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Effect of Fluid Type and Microbial Properties on the Aerosolization of Microorganisms from Metalworking Fluids

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Aerosolization of mist from metalworking fluids (MWFs) has been well characterized in previous studies. Much less is known about the aerosolization of microorganisms, although airborne microbial exposures at MWF sites have been associated with occupational respiratory symptoms and diseases. In this study, the effects of fluid type, microorganism concentration in the liquid, and the microbial species on the aerosolization of microorganisms from MWFs were tested. Three microorganisms were employed to represent different size and surface characteristics: *Bacillus subtilis* bacterial endospores (hydrophobic particles with aerodynamic diameter = 0.9 μm), *Pseudomonas fluorescens* bacterial vegetative cells (hydrophilic, 0.8 μm), and *Penicillium melinii* fungal spores (hydrophobic, 3.1 μm). The testing was first performed using a Collison nebulizer to aerosolize microorganisms from three fluids: water, semisynthetic MWF, and soluble oil. No significant difference in the aerosolization ratio (microbial concentration in the air normalized to the microbial concentration in the liquid) was observed among the three fluids. For all tested microorganisms, the concentration in the air increased proportionally with the increase of the microbial concentration in the liquid. The aerosolization ratio of *B. subtilis* endospores was greater than that of *P. fluorescens* cells and *P. melinii* spores. To explore the aerosolization of microorganisms from MWFs under the conditions that are closer to industrial settings, the tests were conducted with a MWF simulator (a laboratory-scale setup simulating the mist generation during grinding process). Simulator tests showed the same trend with respect to microbial aerosolization as those performed with the Collison nebulizer. This was further confirmed by a separate experiment, in which the Collison nebulizer and the MWF simulator were tested with liquids containing polystyrene latex (PSL) particles. As a result, our study showed that hydrophobic microorganisms were easier to aerosolize from MWFs than hydrophilic microorganisms and that increasing microorganism size was likely to result in decreasing aerosolization ratio.

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INTRODUCTION

Metalworking fluids (MWFs) are used as coolants and lubricants in cutting and forming operations in a variety of industries, e.g., in automotive parts manufacturing. MWFs can be divided into four general categories: straight oils (mineral oils), soluble oils, semisynthetic MWFs, and synthetic MWFs. The last three categories are water-based fluids and are used as suspensions containing up to 95% water by volume.

Since water-based MWFs may become contaminated with bacteria and fungi, the health effects resulting from MWF exposure are of special interest. Health effects associated with MWF exposures include dermatitis, respiratory symptoms, asthma, and in some cases hypersensitivity pneumonitis (HP) (Robertson et al. 1988; Popendorf et al. 1996; Graeves et al. 1997). Although no single etiologic agent has been identified, clusters of respiratory symptoms, work-related asthma, and HP have often been associated with the use of water-based MWFs (NIOSH 1998; Rosenman et al. 1997; Kreiss and Cox-Granser 1997). Antibodies to a common MWF bacterium, *Pseudomonas*, have been found in the serum of MWF-exposed workers, suggesting an association between microbial exposure and allergy-based health effects (Bernstein et al. 1995).

Aerosolization of pure MWF mists in industrial settings (machine centers) has been extensively studied by Thornburg and Leith (2000), Heitbrink et al. (2000), Dasch et al. (2002), and Turchin and Byers (2000). The tool speed has been found to have the largest impact on the mist concentration. Other factors, such as fluid application rate and presence of tramp oil, were also found to have considerable impact on the mist concentration and size distribution.

Most of the studies on MWF contaminated with microorganisms have been focused on identifying the microbial species, determining their concentrations and the growth dynamics in bulk MWFs in the field. Gram-negative bacteria, especially *Pseudomonas* spp., have been found by many investigators to represent the major bacterial contaminants in MWFs (Sondossi and Rossmoore 1985; Bernstein et al. 1995; Lonon et al. 1999). Other microorganisms have also been identified in used industrial MWFs, such as Mycobacteria (Kreiss and Cox-Ganser 1997; Moore et al. 2000), *Aspergillus niger, Staphylococcus capitas*, and *Bacillus pumilus* (Bernstein et al. 1995). A study by Mattsby-Baltzer et al. (1989) showed that bacteria could be present in industrial MWFs at high concentration ($>10^8$ cells/ml) even after large quantities of biocides had been added. Only a few studies have attempted to link the concentrations of microorganisms and endotoxin in the fluid with those in the air (Thorne et al. 1996; Virji et al. 2000). Virji et al. (2000) used a multivariate model to determine the major factors associated with the microbial levels. These investigators found that fluid-related factors (pH level of the fluid and presence of tramp oil) were the most important characteristics related to the microbial levels in the bulk MWF, while process-related factors (worker's distance from the machine, bulk microbial levels, machine enclosures) were the major characteristics associated with the microbial levels in the air. Thorne et al. (1996) monitored microorganisms and endotoxin in the air and in the bulk MWFs in an engine plant for four seasons. The concentration of airborne total bacteria (culturable + nonculturable) varied widely from 5,560 org/m^3 to 468,000 org/m^3. The study revealed that airborne endotoxin concentrations demonstrated significant associations with bulk endotoxin and with bulk total microorganisms. However, there was no clear relationship between airborne and fluidborne total bacteria. This may be due to the considerable temporal and spatial variability of the bioaerosol concentration during the measurements caused by a variety of sources, such as outside air.

We investigated the aerosolization of microorganisms from MWFs using two laboratory-scale setups. First, aerosolization was characterized utilizing a Collison nebulizer, which is commonly used for the aerosolization of bacteria and fungi in laboratory studies. Second, a laboratory-scale setup (MWF simulator) was built and the experiments were repeated to verify the results under conditions that more closely simulate industrial operations.

