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Reponen, T.,[1] Wang, H. X.,[2] and Grinshpun, S. A.,[3]

Effect of Microbial Contamination of Water-Based Metalworking Fluids on the Aerosolization of Particles and Microbial Fragments

ABSTRACT: Aerosolization of particles from metalworking fluids (MWFs) was studied using a laboratory-scale set-up that simulates grinding operations. Semi-synthetic MWF and soluble oil were contaminated with *Pseudomonas fluorescens* and the aerosolized particles were measured using a photometric mass monitor, optical particle counter, and a condensation nucleus counter. Microbial contamination of both semi-synthetic MWF and soluble oil increased the mass concentration as measured by the photometric aerosol mass monitor and the fine particle number concentration as measured by the condensation nucleus counter. These effects were seen most clearly for the fine size range of particles aerosolized from contaminated semi-synthetic MWF. Endotoxin results indicate that the increase in the fine particle concentration occurs at least partially due to the increase in the microbial cell wall components (fragments).

KEYWORDS : metalworking fluid, bioaerosol, bacteria fine particles, fragments.

Introduction

Workers exposed to metalworking fluid (MWF) aerosols may be at increased risk of developing a variety of respiratory and skin diseases (Robertson et al. 1988, Popendorf et al. 1996, Graves et al. 1997, Kriebel et al. 1997, Robins et al. 1997, Kennedy et al. 1999). The aerosolization of mist from metalworking fluids has been studied extensively (e.g., Thornburg and Leith 2000, Heitbrink et al. 2000, Turchin and Byers 2000, Dasch et al. 2002, White and Lucke 2003). It has been reported that increasing tool rotation speed and fluid application rate increases the mist concentration (Heitbrink et al. 2000, Dasch et al. 2002). Furthermore, the presence of contaminants, such as tramp oil, in the MWF has been shown to increase the mist aerosolization (Turchin and Byers 2000, White and Lucke 2003).

Some studies have been focused on the concentrations of microorganisms and endotoxin in bulk fluid whereas much less information is available on the airborne microorganisms in MWF environments. In an effort to understand the aerosolization of microorganisms from MWFs, we have recently built a laboratory-scale set-up, which simulates grinding operations. Using this simulator, we have found that hydrophobic microorganisms were easier to aerosolize from MWFs than hydrophilic ones and that the increase in the size of microorganism resulted in decreased aerosolization rate (Wang et al. 2004a). Furthermore, it was found that microbial contamination of semi-synthetic MWF increased the aerosolization of fine particles (Wang et al. 2004b).

[1]Associate Professor , [2]Graduate Assistant, [3]Professor
University of Cincinnati, Department of Environmental Health, P.O. Box 670056-0056, Cincinnati, Ohio 45267, USA

In this study, we compared the aerosolization of particles from two commonly used MWFs: semi-synthetic MWF and soluble oil. Endotoxin concentration was measured from size-fractionated air samples.

Methods

Experimental Set-up

The experiments were performed using a laboratory-scale set-up, which simulates grinding operations in the field. The set-up has been described in detail by Wang et al. (2004a). In short, it consists of two enclosed chambers, and inner and outer chamber. The aerosolization of MWF mist takes place in the inner chamber (volume = 5 liters) when a liquid pump ejects MWF through a nozzle against a rotating aluminum rod. The rotation speed of the rod can be varied between 200 and 8000 rpm. The particles are aerosolized by centrifugal motion, spray atomization, and by bounce-off after impacting on the rod and chamber walls. The outer chamber has a positive air pressure compared to the inner chamber, which prevents the MWF mist and microorganisms from leaking out form the inner chamber. The aerosol flows from the inner chamber into the measurement chamber, in which samples are drawn into aerosol and bioaerosol instruments. The entire set-up is housed in a biosafety cabinet (Sterilchem-Gard Class II, Type B2, Baker Co., Sanford, ME).

