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Aerosolization of fine particles increases due to microbial contamination of metalworking fluids

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Abstract

Aerosolization of microorganisms from metalworking fluids (MWFs) was studied using a laboratory-scale set-up simulating grinding operations. An optical particle counter (OPC), a condensation nucleus counter (CNC), an electrical low pressure impactor (ELPI), and a photometric aerosol mass monitor were used to measure the airborne particles and microorganisms aerosolized from MWFs. The tests were performed using a semi-synthetic MWF with and without bacterial contamination (*Pseudomonas fluorescens*). Microbial contamination of the MWF increased the number and mass concentrations of aerosolized particles by a factor of 2 (as measured by the OPC and the photometric aerosol mass monitor, respectively). At the same time, there was an up to 50-fold increase in the concentration of fine particles (0.02–1 μm), as measured by the CNC. The data collected with the ELPI showed that the peak of the fine particle number concentration was at 0.37 μm. The results indicate that MWF mist may contain high concentrations of microbial fragments, which may not be detected by traditional microbial analysis methods, such as cultivation or microscopic counting.

Keywords: Grinding simulator; Metalworking fluid; Particle aerosolization; Microbial contamination

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1. Introduction

Metalworking fluids (MWFs) are used in industry in machining and grinding processes to lubricate contact surfaces, to dissipate heat, and to transport material removed from the contact site. MWFs can be divided into four general categories: straight oils (mineral oils), soluble oils, semi-synthetic MWFs, and synthetic MWFs. The last three categories are water-based fluids and are used as suspensions containing up to 95% water by volume. The undiluted water-based MWFs usually contain 5–80% mineral oil, small amount of surfactant, emulsifier, anti-foaming agents and corrosion inhibitor.

Health effects associated with human exposures to MWFs include dermatitis, respiratory symptoms, hypersensitivity pneumonitis and asthma (Robertson, Weir, & Burge, 1988; Popendorf et al., 1996; Graves et al., 1997). About 1.2 million workers in the United States are occupationally exposed to MWFs (NIOSH, 1998). It has been reported that MWF exposure was the second most common cause of work-related asthma in the state of Michigan (Rosenman, Reille, & Kalinowski, 1997). Rosenman et al. (1997) reported an association between the occurrence of work-related asthma and respiratory symptoms among machinists using water-based MWFs.

Water-based MWFs may become contaminated with bacteria, and thus the effect of microbial agents on health, resulting from MWF exposure, is of special interest. The linkage of respiratory complaints to water-based MWF exposure raises concern that these health impairments may be due to the inhalation of specific microbial agents or substances. A study by Mattsby-Baltzer et al. (1989) showed that *Pseudomonas pseudoalcaligenes* could be present in industrial MWFs at very high concentration although large quantities of biocides were added. Later on, several other microorganisms have been identified from water-based MWFs in industrial settings, such as *Mycobacteria* (Kreiss & Cox-Ganser, 1997; Moore et al., 2000), as well as *Aspergillus niger*, *Staphylococcus capitas*, and *Bacillus pumilus* (Bernstein, Lummus, Santilli, Siskosky, & Bernstein, 1995). Antibodies to a common MWF bacterium, *Pseudomonas*, have been found in the serum of MWF exposed workers, suggesting association between microbial exposure and allergy-based health effects related to MWFs mist exposure (Bernstein et al., 1995). Microorganisms contain a variety of substances that can also stimulate inflammatory and toxic reactions when inhaled. Lewis, Janotka, Whimer, and Bledsoe (2001) detected endotoxin (a component of the outer membrane of gram-negative bacteria) from bulk MWF and from MWF aerosols.

Several investigators have studied the factors that affect the aerosolization of mist from pure MWFs. Thornburg and Leith (2000) examined the size distribution of soluble oil and mineral oil mists generated on a lathe during metal machining. Three mechanisms causing aerosol formation were identified: impaction, centrifugal motion, and evaporation/condensation. The investigation found that among the three mechanisms, evaporation/condensation generated the smallest particles, whereas centrifugal forces produced the largest ones. The geometric standard deviation of the MWF mists was not affected by the type of MWF, fluid flow rate, tool rotation speed, or fluid viscosity. Heitbrink, D'Arcy, and Yacher (2000) investigated the particle size and concentration of mist aerosolized from soluble oil as a function of tool rotation speed, fluid application rate, and tool cutting rate in an enclosed machining center. The mist concentration was found to increase with increasing tool speed and fluid application rate. The shape of the size distribution was unaffected by the experimental variables. Dasch, Ang, Mood, and Knowles (2002) investigated the effect of the following variables on the mass concentration of mist generated in a machining center: fluid itself (concentration, type, volatility, age, temperature, and the presence of tramp oil), fluid application (fluid velocity and through-tool application), and machining parameters (tool speed, tool diameter, tool feed, depth-of-cut, and tool wear). It was shown that tool speed had the largest impact on the mist mass concentration. Turchin and Byers (2000) studied the mass concentration of aerosolized mist in a laboratory-scale mist generator, and demonstrated that the presence of fluid contaminants (e.g., tramp oil) had a major effect on the mist level.