MATERIALS AND METHODS

Part I. Aerosolization of Microorganisms and PSL Particles by a Collison Nebulizer

Experimental Setup. The experimental setup utilized in the first part of this study is schematically shown in Figure 1. A six-nozzle Collison nebulizer (BGI Inc., Waltham, MA, USA) generated aerosols either from microorganism suspensions or PSL suspensions at a flow rate of 6 l/min. The aerosol flow was diluted with HEPA-filtered clean air at a flow rate of 30 l/min and entered a test chamber with a Button Inhalable Aerosol Sampler (SKC Inc., Eighty Four, PA, USA) placed in the center. In addition, an optical particle counter (OPC, Model 1.108, Grimm Technologies Inc., Douglasville, GA, USA) was placed

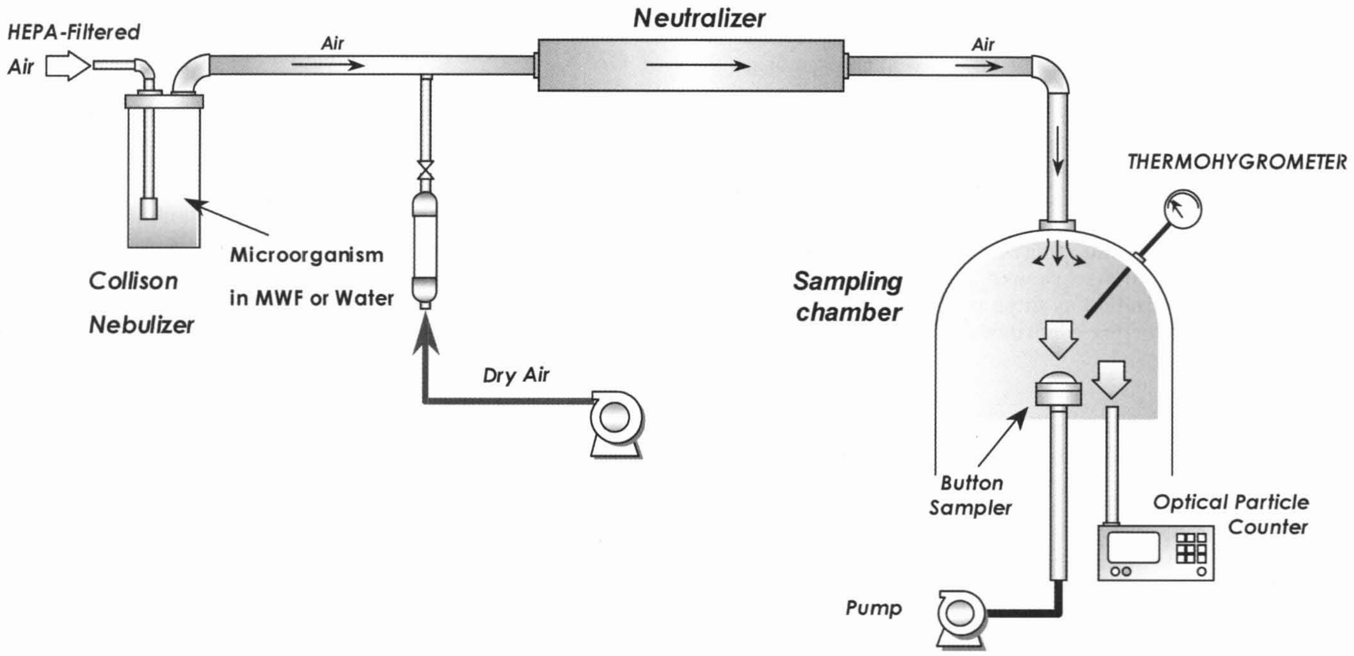

Figure 1. Experimental setup for the aerosolization of microorganisms from metalworking fluids by the Collison nebulizer.

beside the Button Sampler to monitor the stability of the aerosol concentration in the chamber. The entire setup was located in a biological safety cabinet (Sterilchem-Gard Class II, Type B2, Baker Co., Sanford, ME, USA).

Test Microorganisms and Preparation of Microbial Suspensions. The testing was performed with three species of microorganisms: *Bacillus subtilis* bacterial endospores, *Pseudomonas fluorescens* bacterial vegetative cells, and *Penicillium melinii* fungal spores. These microorganisms have been commonly found in used MWFs (Thorne et al. 1996; Mattsby-Baltzer et al. 1989; Lummus et al. 1998; Lonon et al. 1999). They were selected to represent different surface characteristics of microorganisms (hydrophilic or hydrophobic) and different sizes (see Table 1). *B. subtilis* endospores were received from the U.S. Army Edgewood Laboratories (courtesy of Agnes Akiyemi and Dr. Edward Stuebing, Edgewood Research, Development, and Engineering Center, Aberdeen Proving Ground, MD, USA). The spores were activated at 60°C for 25 min and washed twice with sterile deionized water by centrifugation at 7000 rpm for 7 min. *P. fluorescens* (ATCC 13525) culture was obtained from the American Type Culture Collection (Rockville, MD, USA). It was subcultured by incubating in a trypticase soy broth at 28°C for 18 h. The cells were then washed twice, similar to the procedure used for *B. subtilis* spores. *P. melinii* strain had previously been isolated from a moldy building. Prior to their use in the experiments, they were cultured on malt extract agar and subsequently incubated at 25°C for 14 days. Spores were collected from matured sporulating cultures by applying 1 g of dry, autoclaved glass beads (0.45–0.5 mm in diameter, B. Braun Biotech International, Melsungen, Germany) per Petri plate as described by Schmechel et al. (2003). The lid was put back into place and the plates were gently shaken back and forth. This allowed the spores to attach to the beads. The beads were transferred into a 50 ml tube containing sterile deionized water and spores were suspended and separated from the beads by briefly shaking the tube and decanting the spore suspension.

The freshly prepared suspensions of *B. subtilis, P. fluorescens*, and *P. melinii* were diluted with the test MWFs or with sterilized deionized water, depending on the experiment, until desired concentrations were achieved. Two types of MWFs tested in this study, semisynthetic MWF and soluble oil, are commercially available. They were used in this study as 5% water solutions.