Microorganisms and metalworking fluids

Pseudomonas fluorescens (ATCC 13525) was selected as a test microorganism. *Pseudomonas* is most common contaminant in MWFs (e.g., Bernstein et al. 1995, Lonon et al. 1999) and has frequently been used as a simulant for stress-sensitive bacteria in aerosol studies (Wang et al. 2001, Johnson et al., 1999). Furthermore, it is a gram-negative bacterium that contains endotoxin. *P. fluorescens* was grown in trypticase soy broth at 26°C for 18 hours. The cells were then washed twice with sterile deionized water by centrifugation at 7000 rpm for 7 min. Fresh bacterial suspension was prepared daily for the experiments and diluted with the test MWF or with sterile deionized water until a concentration of 10^8 cells/mL was achieved. The cell concentration was measured by microscopic counting using a hemocytometer (Hausser Scientific, Horsham, PA). The following five fluids were tested in this study:

- semi-synthetic MWF,
- soluble oil,
- *P. fluorescens* in sterile water,
- *P. fluorescens* in semi-synthetic MWF, and
- *P. fluorescens* in soluble oil.

Semi-synthetic MWF and soluble oil are both commonly used water-based MWFs. They were both utilized as 5% water solution (5% MWF concentrate and 95% of sterile deionized water) according to the way in which these fluids are applied in industry. No biocide was added for these experiments.

Aerosol measurements

Three aerosol instruments were employed to continuously measure the concentrations and size distributions of aerosolized particles. The inlets were placed isoaxially inside

the measurement chamber. Photometric aerosol mass monitor (Photometer, DustTrak, model 8520, TSI Inc., St. Paul, MN) measured the total particle mass concentration within the particle size range of 0.1 to 10 μm. Optical particle counter (OPC, Portable Dust Monitor model 1.108, Grimm Technologies Inc., Douglasville, GA) measured the concentration and size distribution of particles in 15 size channels between 0.3 and 20 μm. Condenstation nucleus counter (CNC, P-Trak fine particle counter, model 8525, TSI Inc.) measured the number concentration of fine particles (0.02 - 1 μm). All instruments operated continuously with one minute averaging time.

Additionally, an electrical low pressure impactor (ELPI; 3935 series, Dekati Ltd., Tampere, Finland) was utilized to collect selected size fractions of particles for endotoxin analysis. The ELPI measured the aerodynamic particle size (d_a) and collected particles onto 13 different size fractions in the size range of 0.029 to 10.18 μm. Semi-synthetic MWF contaminated with *P. fluorescens* was tested in this experiment. We have shown earlier that the peak in the particle size distribution is of d_a= 0.66 μm determined for particles aerosolized from pure *P. fluorescens* suspension (bacteria in water) decreases to 0.36 μm when the water was changed to semi-synthetic MWF (Wang et al., 2004b). Therefore, these two size fractions were selected for endotoxin analysis to represents fragments and intact bacterial cells, respectively.

The endotoxin analysis was performed with Kinetic-QCL® instrumentation using limulus amebocyte lysate (LAL) assay method.

Statistical analysis

Statistical analysis was performed using SAS software (version 8.02 for Windows, SAS Institute, Inc., Gary, NC). The differences in particle concentrations aerosolized from five different fluids were compared using ANOVA followed by the Scheffe's test.

Results and Discussion

Figure 1 presents the concentration of particles aerosolized from soluble oil as a function of tool rotation speed. As shown, all the three aerosol instruments (photometer, OPC, and CNC) showed an increase in the mist concentration when the tool rotation speed increased from 800 to 8000 rpm. This finding agrees with previous results reported by Heitbrink et al. (2000) and Dasch et al. (2002) indicating that our set-up appropriately simulates the aerosolization of mist. The largest increase (by a factor of 200) was seen in the fine particle number concentrations measured by the CNC.

Figure 2 compares the particle concentrations aerosolized from five different fluids when the tool rotation speed was 8000 rpm. Three different particle concentrations are presented: (A) mass concentration measured with the photometer, (B) total particle number concentration measured with the OPC, and (C) fine particle number concentration measured with the CNC. The mass concentrations of particles aerosolized from the five different fluids were statistically significantly different from each other ($p<0.001$) (Figure 2A). The lowest mass concentration was measured for particles aerosolized from bacterial suspension in water. The highest mass concentrations were measured for pure soluble oil and for soluble oil contaminated with bacteria. The microbial contamination increased the mass concentration of particles aerosolized from both semi-synthetic and soluble oil. Bacterial contamination had a more pronounced effect for the mass concentration of particles aerosolized from semi-synthetic MWF (increase by a a factor of 1.8) than from soluble oil (increase by a factor of 1.02).

