EVALUATION OF THE CYCLEX IMPACTOR

PHASE 2:

COLLECTION EFFICIENCY EVALUATION WITH MICROBIAL SPORES (IMPACTOR'S CONFIGURATIONS 1, 2, AND 3)

REPORT 3

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OBJECTIVE

The objective of Phase 2 was to evaluate the collection efficiency of the new EMS Impactor (Environmental Monitoring Systems, Inc.) through laboratory experiments with actual microbial spores, including two fungal and one bacterial species. The sampler was initially designed for fungal spore collection. Three different configurations of the collection unit of the EMS Impactor were tested, all with the sampling flow rate of 20 Lpm (same as in most of the tests of Phase 1).

METHODS

The tests were performed with fungal spores of *Cladosporium cladosporioides* and *Aspergillus versicolor* as well as with bacterial endospores of *Bacillus subtilis* var. *niger*. These microorganisms commonly occur in indoor and outdoor environments in various climate zones worldwide. The aerodynamic sizes of fungi are 1.8 μm for *C. cladosporioides* and 2.5 μm for *A. versicolor*. *B. subtilis* is a gram-positive bacterium, which have an aerodynamic diameter of approximately 0.9 μm (Reponen et al., 1996).

Prior to the experiments, *C. cladosporioides* and *A. versicolor* were cultured in dispersion tubes containing malt extract agar (MEA), and were then incubated at 25°C for 7 days. The dispersion tubes were inserted into an agar-tube disperser for dry spore generation, as previously described by Reponen et al. (1997). *B. subtilis* endospores were washed twice (with sterile deionized water by centrifugation at 7,000 rpm for 7 minutes) before their generation from water suspension by Collison nebulizer (BGI Inc., Waltham, MA).

The test system for determining the spore collection efficiency is schematically presented in Figure 1. The fungal spores were generated from an agar-tube disperser by passing HEPA-filtered air through this device. The test aerosol was additionally diluted with HEPA-filtered compressed air, Q_{DIL}. The diluted fungal aerosol entered the test chamber housing the EMS Impactor. The bacterial spores were generated using a Collison nebulizer, diluted with HEPA-filtered compressed air, and charge neutralized using a 10-mCi ^{85}Kr electrostatic charge equilibrator (Model 3012, TSI Inc., St. Paul, MN). The upstream aerosol concentration, C_{UP}, and the downstream aerosol

concentration, C_{DOWN}, were measured using an optical particle counter (Model 1.108, Grimm Technologies, Inc., Douglasville, Georgia) operated at a flow rate of 1.2 Lpm.

The EMS Impactor was tested in three different configurations referred further as Configuration 1, Configuration 2, and Configuration 3, which respectively have jet-to-plate distances of 0.006", 0.012", and 0.018" (non-dimensional S/W = 0.033, 0.066, and 0.099, respectively). Each configuration was tested with three above-listed microbial species, and each experiment was done in three replicates. During these tests, the EMS Impactor was operated at a flowrate Q_S=20 Lpm for up to 5 min. From the obtained experimental data, the average value of the collection efficiency and the standard deviation were calculated. Using the particle concentration and size distribution measured upstream and downstream of the sampler, we determined the overall capture efficiency of the Impactor, E_{Copt}:

$$E_{Copt} = (1 - C_{DOWN}/C_{UP}) \times 100\% \quad (1)$$

This efficiency represents the fraction of particles of a given size that were captured by the sampler, irrespective of the location of their collection: some of them are collected on the slide and thus be subjected for the microscopic or other analysis, and some is considered as interior losses.

By counting the number of spores collected on a slide and then comparing this number with spore concentration upstream of the sampler, we determined the actual collection efficiency, $E_{Cmicroscope}$:

$$E_{Cmicroscope} = (1 - C_{SLIDE}/C_{UP}) \times 100\% , \quad (2)$$

which is more adequate performance characteristic of a bioaerosol sampler. The difference between the collection efficiencies of E_{Copt} and $E_{Cmicroscope}$ represents the particle losses inside the impactor. Both efficiencies were determined for each of the three Impactor's configuration and for each test microorganism.

RESULTS

The capture and actual collection efficiencies are presented side by side in Figure 2 (Configuration 1), Figure 3 (Configuration 2), and 4 (Configuration 3).

All three configurations were found adequate to collect fungal spores with the actual efficiency exceeding 50% (50% is a minimum requirement since the aerosol impactors

are usually characterized by the particle cut-off size, d_{50}). For the tested fungal spores (one below 2 μm and one above 2 μm), **the EMS Impactor collection efficiency was found to be higher than that of the Air-O-Cell and Burkard samplers** (e.g., both collect *C. cladosporioides* with the efficiency lower than 50%). For bacterial spores (below 1 μm), the collection efficiency was below 50% that shows the sampler limitations (it seems to be efficient for fungal spores but may not be as good for smaller bacteria). The differences between the overall capture and the actual collection efficiencies were statistically insignificant in most of the cases (see error bars in Figures 2-4).