Measurement of Microbial Concentration in the Air

Button sampler. The Button Personal Inhalable Aerosol Sampler operating at a flow rate of 4 l/min collects particles on a 25 mm membrane filter. The filter is located behind the porous metal inlet screen, which has a spherical surface with a subtended angle of 160° and a porosity of 21%. Orifices of 381 μm diameter are evenly spaced throughout the inlet surface, resulting in uniform particle deposition on the entire filter area. The Button Sampler was found to be significantly wind insensitive under laboratory conditions (Aizenberg et al. 2000) as well as outdoor field conditions (Adhikari et al. 2003). Black polycarbonate filters with a pore size of 0.2 μm (Osmonics Inc. Westborough, MA, USA) were employed for the collection of *P. fluorescens* and *B. subtilis* at a sampling time of 10 min. Black polycarbonate filter with a pore size of 3.0 μm was employed for the collection of *P. melinii* to allow a longer sampling time (30 min) without blockage of the filter. Although *P. melinii* spores were extracted directly from culture plates, we found it challenging to obtain a large amount of spores for the liquid suspension. As a result, the number of *P. melinii* spores aerosolized from the liquid into the air was also relatively low. In order to collect a sufficient number of fungal spores that could be adequately quantified by microscopic counting, an extended sampling time was required for *P. melinii*. Lee et al. (2004) reported that the collection efficiency of 3.0 μm particles on a polycarbonate filter with 3.0 μm pores was 95% at a flow rate of 7.2 l/min.

Total microbial concentration in the air. For determining the total microbial concentration in the air, the black polycarbonate filter was removed from the Button Sampler and directly stained by the acridine orange method, as described by Wang et al. (2001). An epifluorescence microscope was used to count the microorganisms on the filter at a magnification of 1000× (Model Laborlux S; E. Leitz, Inc., New York, NY, USA). Forty randomly chosen microscopic fields were counted.

The total microbial concentration in the air, C_{total_air} (org/m^3), was determined as follows:

$$C_{total\_air} = N_1 A_1 / (A_2 Q t), \quad [1]$$

where N_1 is the average microbial count on each microscope field in the air sample, A_1 is the effective collection area of the Button Sampler filter (360 mm^2, i.e., the area on which particles are deposited, which is 22 mm in diameter on the entire 25 mm filter), A_2 is the area of the microscopic field (0.02404 mm^2), Q is the air flow rate of the Button Sampler (m^3/min), and t is the sampling time (min).

Table 1
The physical characteristics of the tested microorganisms

Test organism	Type	Aerodynamic size (Reference)	Cell surface hydrophobicity
P. melinii	Fungal spore	3.1 μm (Aizenberg et al. 2000)	Hydrophobic (Wösten and Vocht 2000)
P. fluorescens	Bacterial vegetative cell	0.8 μm (Willeke et al. 1996)	Hydrophilic (Madigan et al. 1996)
B. subtilis	Bacterial endospore	0.9 μm (Aizenberg et al. 2000)	Hydrophobic (Doyle and Rosenberg 1990)

Measurement of Microbial Concentration in the Fluid. For determining the total microbial concentration in the fluid, 1 ml of the fluid sample was stained by the acridine orange method and filtered through a 25 mm diameter black polycarbonate filter, as described by Wang et al. (2001). The epifluorescence microscope was used to count the microorganisms on the filter at a magnification of 1000×. Forty randomly chosen microscopic fields were counted.

The total microbial count in the fluid, C_{total_fluid} (org/ml), was determined as follows:

$$C_{total\_fluid} = N_2(A_3/A_2V), \quad [2]$$

where N_2 is the average microbial count on each microscope field in the fluid sample, A_3 is the effective filtration area of the 25 mm filter (210 mm^2), V is the volume of the suspension that was stained and filtered (1 ml).

The aerosolization ratio [(org/m^3 in the air)/(org/ml in the liquid)] of each microbial species is defined as the total concentration in the air (C_{total_air}, org/m^3) divided by the total concentration in the fluid (C_{total_fluid}, org/ml):

$$\text{Aerosolization ratio} = C_{total\_air}/C_{total\_fluid}. \quad [3]$$

Experiments with PSL Particles

PSL particles and preparation of the suspension. PSL particles (Bangs Laboratories Inc., Fishers, IN, USA) of two aerodynamic sizes, 1.0 μm and 3.4 μm, were used in this study to investigate the effect of aerodynamic size on the aerosolization of particles with the same surface characteristics (hydrophobic). These two sizes were selected to simulate the spores of two of our three test microorganisms, *B. subtilis* and *P. melinii*, which are both hydrophobic and have the aerodynamic diameters of 0.9 μm and 3.1 μm, respectively. Thirty microliters of PSL suspension were diluted with deionized water until a desired concentration was reached.

PSL particle concentrations in the air. The OPC was used to measure the PSL particle concentration in the air. During our preliminary experiments, the aerosolization of 1 μm PSL particle solution resulted in a concentration peak between 0.5 and 1.0 μm, as measured by the OPC. This is different from the actual aerodynamic size of the PSL particles (1 μm) since the OPC determines the optical diameter. Thus, the OPC count of particles ranging from 0.5 to 1.0 μm was used for determining the aerosol concentration of 1 μm PSL particles. Similarly, the particle count recorded by the OPC within the size range of 2.0–5.0 μm represented the 3.0 μm PSL particles as their concentration peak was observed in this size range.

Measurement of PSL particle concentration in the fluid. PSL particle concentrations in the liquid were measured by a hemacytometer (Hausser Scientific, Horsham, PA, USA). The test liquid was placed on the hemacytometer chamber and mounted using a cover glass. A light microscope was used to count the PSL particles on the hemacytometer at a magnification of 400× (Model Laborlux S; E. Leitz, Inc., New York, NY, USA). Eighteen squares were counted on both upper and lower chambers of the hemacytometer. The total concentration of PSL particles in the fluid, P_{total_fluid} (particles/ml), was determined as follows:

$$P_{total\_fluid} = f \times N_3, \quad [4]$$

where N_3 is the average PSL count on the squares and f is the hemacytometer constant (2.5×10^5).