The total particle number concentrations as measured by the OPC are presented in Figure 2B. Multiple comparisons with the Scheffe's test revealed that the concentrations of particles aerosolized from pure soluble oil and from soluble oil contaminated with bacteria did not significantly differ from each other. The concentrations of particles aerosolized from the three other fluids were significantly different ($p<0.001$). Bacterial contamination increased the total number concentration of particles aerosolized from semi-synthetic MWF by a factor of 2.0.

Figure 2C presents the concentration of fine particles measured with the CNC. The lowest concentration of fine particles was aerosolized from pure semi-synthetic and pure soluble oil. Microbial contamination of these fluids increased the concentration of fine particles by a factor of 52 and 15, respectively ($p<0.001$). Thus, bacterial contamination had more pronounced increase for the fine particles aerosolized from semi-synthetic MWF than for those aerosolized from soluble oil. Furthermore, number concentrations of fine particles exhibited greater increase compared to the total particle number concentrations (measured by the OPC) or the mass concentrations. This can be explained by the physical characteristics of fine particles: due to their small size, particles in this range are not detected by the OPC and do not contribute significantly to the mass concentration despite their high number concentration.

Results of the endotoxin analysis show that the particle size ranges representing fragments and intact cells contain about the same amount of endotoxin (Table 1). This suggests that the size fractions below the size of intact bacterial cells contain cell wall components of bacteria.

TABLE 1—Endotoxin concentration of particles aerosolized from semi-synthetic MWF contaminated with bacteria. Particle number concentration was measured using the ELPI direct-reading capability and endotoxin analysis was performed from samples collected on two different ELPI stages. The results represent and average and standard deviation of three repeats.

	Particle size fraction	
Concentration	Fragments (d_a = 0.36 μm)*	Intact bacteria (da = 0.66 m)*
Particle number concentration (#/m^3)	1.7×10^9	9.3×10^8
Endotoxin concentration (EU/m^3)	13.1 ± 6.0	13.9 ± 4.4

*d_a =aerodynamic particle size

Conclusions

Our results confirm the previous reports that increasing tool rotation speed in grinding operations increases the mist concentrations. An original finding of this study was that microbial contamination of MWF increases the aerosolization of particles. This effect was seen more clearly with semi-synthetic metalworking fluid and in the fine particle size range. Endotoxin results suggest that these fine particles contain microbial cell wall components. Due to their small size and high aerosol concentration, microbial fragments may play a crucial role in the health effects caused by MWF mist exposures.