While several studies have targeted the concentration of microorganisms and endotoxin in the bulk MWFs or in the air (Mattsby-Baltzer et al., 1989; Rossmoore, Moo-Young, Cooney, & Humphres, 1986; Woskie et al., 1996; Lonon, Ababto, & Findlay, 1999; Abrams et al., 2000; Lewis et al., 2001), only a few attempted to make a link between the concentration/composition of fluidborne and airborne microorganisms (Thorne, Dekoster, & Subramanian, 1996; Virji, Woskie, Sama, Kriebel, & Eberiel, 2000). In this study, a laboratory-scale simulator was employed to investigate the aerosolization of MWFs into mists containing microorganisms.

2. Materials and methods

2.1. Experimental set-up

The laboratory-scale metalworking simulation facility has been described in detail in Wang, Reponen, Adhikari, Willeke, and Grinshpun (2004). This description is summarized here. The simulator was made of two enclosed chambers, an inner chamber (5 l) and an outer chamber (120 l). The aerosol was generated in the inner chamber where a liquid pump ejected MWF through a nozzle against a rotating aluminum rod (3.8 cm diameter). The fluid application rate varied from 0.4 to 1.6 $\mathrm{l\,min^{-1}}$. The rotation speed of the rod varied from 800 to 8000 rpm as measured by a tachometer (Monarch Instrument, Amherst, NH). The resulting surface speed of the rod varied from 318 to 3180 $\mathrm{cm\,s^{-1}}$. The particle generation involved mechanisms, such as the centrifugal motion, spray atomization and impaction on the rod and chamber walls. The MWF was recirculated by a variable flow chemical transfer pump (Control Company, Friendswood, TX). A motor (Milwaukee Electric Tool Ltd., Milwaukee, WI) driving the rod was placed in the outer chamber to prevent its contamination by MWF mists.

The total airflow rate into and out of the inner chamber of the simulator was 100 $\mathrm{l\,min^{-1}}$. The aerosol flow from the inner chamber was drawn past a baffle for removal of larger droplets before entering the sampling chamber. There, sampling probes for aerosol instruments were placed isoaxial to the air flow. The remaining aerosol flow ($Q = 100 - 1.2 - 2 - 0.7 - 30 = 66.1\,\mathrm{l\,min^{-1}}$) was exhausted from the sampling chamber through a HEPA-filter into a biological safety hood (Sterilchem-Gard Class II, Type B2, Baker Co., Sanford, ME).

2.2. Preparation of MWFs

A commercially available water-based MWF was selected for this study. The selected fluid was a semi-synthetic MWF commonly used in factories as a 5% water solution. The semi-synthetic MWF was chosen to represent water-based fluids, which are known to be prone to microbial contamination (NIOSH, 1998; Moore et al., 2000). *Pseudomonas fluorescens* was used as the test bacterium. Numerous studies have found that gram-negative bacteria, especially *Pseudomonas* spp. represent the major bacterial contaminants in MWFs (Sondossi & Rossmoore, 1985; Bernstein et al., 1995; Lonon et al., 1999). *P. fluorescens* (ATCC 13525) culture was obtained from the American Type Culture Collection (Rockville, MD). It was subcultured by incubating in a trypticase soy broth at 26 °C ± 2 °C for 18 h. The cells were then washed twice with sterile deionized water by centrifugation at 7000 rpm for 7 min. Then the *P. fluorescens* suspension was diluted with the test MWF or with sterilized deionized water, depending on the experiment, until the desired concentration was achieved (10^8 cells ml^{-1}). A fresh bacterial suspension was prepared daily for the test.

The following three fluids were tested in this study:

- Semi-synthetic MWF (consisting of 95% water and 5% commercial semi-synthetic MWF concentrate, no biocide added),
- *P. fluorescens* suspension in water (10^8 cells ml^{-1}) and
- *P. fluorescens* suspension in semi-synthetic MWF (10^8 cells ml^{-1}).

2.3. Experimental procedures

In addition to the fluid type, the tool rotation speed and the fluid application rate were selected as the major variables in this study. Previous studies have shown that these three variables have the greatest effect on mist generation (Thornburg & Leith, 2000; Heitbrink et al., 2000; Dasch et al., 2002).

Test fluids were prepared on the same day prior to the experiment. One liter of the test fluid was transferred into the inner chamber at the beginning of the experiment. Each experimental run lasted 13 min. The experiment started within 5 min after the bacteria was introduced into the MWF. At first, the rotation rod was adjusted to the assigned speed using a Variac (Staco Energy Products Co., Dayton, OH) that was connected to the motor. Five rotation speeds were used in the tests: 800, 2000, 4000, 6000, and 8000 rpm. As the diameter of the rod is 3.8 cm, the tool rotation speed of 8000 rpm corresponds to a surface speed of 3180 cm s^{-1}, which represents the typical surface speed for grinding process.

A time period of 5 min was sufficient to stabilize the rotation speed. Then a chemical pump was turned on to apply the test fluid to the rotating rod. Five fluid application rates were tested: 0.4, 0.7, 1.0, 1.3, and 1.6 l min^{-1}. Preliminary measurements made with an optical particle counter (OPC) indicated that the mist concentrations required 3 min to reach steady state. During our experiments, therefore, we waited 3 min and then continuously recorded the particle concentrations for 5 min, as described below.