The aerosolization ratio of PSL particles, defined as the ratio of particles/m^3 in the air to particles/ml in the liquid, was determined as:

$$\text{Aerosolization ratio} = P_{total\_air}/P_{total\_fluid} \quad [5]$$

Viscosity Measurement of Microorganism Suspensions in MWFs. The viscosities of our test fluids were measured using a Cannon-Fenske Routine viscometer (Cannon Instrument Company, State College, PA, USA). Ten milliliters of the test fluid were pipetted into the viscometer. Then the viscometer was vertically aligned in a water bath. The sample was kept in the water bath for 10 min, which allowed it to stabilize to the bath temperature (25°C). Suction was applied to draw the liquid above a specified mark of the viscometer and then the liquid was let to flow down. The time for the liquid to flow a specified distance (approximately 4 cm) was recorded as the efflux time. The kinematic viscosity (mm^2/s) of the sample was calculated by multiplying the efflux time (in seconds) by the viscometer constant (0.1569 mm^2/s^2).

Part II. Aerosolization of Microorganisms and PSL Particles by the MWF Simulator

Experimental Setup. To study the aerosolization of microorganisms under more realistic conditions that occur in industrial settings, a laboratory-scale setup that simulates the mist generation during grinding process (MWF simulator) was built. Similar type of approach has previously been used for studying mist generation by Turchin and Byers (2000), Thornburg and Leith (2000), and White and Lucke (2003). As our simulator was developed primarily for the investigation of aerosolization of microorganisms from MWFs, one of the main goals in the design was to avoid contamination of laboratory air and cross-contamination of the setup between experiments with different microorganisms. The MWF simulator is schematically shown in Figure 2. It was made of two enclosed chambers, an inner chamber and an outer chamber. The aerosol was generated in the inner chamber (5 l) when a liquid pump ejected MWF through a nozzle against a rotating aluminum rod (3.8 cm diameter). The actual metal grinding was intentionally omitted in this experimental design. An earlier study (Turchin and Byers 2000) showed that mist levels were higher when the machine was idling with the fluid flowing, but no metal was being ground. Apparently, the metal part tended to block the fluid spray and minimize misting. Thus, in a laboratory-scale test of mist aerosolization, the

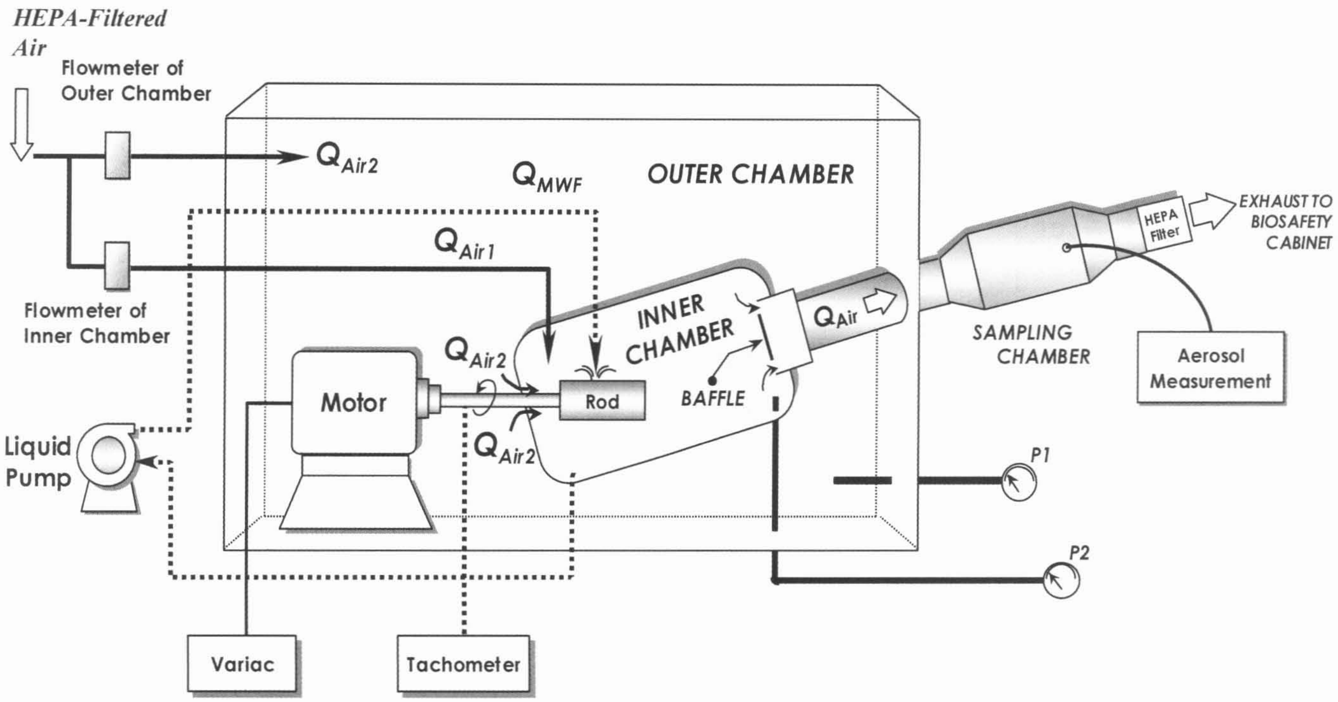

Figure 2. Laboratory-scale MWF simulator for the aerosolization of microorganisms from metalworking fluids.

grinding wheel appears to be the most critical part, particularly as the focus was the aerosolization of microorganisms, not metallic particles. The fluid flow rate was 1 l/min, which resulted in an ejection velocity of the fluid of 85 cm/s. This value was close to the lowest fluid velocity used in the study of Dasch et al. (2002). The rotation speed of the rod was 8000 rpm, resulting in a surface speed of 4064 cm/s, which is a typical speed for grinding processes. The rod rotation speed was measured by a tachometer (Monarch Instrument, Amherst, NH, USA). The largest fluid droplets hit the walls of the inner chamber, drained downward, and were collected at the bottom of the chamber, from where the fluid was recirculated by a variable flow chemical transfer pump (Control Company, Friendswood, TX, USA). A motor (Milwaukee Electric Tool Ltd., Milwaukee, WI, USA) driving the rod was placed in the outer chamber (120 l) to prevent its contamination by MWF mists. A small opening was drilled into the wall of the inner chamber to allow the motor's shaft to enter the chamber.