Acknowledgements

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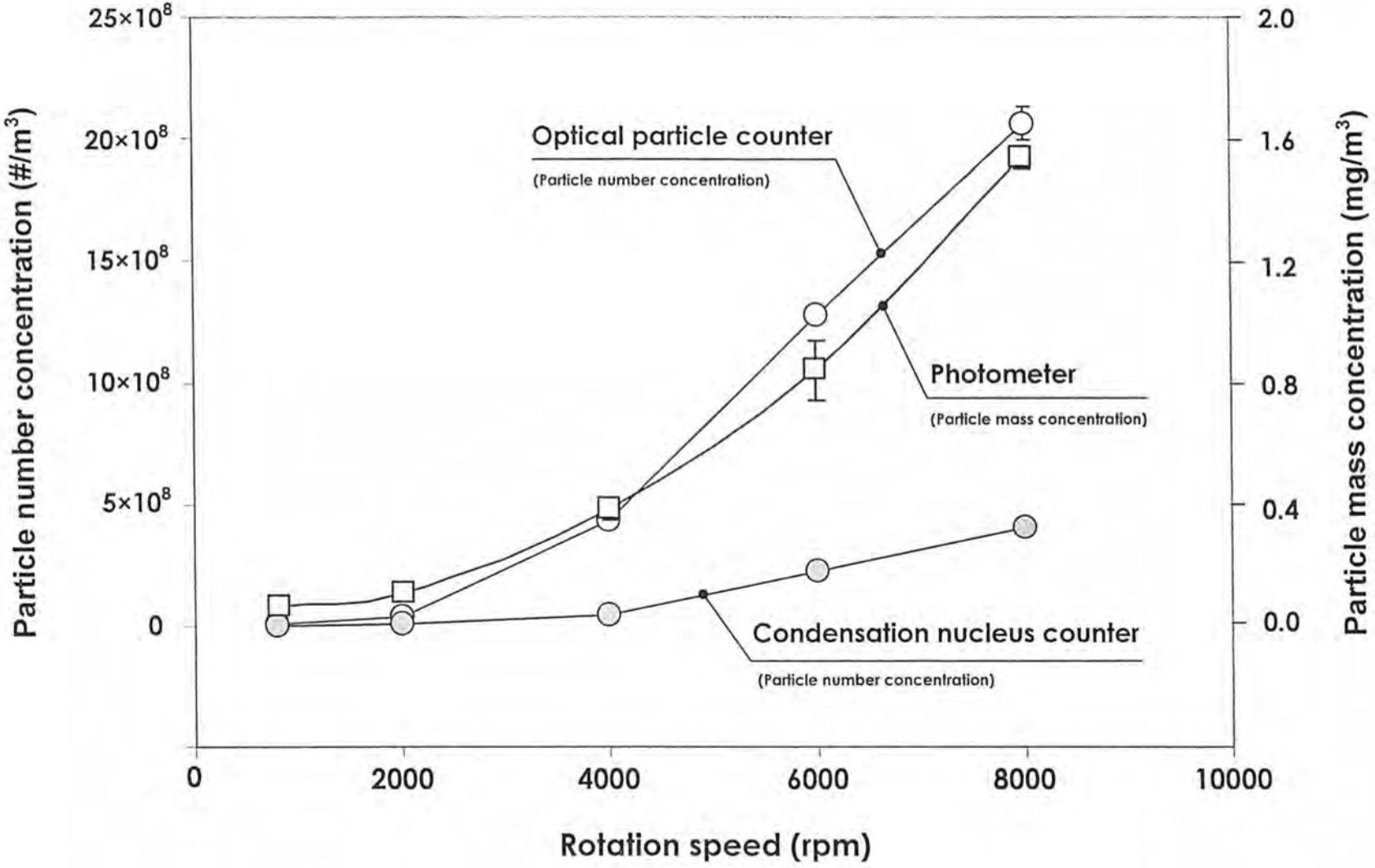
Reponen et al. - Figure Captions

Figure 1. The effect of rotation speed on the concentrations of particles aerosolized from soluble oil. Particle concentrations measured by (A) Photometer, (B) Optical Particle Counter, and (C) Condensation nucleus counter. Each data point represents an average of five consecutive one-minute measurements. The error bars represent standard deviations.

Figure 2. The effect of fluid type on the particle concentrations at 8000 rpm. The particle concentrations measured by (A) Photometer, (B) Optical Particle Counter, and (C) Condensation nucleus counter. Each bar represents an average of five consecutive one-minute measurements. The error bars represent standard deviations.



