the AGI-30. For *B. subtilis* aerosols the mean value of the ratio of N^V to N^C reached 0.78 ± 0.20. These results indicate that the UVAPS data on the fluorescence intensity and the size distribution of fluorescent particles can be used to estimate the concentration of airborne viable organisms.

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