HEPA-filtered air entered the inner chamber either directly (Q_{Air1}) or by passing air (Q_{Air2}) into the outer chamber and then through the hole surrounding the rotating shaft into the inner chamber. The flow rates ($Q_{Air1} = 70$ l/min and $Q_{Air2} = 30$ l/min) were constant throughout the experiment. The airflow rate into and out of the test environment of the MWF simulator was therefore 100 l/min. The flow of Q_{Air1} passing from the outer chamber through the opening for the motor's shaft to the inner chamber created a positive pressure difference between the outer and inner chambers. This prevented the mists from passing into the outer chamber through the small opening. Before applying this positive pressure difference, we had frequently noticed mists in the outer chamber when the rod rotated at a high speed. The air flow rates of each airway were monitored by calibrated rotameters (Dwyer Instrument, Inc., Michigan City, IN, USA). The air pressures in the inner and outer chambers were monitored by two pressure meters, P_1 and P_2, respectively (Dwyer Instruments Inc, Michigan City, IN, USA).

The aerosol flow from the inner chamber was drawn past a baffle for removal of the larger droplets before entering the sampling chamber. One Button Sampler at a time was placed in the sampling chamber facing the air flow. The sampling probe for the OPC also intruded into the sampling chamber parallel to the air flow. The remaining aerosol flow was exhausted from the sampling chamber through a HEPA-filter into the surrounding biological safety cabinet. The OPC body was also placed inside the biosafety cabinet to avoid biocontamination.

The concentrations of microorganisms and PSL particles in the air and in the liquid were determined as described in Part I.

Statistical Analyses. Three replicates were obtained for each experiment. The Kruskal-Wallis test was used to compare the aerosolization ratios between the three test fluids or between the three microorganisms. The Wilcoxon test was used to compare the aerosolization ratio of PSL particles between the Collison nebulizer and the MWF simulator.

RESULTS AND DISCUSSION

Figure 3 shows the effect of fluid type on the aerosolization of microorganisms by the Collision nebulizer. Three fluids were tested, *P. melinii* in water, *P. melinii* in semisynthetic MWF, and *P. melinii* in soluble oil. *P. melinii* was selected as the test

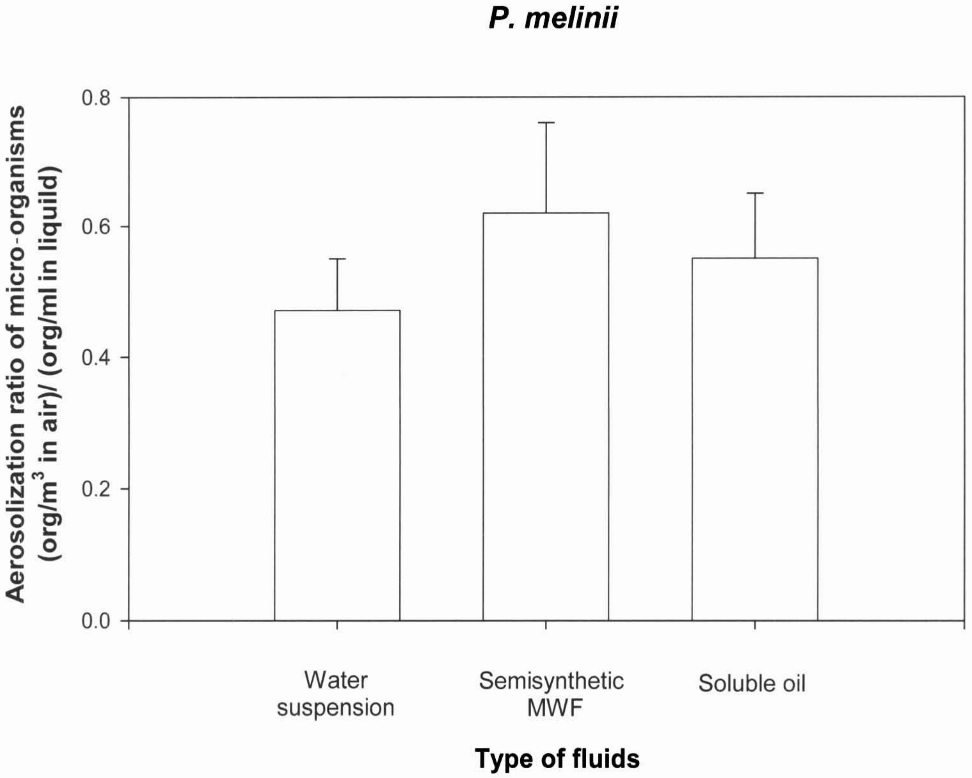

Figure 3. Effect of the fluid type on the aerosolization of *P. melinii* spores (Collison nebulizer, air pressure = 15 psi).

microorganism because it allowed reliable testing of all the three fluids with the same microorganism. Soluble oil is very viscous and has a milk-white color, which made it difficult to enumerate small *B. subtilis* spores and *P. fluorescens* cells under the microscope. For all three fluids, the *P. melinii* concentrations in the liquid were about 10^7 org/ml. For the *P. melinii* in water suspension, the aerosolization ratio was 0.47 ± 0.08 [(org/m^3 in air)/(org/ml in liquid)]. The respective value was 0.62 ± 0.14 for the *P. melinii* in semisynthetic MWF and 0.55 ± 0.1 for the *P. melinii* in soluble oil. There was no statistically significant difference (Kruskal-Wallis, $p = 0.23$) in the aerosolization ratio between these 3 fluids. We speculate that this is due to the characteristics of MWFs, which were close to water suspensions since MWFs contain 95% water and 5% MWF concentrate. Table 2 shows the viscosity of microorganism suspensions in 2 types of MWFs and in water as measured by a viscometer. The efflux time and kinematic viscosity were measured for 3 types of fluids: microorganisms in water, microorganisms in semisynthetic MWF, and microorganisms in soluble oil. The result shows that the differences of the viscosities between these fluids were less than 20%.