2.4. Particle concentration measurements

The aerosol concentration in the sampling chamber was continuously monitored using an OPC, a photometric aerosol mass monitor, a condensation nucleus counter (CNC), and an electrical low pressure impactor (ELPI). A one-minute averaging time was used for all the instruments.

The OPC (Portable Dust Monitor model 1.108, Grimm Technologies Inc., Douglasville, GA) continuously monitored the particle number concentration and size distribution. The Grimm counter utilized an 8 cm long, 3 mm diameter probe to sample at a flow rate of 1.2 l min^{-1}. The particles were size-classified into 15 channels: >0.3, >0.4, >0.5, >0.65, >0.8, >1.0, >1.2, >2.0, >3.0, >4.0, >5.0, >7.5, >10.0, >15.0 and >20.0 μm. The particle number concentration was converted to mass concentration using the following equation:

$$C_\mathrm{m} = \sum_{i=1}^{n} [\pi d_i^3 \rho_\mathrm{p}/6] \times C_i, \tag{1}$$

where C_i is the particle number concentration in each channel, d_i is the average particle diameter of each channel, and ρ_P is particle density (assumed $\rho_P = 1\,\mathrm{g\,ml^{-1}}$).

The photometric aerosol mass monitor (DustTrak aerosol monitor, model 8520, TSI Inc., St. Paul, MN) continuously monitored the total particle mass concentration within the particles size range of 0.1–10 μm. It sampled through an 8 cm long, 6 mm diameter probe at a flow rate of $2\,\mathrm{l\,min^{-1}}$.

The CNC (P-trak fine particle counter, model 8525, TSI Inc., St Paul, MN) was used to continuously monitor the total number concentration of fine particle. It sampled through an 8 cm long, 3 mm diameter probe at a flow rate of $0.7\,\mathrm{l\,min^{-1}}$. The P-trak can measure particles ranging from 0.02 μm to those exceeding 1 μm.

The ELPI (Dekati Ltd., Tampere, Finland) was used to continuously monitor the particle concentration and aerodynamic size distribution in a size range that included the fine and coarse aerosol particle fractions. It has an inlet of 10 cm length, 13 mm in diameter, and operates at a flow rate of $30\,\mathrm{l\,min^{-1}}$. The 50% cut diameters for the 13 impactor stages are 0.029, 0.059, 0.103, 0.165, 0.254, 0.392, 0.636, 0.99, 1.61, 2.45, 3.97, 6.58 and 10.18 μm. Unlike the Grimm OPC, it allows the measurement of particles below 0.3 μm. The benefit of using the ELPI over the CNC is that the ELPI records particle size-selective data. The inlet efficiency (including the aspiration and transmission) in the sampling chamber for all the instruments was calculated according to the methods described by Baron and Willeke (2001). For particles < 1 μm, the inlet efficiency ranged from 0.981 to 0.999. The lowest inlet efficiency was calculated for the combination of OPC and 5-μm particles (0.883). As the focus of this study was on fine particles, we concluded that the sampling in the sampling chamber was representative. The representative of the sampling was also assured by positioning the four inlets at a distance of at least 5 inlet diameters from each other.

2.5. *Viability of P. fluorescens in the test fluids*

To investigate possible changes in the characteristics of *P. fluorescens* during the test, the viability of *P. fluorescens* was examined by measuring the culturable and total bacterial count at the following time points after the preparation of the test fluids: 0, 0.5, 1, 2, and 3 h.

2.5.1. *Culturable microbial count in the fluid*

Immediately after taking the fluid sample, 1 ml of the fluid was diluted and cultivated on trypticase soy agar (DIFCO Laboratories, Detroit, MI) in triplicate. The culture plates with *P. fluorescens* were incubated at 26 °C ± 2 °C for 48 h. The colony-forming units on plates were counted and an average of the three repeat cultures was used for the data analysis.

The culturable microbial count in the suspension was calculated as follows:

$$C_{\mathrm{cfu}} = (\mathrm{cfu}/10^{-n}) \times (V_1/V_2), \tag{2}$$

where cfu is the average number of total colony-forming units on three culture plates, n is the dilution factor, V_1 is volume of the suspension analyzed (1 ml), and V_2 is the volume of diluted suspension spread on each plate (0.1 ml).

2.5.2. *Total microbial count in the fluid*

For determining the total count, 1 ml of the fluid sample was stained by the acridine orange method and filtered through a black polycarbonate filter, as described by Wang, Reponen, Grinshpun, Górny, and Willeke (2001). An epifluorescence microscope was used to count the microorganisms on the filter at a magnification of 1000× (Model Laborlux S; E. Leitz, Inc., available from W. Nuhsbaum Inc., McHenry, IL). Forty randomly chosen microscopic fields were counted.

The total microbial count in the fluid, C_{total}, was determined as follows:

$$C_{total} = N(\pi R^2/AV_3), \tag{3}$$

where N is the average microbial count on each microscope field, R is the effective radius of the filter (8.5 mm), A is the area of the microscopic field ($0.02404\,mm^2$) and V_3 is the volume of the suspension that was stained and filtered (1 ml).