For *B. subtilis* spores, the comparison of E_{Copt} and $E_{Cmicroscope}$ shows that the highest collection efficiency was observed for Configuration 1 ($E_{Copt}=37\pm\ldots\%$ and $E_{Cmicroscope}=27\pm\ldots\%$). The efficiencies determined for Configurations 2 and 3 from the optical particle counter reading, see Eq. (1), and microscopic slide counting, see Eq. (2), were lower than those determined for Configuration 1.

For *C. cladosporioides,* the most efficient collection was achieved with Configuration 1 ($E_{Copt}=74\pm\ldots\%$ and $E_{Cmicroscope}=86\pm\ldots\%$). Configurations 2 and 3 revealed about 18-23% decrease in the collection efficiency when compared to Configurations 1.

A. versicolor spores were also efficiently collected (>50%). Surprisingly, however, for Configuration 1, the actual collection efficiency of *A. versicolor* was slightly lower than that of *C. cladosporioides* (although *A. versicolor* spores are larger, has higher inertia, and hence should impact on the slide more efficiently). This finding will be explained in the Discussion section.

The comparison of the collection efficiencies of the three tested spores with the collection efficiencies of the oleic acid and PSL particles (determined in Phase 1) showed that:

- for Configuration 1: collection efficiencies of *B. subtilis* and *C. cladosporioides* spores are in a good agreement with the data obtained with PSL particles and were below values obtained for oleic acid particles. The capture efficiency, E_{Copt}, and the actual collection efficiency, $E_{Cmicroscope}$, of *A. versicolor* spores were below the values previously reported for both oleic acid and PSL particles;

- for Configuration 2: E_{Copt} and $E_{Cmicroscope}$ for *B. subtilis* agree well with the data obtained with inert particles. Collection efficiency values for fungal spores were somewhat below the oleic acid and PSL data points;
- for Configuration 3: E_{Copt} and $E_{Cmicroscope}$ for *B. subtilis* were higher than the collection efficiencies obtained with inert particles. E_{Copt} and $E_{Cmicroscope}$ values for *C. cladosporioides* spores were between the curves obtained with oleic acid and PSL particles respectively. E_{Copt} and $E_{Cmicroscope}$ values for *A. versicolor* spores were below these curves.

DISCUSSION

The experiments performed with *B. subtilis* and *C. cladosporioides* spores showed that the highest collection efficiency was achieved with Configuration 1, which has the smallest jet-to-plate distance of the three configurations. Besides, for all three configurations, no statistical difference was observed between the overall capture efficiency determined using the optical particle counter measurements, E_{Copt}, and the collection efficiency based on the microscopic counting on a slide, $E_{Cmicroscope}$, when collecting *B. subtilis* and *C. cladosporioides* spores. These results are different for the collection of *A. versicolor* spores. For *A. versicolor*, the overall capture efficiency, E_{Copt}, was somewhat higher than the collection efficiency based on the microscopic counting on a slide, $E_{Cmicroscope}$.

For *A. versicolor*, the spores are released as single spores and agglomerates. It is known that *A. versicolor* can release spore chains of up to 60-80 spores in a chain (Górny et al., 2001). The big agglomerates released from an agar surface impact on the slide and may broke up into smaller particles-fragments. Some of these particles remain on sticky slide surface, while the other are reaerosolized and then may be collected inside the housing of the Impactor. These internal losses contribute to E_{Copt} values, thus the collection efficiency obtained from optical particle count reading outnumbers the collection efficiency based on microscopic slide counting.