Since the fluid type had no significant effect on the aerosolization ratio of the microorganisms, the effects of microorganism species and microbial concentration in the liquid on aerosolization were tested using water suspensions in the Collison nebulizer. The results are shown in Figure 4. The microorganism concentrations in the liquid varied from 10^7 to 10^9 org/ml, and the microorganism concentrations in the air varied from 10^6 to 10^8 org/m^3. A linear regression line was drawn to fit the data points for each microorganism species. When the microorganism concentration in the liquid is zero, the microorganism concentration in the air should also be zero. Moreover, the microorganism concentration in the air should always be positive. Therefore, the regression lines were forced to go through the origin according to a method described by Armitage (1971). For all test species, a significant linear relationship was found between the microorganism concentration in the air and the microorganism concentration in the liquid ($p < 0.001$ for *B. subtilis*, $p < 0.001$ for *P. fluorescens*, and $p = 0.017$ for *P. melinii*). It demonstrates that the microbial concentration in the liquid does not affect the aerosolization ratio of microorganisms.

Table 2
Viscosity of microorganism suspensions in two types of MWFs and in water as measured by a viscometer (25°C)

Fluid type	Efflux time (s)	Kinematic viscosity (mm^2/s^2)
Microorganism in water	62 ± 1	0.9728 ± 0.0157
Microorganism in semisynthetic MWF	66 ± 1	1.0355 ± 0.0157
Microorganism in soluble oil	73 ± 1	1.1454 ± 0.0157
Distilled H_2O	61 ± 1	0.9571 ± 0.0157

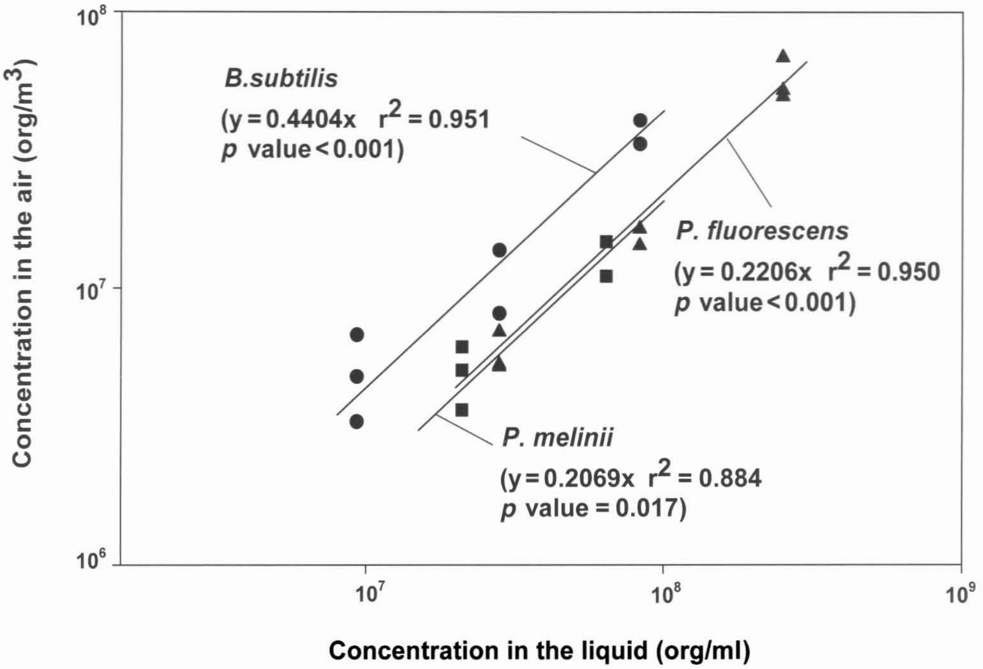

Figure 4. Aerosolization of microorganisms from water suspension of different microbial concentrations (Collison nebulizer, air pressure = 12 psi).

The aerosolization ratio was 0.44 ± 0.14 [(org/m^3 in the air)/(org/ml in the liquid)] for *B. subtilis*, 0.23 ± 0.04 for *P. fluorescens*, and 0.20 ± 0.04 for *P. melinii*. Wilcoxon tests showed that the aerosolization ratio of *B. subtilis* was significantly different from that of *P. fluorescens* and of *P. melinii* ($p < 0.001$ for both). The aerosolization ratios obtained for *P. fluorescens* and *P. melinii* were not significantly different (Wilcoxon, $p = 0.556$). For the same concentration in the liquid, the *B. subtilis* concentration in the air was much higher than that of *P. fluorescens* or *P. melinii*. *B. subtilis* spores and *P. fluorescens* cells have approximately the same aerodynamic sizes (0.9 μm for *B. subtilis* and 0.8 μm for *P. fluorescens*), but different physical surface characteristics (*B. subtilis* spores are hydrophobic and *P. fluorescens* cells are hydrophilic). Thus, our results indicate that hydrophobic microorganisms are easier to aerosolize with the Collison nebulizer than hydrophilic ones. *B. subtilis* spores and *P. melinii* cells have the same surface characteristics (both are hydrophilic) but different sizes (0.9 μm for *B. subtilis* and 3.4 μm for *P. melinii*). The particle size seems to play a role as well, since the larger microorganisms were more difficult to aerosolize than the smaller ones. The data show that *P. fluorescens* and *P. melinii* had similar aerosolization ratios, although they have different sizes and different surface characteristics. It appears that the effect of size and surface characteristics compensated each other for these two species. All these experiments were conducted with the air pressure = 12 psi (82.7 kPa) for the Collison nebulizer. This resulted in a lower aerosolization ratio for *P. melinii* compared to the one presented in Figure 3, for which the air pressure = 15 psi (103.4 kPa). At the same concentration of particles in the liquid, the concentration of aerosolized particles increases with increasing air pressure in the Collison nebulizer (Sterk et al. 1983).