The viability of *P. fluorescens* in the fluid is defined as the culturable count (C_{cfu}) divided by the total count (C_{total}):

$$\text{Viability} = C_{cfu}/C_{total}. \tag{4}$$

2.6. Validation of the laboratory simulator through field sampling

Prior to the laboratory experiments, the simulator was validated by comparing the particle size distributions generated by the device and measured with the OPC under laboratory conditions to the data obtained in the working environment. Field sampling was conducted at a grinding site in a plant, where semi-synthetic MWF was applied during the machining operation. The samples were collected at a stationary site as close to the workers' breathing zone as possible without disturbing the normal working activities. The particle size distributions and number concentrations of the aerosol generated in the simulator were compared with those measured with the OPC in the field.

3. Results and discussion

Table 1 shows the size distribution characteristics of geometric mean diameter (d_g) and geometric standard deviation (σ_g) of the particles aerosolized in the simulator and measured in the field. The two size distributions were compared by comparing their geometric mean diameters using a student's t-test as described by Baron and Willeke (2001). The result showed that there is no significant difference between them ($p = 0.25$) indicating that particles generated in the simulator well represent those in the field.

After the initial validation, the simulator was used for a detailed study on the properties of airborne particles aerosolized from MWF with and without microbial contamination. The results on the aerosolization

Table 1
Comparison of the geometric mean (d_g) and geometric standard deviation (σ_g) of the particles generated by the simulator in the laboratory with those measured in the field by an optical particle counter

Site	d_g (μm)	σ_g
Laboratory simulator[a]	0.54	1.80
Field (grinding area)	0.62	1.96

[a] Rotation speed of 8000 rpm, semi-synthetic MWF.

from pure semi-synthetic MWF are presented in Figs. 1–3. Fig. 1 shows the effect of the tool rotation speed on the number concentration and size distribution of particles aerosolized from the pure MWF. The particle number concentration, measured by the OPC is presented as a function of the particle optical diameter. As seen from Fig. 1, the increase of the tool rotation speed increased the particle number

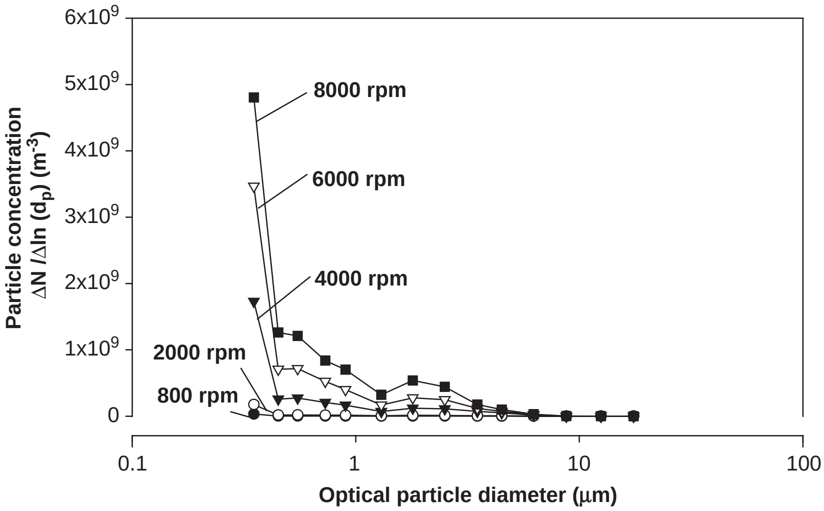

Fig. 1. The effect of tool rotation speed (rpm) on the number concentration and size distribution of particles aerosolized from semi-synthetic MWF. The data were collected with an optical particle counter. Each data point indicates an average of 5-min measurement. Standard deviations are so small that they do not show in the figure.

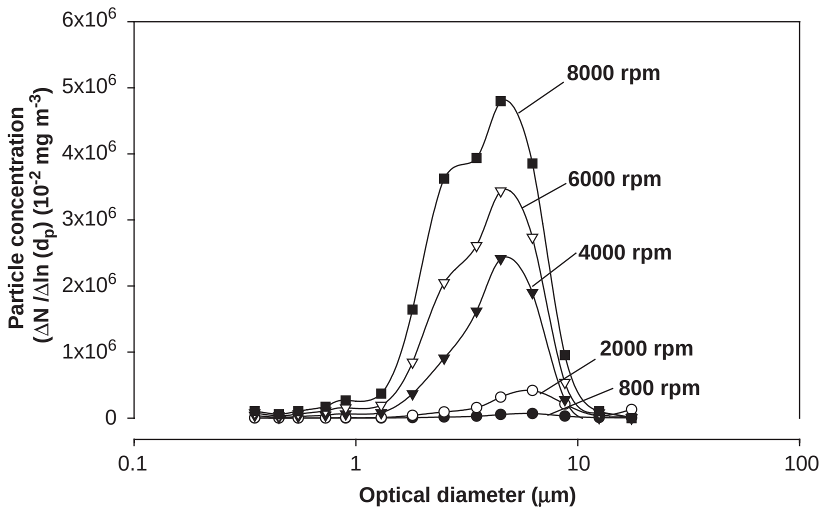

Fig. 2. The effect of tool rotation speed (rpm) on the mass concentration and size distribution of particles aerosolized from semi-synthetic MWF. The data resulted from converting the number concentration, measured by an optical particle counter, to the mass concentration assuming that the density of the particles is $1\,g\,cm^{-3}$. Each data point indicates an average of 5-min measurement. Standard deviations are so small that they do not show in the figure.