REFERENCES

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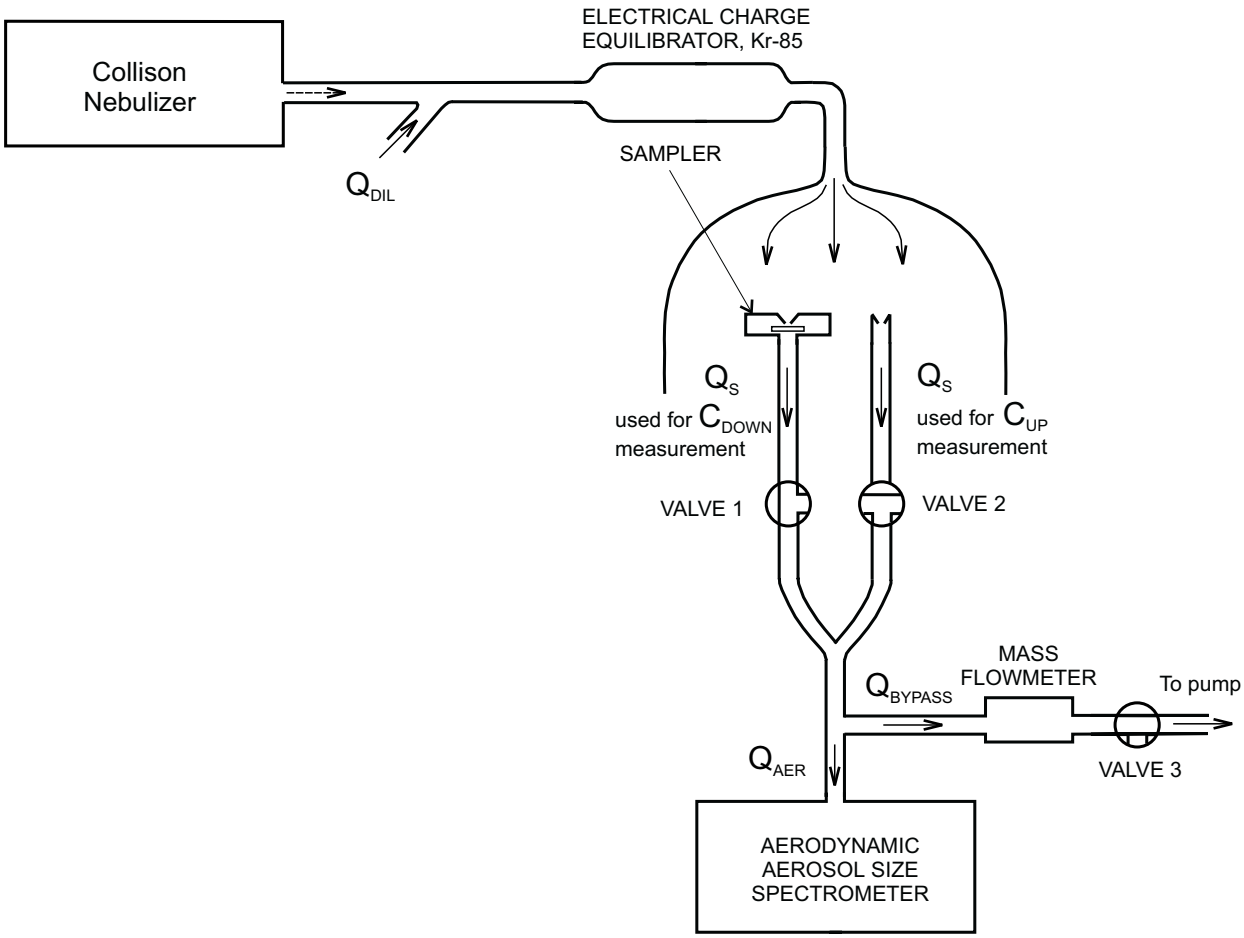

FIGURE 1. EXPERIMENTAL SETUP.