To confirm the results under conditions that would be closer to field settings, tests were also conducted with our MWF simulator that simulates the mist generation in grinding process in manufacturing. The relationship to industrial operations was validated by comparing the particle size distributions generated by the MWF simulator and measured with the OPC to similar data obtained in working environments (Wang et al. 2004). The three microorganisms in semisynthetic MWF were tested and the results are shown in Figure 5. The microorganism concentrations in the liquid were about 10^7org/ml for all three test fluids. The aerosolization ratio of *B. subtilis* in MWF was significantly higher than that of *P. melinii* (Kruskal-Wallis, $p < 0.001$). *P. melinii* and *P. fluorescens* had similar aerosolization ratio (Wilcoxon, $p = 0.227$). The MWF simulator tests showed the same trend as the Collison nebulizer tests (Figure 4): *B. subtilis* was easier to aerosolize than *P. fluorescens* and *P. melinii*. The results demonstrate that aerosolization of microorganisms in an industrial setting showed the same trend as in a laboratory setting: hydrophobic microorganisms tend to be easier to aerosolize than hydrophilic microorganisms, and small microorganisms are easier to aerosolize than large microorganisms. It should be pointed out that the aerosolization of microorganisms by our MWF simulator in the laboratory is not fully identical to the aerosolization during grinding operation in the field. For example, in the MWF simulator, a baffle was installed in front of the sampling chamber to remove large particles. It could cause the

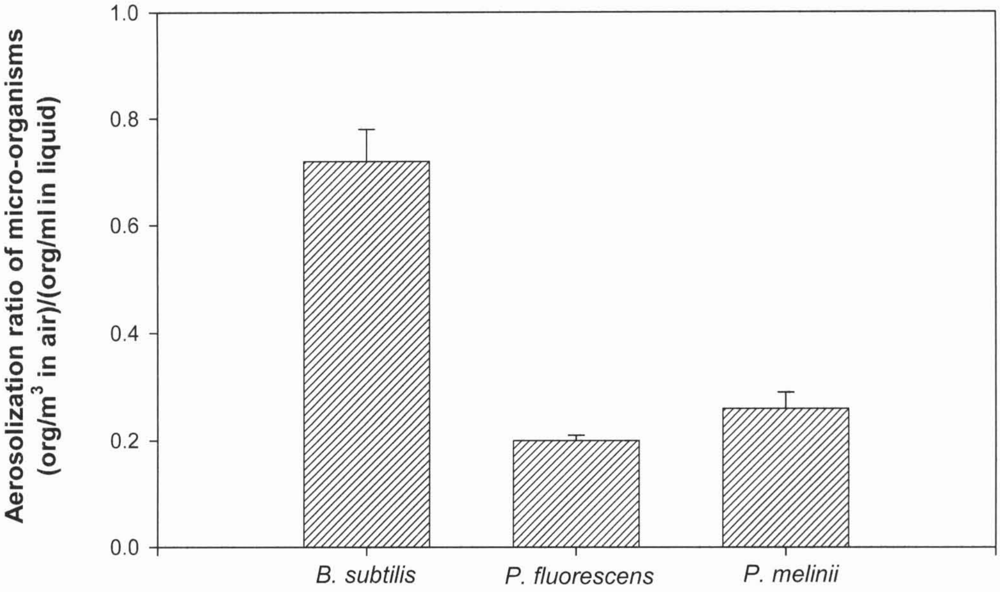

Figure 5. Effect of microorganism type on the aerosolization from semisynthetic MWF (MWF simulator).

loss of the relatively large *P. melinii* spores. However, no changes were observed in the trend of the microorganism aerosolization as the calculated loss of 3.1 μm particle by the baffle in the MWF simulator was found to be below 1% (Stk = 0.0006).

To further examine the effect of particle size on the aerosolization ratio, 1 μm and 3.4 μm hydrophobic monodisperse PSL particles (surrogates for *B. subtilis* and *P. melinii* spores, respectively) were aerosolized from water suspensions by the Collison nebulizer and the MWF simulator. The results are shown in Figure 6. For the Collison nebulizer, the aerosolization ratio of 1 μm PSL particles was approximately 7 times higher than that of 3 μm PSL particles and for the MWF simulator, it was 20 times higher. Thus, similar trend was found with PSL particles than with microorganisms (Figures 4 and 5): smaller particles are easier to aerosolize than larger particles. There might be two reasons for the more pronounced effect of the particle size on the aerosolization of PSL particles than microorganisms. First, PSL particles have more uniform size than microorganisms (we observed their size variability under the microscope). As a result, PSL particles of different sizes demonstrated a more distinct difference in their aerosolization ratio. Second, the concentrations of PSL particles and microorganisms were measured with different methods. For the microorganisms, the particle concentrations in the air and in the fluid were measured by acridine orange staining and epifluorescence microscope counting. For the PSL particles, the concentration in the air was measured by the OPC whereas the concentration in the liquid was measured by the hemacytometer method.

Both MWFs and PSL suspensions contain small amount of surfactants. The surfactants in MWFs work as corrosion inhibitor and emulsifier. Surfactants in the PSL suspension serve as a stabilizer to prevent PSL particle flocculation. The surface active ability of the surfactants could decrease the surface tension of the liquid. Surfactants could also interact with particles in the fluids and consequently change their surface characteristics. In this study, surfactants did not seem to play an important role in affecting the aerosolization of particles from suspension. This may be due to the fact that both MWFs and the PSL suspensions contained high concentration of particles (10^7 #/ ml), and the amount of surfactants in the fluids was not sufficient to change the trend of the particle aerosolization.