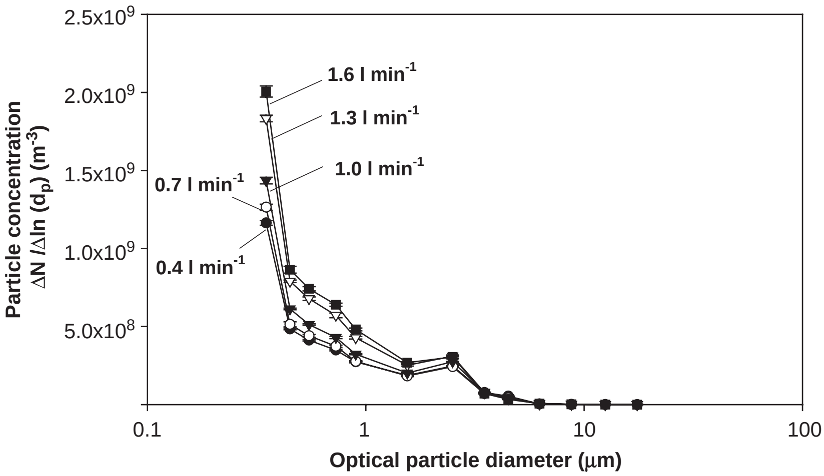

Fig. 3. The effect of fluid application rate on the number concentration and size distribution of particles aerosolized from semi-synthetic MWF, as measured by an optical particle counter. Each data point indicates an average of 5-min measurement and the error bars indicate one standard deviation.

concentration. For a specific rotation speed, the aerosol particle concentration increased with decreasing particle size in the measured particle size range of 0.3–20 μm. The trend was more pronounced with higher rotation speeds.

The effect of the tool rotation speed on the mass concentration of particles aerosolized from semi-synthetic MWF was evaluated by converting the particle number concentration measured by the OPC into particle mass concentration (C_m) through the use of Eq. (1). Fig. 2 shows the effect of tool rotation speed on the particle mass concentration and size distribution. Similar to the number concentration, the particle mass concentration increased with increasing rotation speed. Dasch et al. (2002) has shown a similar trend with soluble oil. As seen in Fig. 2, the shape of the mass size distribution is essentially unaffected by the tool rotation speed, as was also reported by Heitbrink et al. (2000). The mode of the mass size distributions shown in Fig. 2 was approximately 4.5 μm, which is lower than the 8–10 μm mode obtained in Heitbrink's study. This may be caused by the different fluid type and different measurement instruments (Heitbrink et al. used soluble oil and performed their measurements with the aerodynamic particle sizer and MOUDI). The difference may also be associated with machining parameters, such as the fluid application rate and the tool diameter. Fig. 3 shows that the number concentration of the aerosolized particles increased with increasing fluid application rate. At the same time, the particle number size distribution was not affected by the fluid application rate. This is consistent with the results reported by Heitbrink et al. (2000).

Our experiments conducted with the pure semi-synthetic MWF showed the same trends as was reported in previous studies regarding the effect of tool rotation speed and fluid application rate on the mist concentration and size distribution (Heitbrink et al., 2000; Thornburg & Leith, 2000; Dasch et al., 2002). Thus, we concluded that our simulator was suitable to represent the aerosolization of mist during machining operations. Following this conclusion, experiments with microbiologically contaminated MWFs were initiated.

The properties of particles that were aerosolized from *P. fluorescens* suspension in semi-synthetic MWF and in water, respectively, were examined and compared with those obtained with pure semi-synthetic MWF not containing microbial contamination. The results are shown in Figs. 4–6. Fig. 4 presents the effect of the tool rotation speed (rpm) on the total particle number concentration for different fluids as

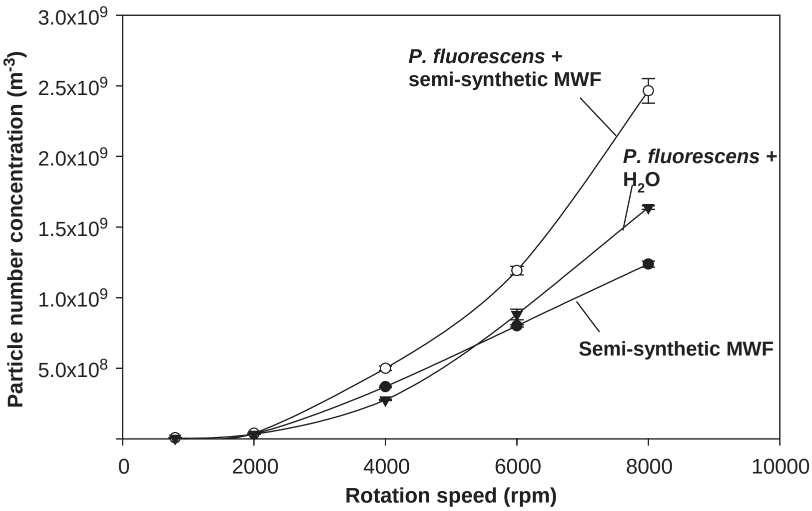

Fig. 4. The effect of tool rotation speed (rpm) on the total number concentration of particles aerosolized from different fluids as measured by an optical particle counter. Each data point indicates an average of 5-min measurement and the error bars indicate one standard deviation.