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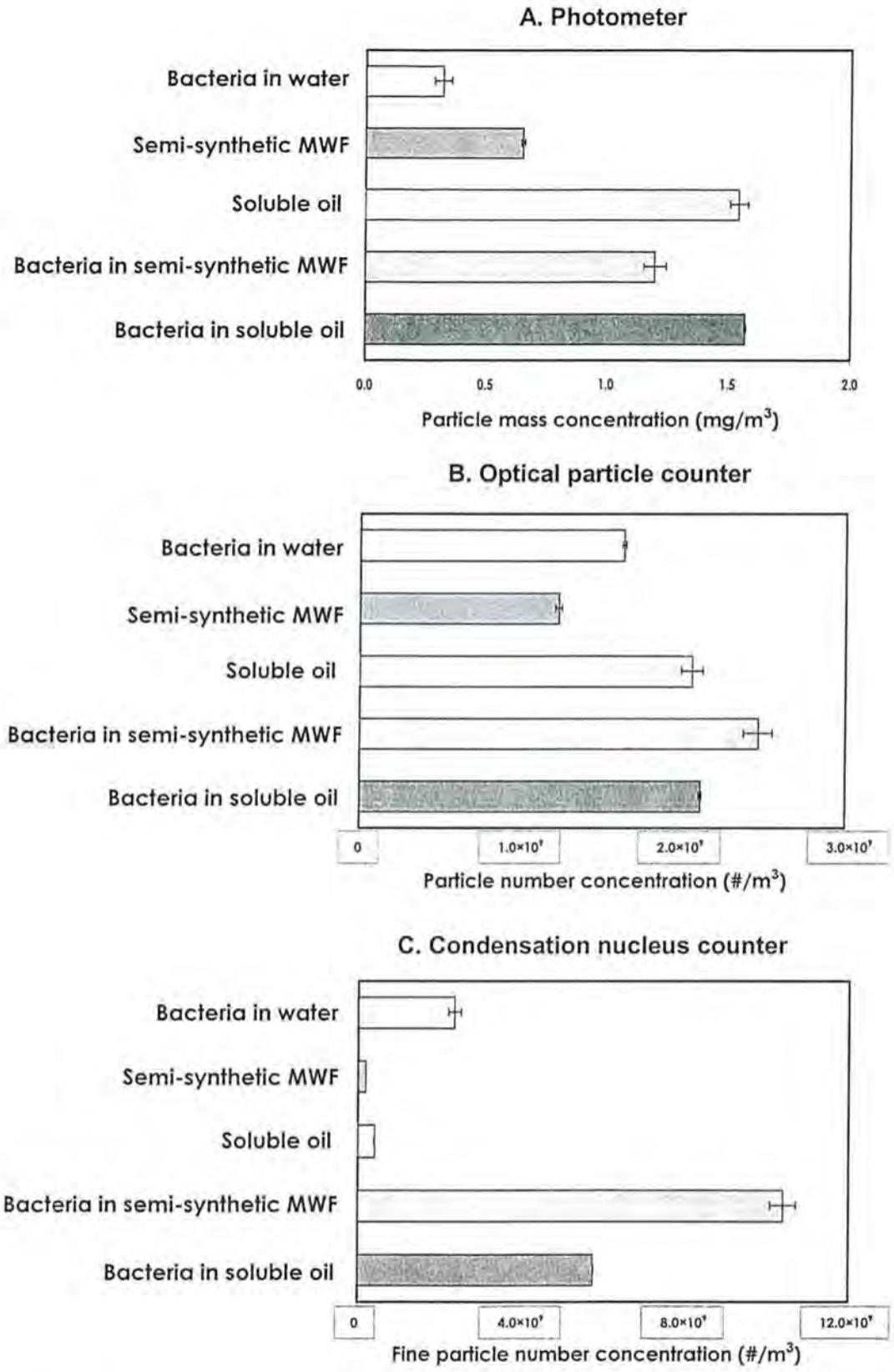

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Reponen, T
University of Cincinnati, Department of Environmental Health, Cincinnati, Ohio, USA

Wang, HX
University of Cincinnati, Department of Environmental Health, Cincinnati, Ohio, USA

Grinshpun, SA
University of Cincinnati, Department of Environmental Health, Cincinnati, Ohio, USA

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Abstract
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Keywords:
bacteria fine particles, bioaerosol, fragments, metalworking fluids

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Author Reponen, T Affiliation University of Cincinnati, Department of Environmental Health, Cincinnati USA Author Wang, HX Affiliation University of Cincinnati, Department of Environmental Health, Cincin USA Author Grinshpun, SA Affiliation University of Cincinnati, Department of Environmental Health, Ci Ohio, USA Title Effect of Microbial Contamination of Water-Based Metalworking Fluids on the Aerosoliz

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