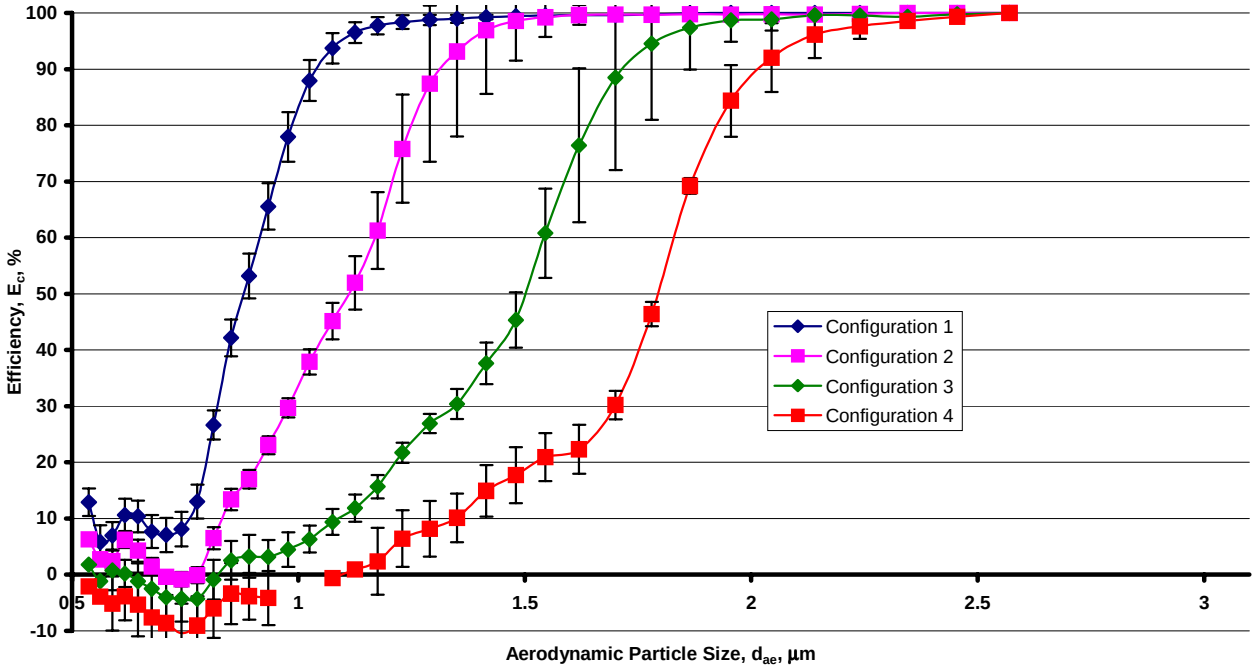

FIGURE 2. EMS IMPACTOR EFFICIENCY FOR DIFFERENT CONFIGURATIONS MEASURED WITH **OLEIC ACID PARTICLES** AT 20 LPM.

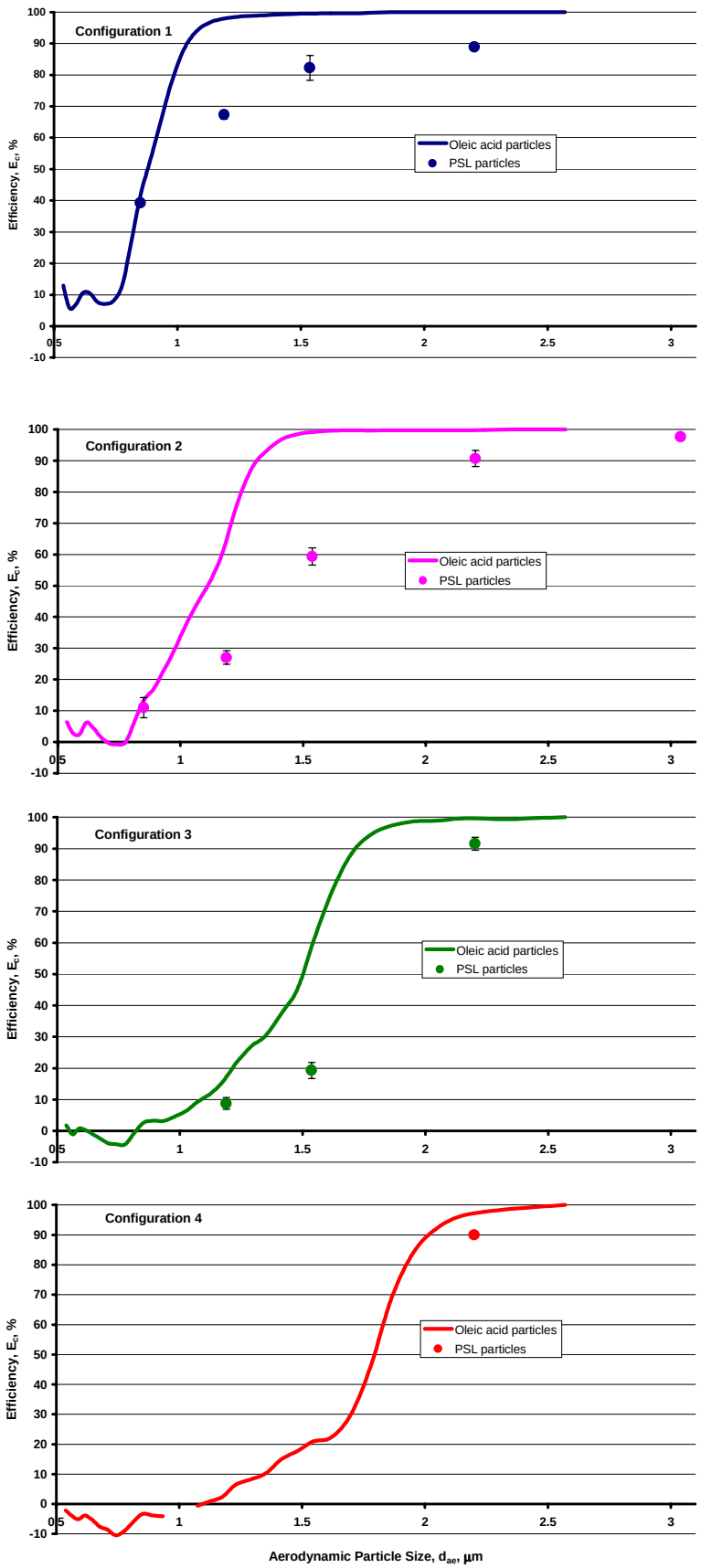

FIGURE 3. EMS IMPACTOR EFFICIENCY FOR DIFFERENT CONFIGURATIONS MEASURED WITH **OLEIC ACID AND PSL PARTICLES** AT 20 LPM.