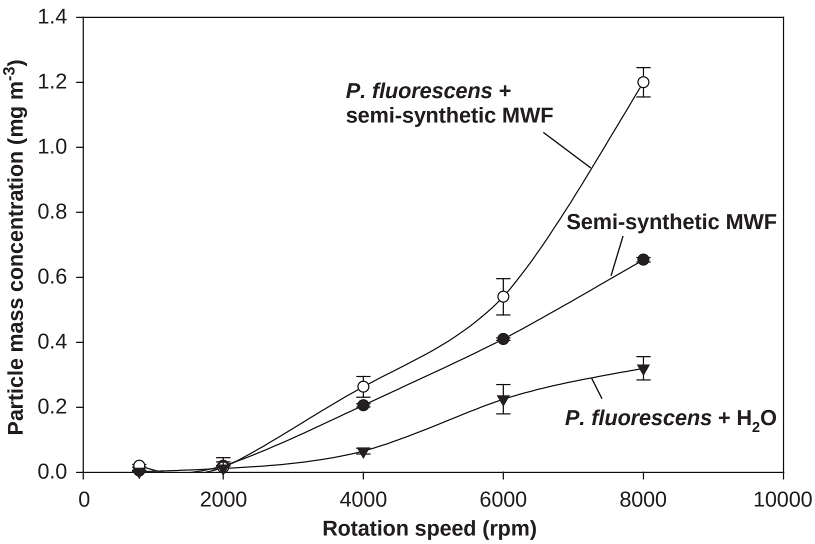

Fig. 5. The effect of tool rotation speed (rpm) on the mass concentration of particles aerosolized from different fluid, as measured by a photometric aerosol mass monitor.

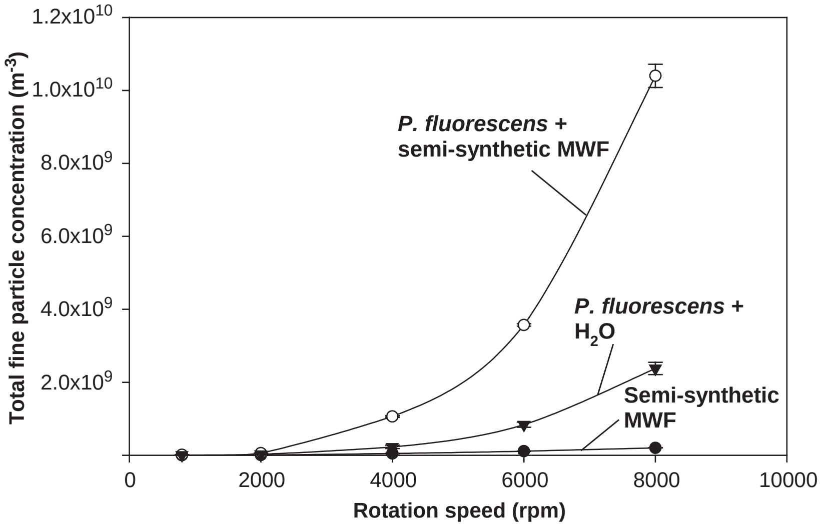

Fig. 6. The effect of tool rotation speed (rpm) on the number concentration of particles aerosolized from different fluids as measured by a condensation nucleus counter. Each data point indicates an average of 5-min measurement and the error bars indicate one standard deviation.

measured by the OPC. The test results were compared for three fluids: pure semi-synthetic MWF, *P. fluorescens* suspension in water, and *P. fluorescens* suspension in semi-synthetic MWF. For a specific fluid type, the total number concentration of the aerosolized particles increased with increasing rotation speed. Among the different fluids, the total particle number concentrations were very close to each other and close to zero at speeds below 2000 rpm. The difference among the fluids increased with increasing the tool rotation speed. The lowest total particle number concentration was observed with pure semi-synthetic MWF and the highest with *P. fluorescens* suspension in semi-synthetic MWF. At 8000 rpm the total number concentration aerosolized from *P. fluorescens* suspension in semi-synthetic MWF was about 2 times of that from pure semi-synthetic MWF.