Aerosol generation from pure liquid (without any microorganisms or solid particles) by the Collison nebulizer has been explored quite extensively (May, 1973; Gussman, 1984). In the Collison nebulizer, a gas is used to aspirate the liquid into a sonic velocity gas jet. The jet then impacts against the inside of the jar to remove the larger droplets. However, the aerosolization of microorganisms or solid particles from the Collison nebulizer suspension has not been quantitatively characterized. We assume that, in the Collison nebulizer jar, hydrophobic particles are easier to get into the liquid/gas jet since the force between the particles and the liquid in the jar is lower. The large droplets will be removed by impaction onto the inner wall of the jar and the smaller droplets will enter the sampling chamber (these droplets may or may not contain microorganisms). As a result, hydrophobic particles have a higher aerosolization ratio than hydrophilic particles. Larger particles are easier to remove by impaction onto the wall of the jar. Therefore, the larger particles have a lower aerosolization ratio than the smaller ones.

In field situations, MWF mists can be generated by centrifugal forces on the liquid coating the turning rod, by impaction of the liquid on walls, or by condensation of the vapors produced by the high temperature in the metal being cut (Thornburg and Leith

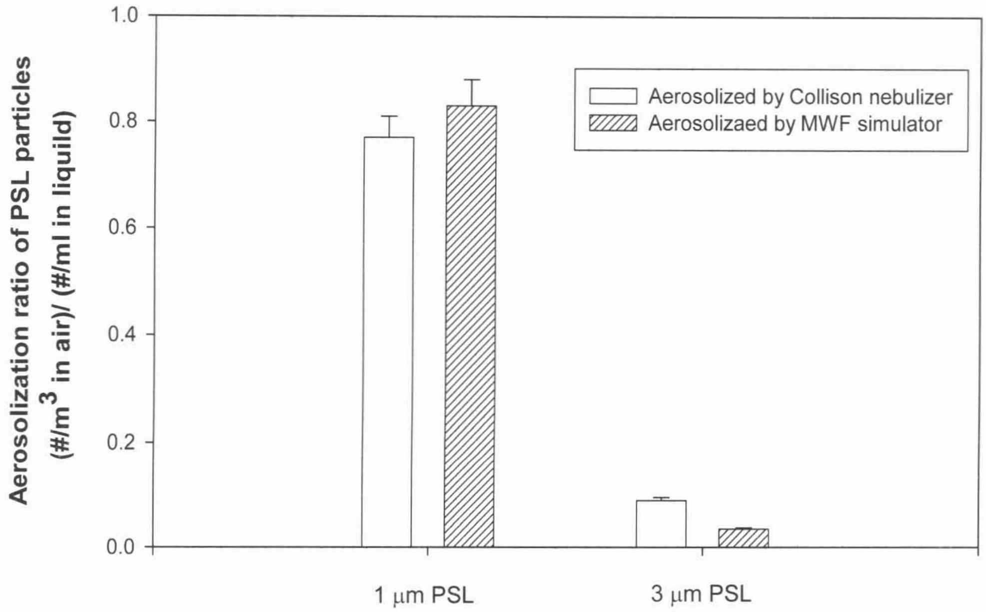

Figure 6. Effect of particle size on the aerosolization of polystyrene latex (PSL) particles from water suspension by the Collison nebulizer and by the MWF simulator.

2000). Our MWF simulator included the first two mechanisms of aerosol generation, the centrifugal force being the predominant one. The evaporation/condensation mechanism is not relevant to the aerosolization of intact microbial cells or spores. Hence, this mechanism was not considered when designing the MWF simulator. When the fluid is continuously fed to the rotating rod, a liquid film develops on the body of the rod. With time, this film grows in thickness until the centrifugal forces overcome the surface tension of the liquid. Part of the film then spins off in long threads that look like tadpoles. The heads of the "tadpoles" subsequently break off as primary droplets while the long tails break into several smaller satellite droplets. Each droplet may contain one or more microorganisms or solid particles, depending on the droplet and microorganism size. Many or most of the droplets may not contain any microorganisms. Since MWF is water-based, the water evaporates quickly from the droplet. The presence of microorganisms may change the aerosol characteristics of the mists. Hydrophobic particles have lower surface tension than hydrophilic particles. Consecutively, hydrophobic particles are easier to detach by centrifugal forces and easy to spin off in the MWF simulator compared to hydrophilic ones. A similar role was played by particle size on the aerosolization of particles as the one described for the Collison nebulizer.

CONCLUSIONS

The effects of fluid type, microorganism concentration in the liquid, and microbial species on the aerosolization of microorganisms from MWFs were examined. Three microorganisms were employed to represent different particle sizes and surface characteristics: *B. subtilis* bacterial endospores (hydrophobic with an aerodynamic diameter of 0.9 μm), *P. fluorescens* bacterial vegetative cells (hydrophilic, 0.8 μm), and *P. melinii* fungal spores (hydrophobic, 3.1 μm). The testing was first performed using a Collison nebulizer. It was observed that the fluid type and the microorganism concentration in the liquid did not affect the aerosolization of microorganisms. In contrast, the microorganism species showed an effect. *B. subtilis* was easier to aerosolize than *P. fluorescens* and *P. melinii*. To confirm the results under conditions that are closer to an industrial setting, the three microorganisms were tested with the MWF simulator. The results showed the same trend. Experiments with PSL particles confirmed that aerosolization mechanisms employed by both the Collison nebulizer and by the MWF simulator resulted in a higher aerosolization of small particles (representing the bacterial size range) than larger ones (representing the fungal spore size range). Summarizing the findings in both the laboratory environment and in an industrial setting, our study showed that hydrophobic microorganisms were easier to aerosolize from MWFs than hydrophilic microorganisms and that increasing microorganism size was likely to result in a decrease of the aerosolization ratio.

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