Fig. 5 shows the effect of the tool rotation speed on the total mass concentration of particles aerosolized from the three different fluids as measured by the photometric aerosol mass monitor. For a specific fluid type, the particle mass concentration increased with increasing tool rotation speed. The highest total particle mass concentration was measured with *P. fluorescens* suspension in semi-synthetic MWF and the lowest with *P. fluorescens* suspension in water. At 8000 rpm, the total mass concentration aerosolized from *P. fluorescens* suspension in semi-synthetic MWF was about 2 times of that from pure semi-synthetic MWF. As the diameter of the rod is 3.8 cm, the tool rotation speed of 8000 rpm corresponds to a surface speed of 3180 cm s^{-1}. The total mass concentration of particles generated in our simulator at this velocity and a fluid application rate of 1 l min^{-1} was 0.65 mg m^{-3}. This is close to the value that has been obtained by Dasch et al. (2002) under similar laboratory conditions. In that study, the total mass concentration was 0.36 mg m^{-3} at a fluid application rate of 4.9 l min^{-1} and tool rotation speed of 2320 cm s^{-1}. Our mass concentration data are also consistent with those reported by Ball (1997) based on the measurements conducted in eight manufacturing plants: the mean value of the MWF mist concentration obtained in her study was 0.85 mg m^{-3}.

Fig. 6 shows the effect of the tool rotation speed on the concentration of fine particles aerosolized from the three different fluids as measured by the CNC. Similar to the findings presented in Figs. 4 and 5, within the fluid type, the fine particle concentration increased with increasing rotation speed. However, there was a more pronounced difference among the fluid types than observed with the OPC and the photometric aerosol mass monitor. A considerable increase occurred in the number of fine particles aerosolized from semi-synthetic MWF after the fluid was inoculated with *P. fluorescens*. For example, at 8000 rpm the fine particle number concentration from *P. fluorescens* suspension in semi-synthetic MWF was 50 times higher than for the pure semi-synthetic MWF.

To further explore the properties of the fine particles aerosolized from contaminated semi-synthetic MWF, the ELPI was used to measure the size distribution of aerosolized particles in the size range of 0.029–10.18 μm. The results are presented in Fig. 7. The horizontal axis presents the aerodynamic particle diameter as measured by ELPI, in contrast to previous figures, which referred to the optical diameter measured by optical instruments. To facilitate the comparison of the size distributions of particles generated from two suspensions (*P. fluorescens* in semi-synthetic MWF and *P. fluorescens* in water), each size distribution was normalized by its highest concentration. In this experiment, the tool rotation speed was 8000 rpm and the fluid application rate was $1\,l\,min^{-1}$. The mode of the size distribution of particles aerosolized from the *P. fluorescens* suspension in water was 0.66 μm. This is consistent with the result of Qian, Willeke, Ulevicius, Grinshpun, and Donnelly (1994), who reported that the aerodynamic diameter of *P. fluorescens* was 0.7 ± 0.1 μm. The mode of the other size distribution (representative *P. fluorescens* suspension in MWF) was about 0.37 μm. The two size distributions were compared using a chi-square test as described by Baron and Willeke (2001). The test showed a significant difference between them ($p < 0.01$). The results of Figs. 6 and 7 thus indicate that the microbial contamination of MWFs resulted in a shift in the size distribution of aerosolized particles towards a smaller particle size.

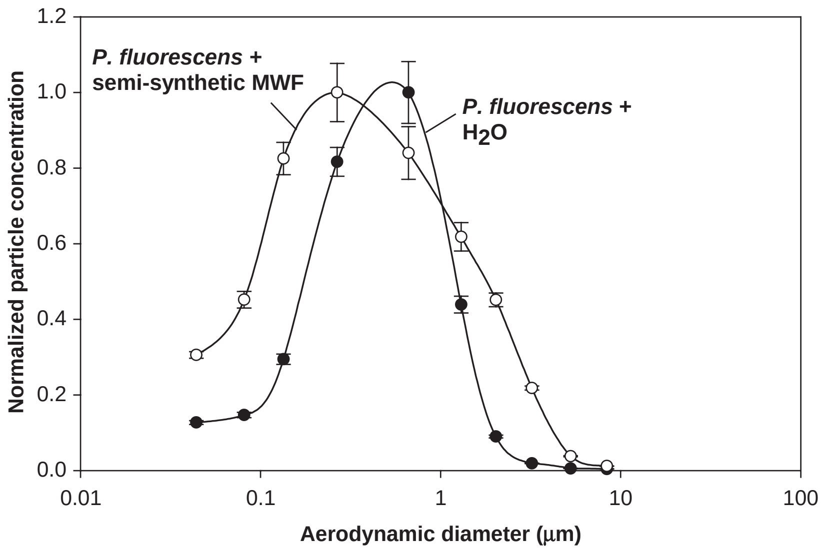

Fig. 7. The normalized number size distribution of particles aerosolized from different fluids at a tool speed of 8000 rpm, as measured by an electrical low pressure impactor. Each size distribution was normalized by its highest concentration. Each data point indicates an average of 5-min measurement and the error bars indicate one standard deviation.

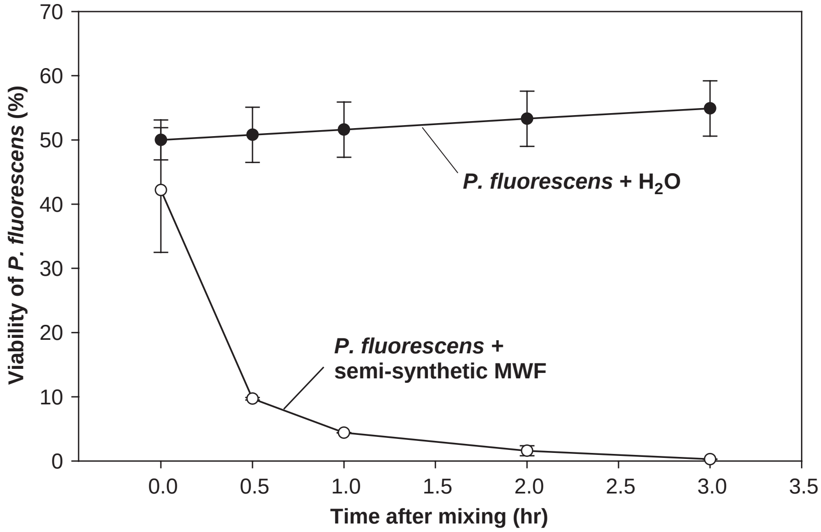

Fig. 8. Viability of *P. fluorescens* cells after mixing with water and semi-synthetic MWF. Each data point indicates an average of three repeats and the error bars indicate one standard deviation.

We had anticipated that a high number of fine particles would be aerosolized from the *P. fluorescens* suspension in semi-synthetic MWF as a result of cell fragmentation and other processes associated with cell death after *P. fluorescens* was mixed with semi-synthetic MWF. The viability of *P. fluorescens* was therefore tested at $t = 0$, 0.5, 1, 2 and 3 h after MWF was inoculated with the bacteria. The results are shown in Fig. 8. The percentage of viable *P. fluorescens* cells in the water suspension remained around 50–55% during the entire testing period. When mixed with MWF, the viability of *P. fluorescens* was around 42% at $t = 0$. A half hour after inoculation the viability had dropped to 10%. After 3 h, the viability had decreased to 0.3%. These data show that the viability of *P. fluorescens* in the suspension decreased very quickly after the cells were mixed with MWF (in contrast to water). This shows that MWF could kill bacteria in a very short time. However, the total microbial count of *P. fluorescens* in the MWF remained constant during the three-hour testing period. It was 3.08×10^8 cells ml^{-1} at $t = 0$ and 3.70×10^8 cells ml^{-1} at $t = 3$ h. The slight increase in the total bacterial count may be due to bacterial deagglomeration during mixing. However, the increase is within the measurement error range. As the total count did not significantly change, cell rupture in the suspension was not likely the source of fine aerosol particles.

A study by Terzieva et al. (1996) also showed an increased amount of cell fragments detected in the air due to continuous bacterial nebulization. The increase of fine particles may be an indicator of bacterial death or injury that is likely to occur in the grinding process causing bacterial slime, capsular material, cell wall, or cell membrane to be broken from the cells and subsequent leakage of intracellular components, such as DNA, RNA, Mg^{2+}, polysaccharides, proteins, and other nutrients (Ray, 1989; Terzieva et al., 1996). Another reason might be that MWFs contain small amount of surfactants. Surfactants can release material from bacteria by making the cell wall highly porous. After adding bacteria into the MWF, its surface tension may be changed. This might contribute to the increasing aerosolization of fine particles.

4. Conclusions

A laboratory-scale simulator was used to investigate the properties of particles aerosolized from microbiologically contaminated MWFs. The method was validated by comparing the size distributions of particles generated with the simulator in the laboratory to those obtained in a machining plant where MWF was applied during a grinding procedure. After this, we used the simulator to examine the effects of tool rotation speed (ranging from 800 to 8000 rpm) and fluid application rate (ranging from 0.4 to $1.6\,\mathrm{l\,min^{-1}}$) on the concentration of particles aerosolized from a pure semi-synthetic MWF (without microbial contamination). As expected, the concentration of aerosolized particles increased with increasing tool rotation speed and fluid application rate. The shape of the particle size distributions was not affected by these variables. After the testing with pure MWF was completed, the aerosolization from MWF contaminated by *P. fluorescens* bacterial cells was studied. While there was a factor of 2 increase in the total particle number concentration and mass concentration above 0.3 μm, there was a very pronounced increase in the fine particle concentration (0.02–1 μm). This phenomenon was observed when the source (MWF suspension) was contaminated with *P. fluorescens*. The experiments with the ELPI showed that the mode of the size distribution of the fine particles was around 0.37 μm. The significant increase in the concentration of fine particles that was found at the tool rotation speed of 8000 rpm and fluid application rate of $1\,\mathrm{l\,min^{-1}}$ may be caused by cell death or injury. The latter was likely to result from the interaction of *P. fluorescens* with MWF, which might have caused bacterial slime, capsular material, as well as cell wall, or membranes to be broken from the cells. This in turn led to the leakage of intracellular components and thus contributed to the fine fraction after aerosolization from the fluid. This hypothesis is supported by the experimental evidence that cell viability decreased quickly after *P. fluorescens* was mixed with MWF. The result of this study indicates that the fine particle size fraction of the MWF mist may contain microbial fragments that cannot be detected by traditional microbiological methods, such as microscopic counting and cultivation.

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