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Microbial contamination and biofilms on machines of metal industry using metalworking fluids with or without biocides

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ABSTRACT

Microbial contamination is commonly found in metalworking fluids (MWFs) used in metal manufacturing industry. The aim of this study was to determine whether the use of MWF containing a biocide reduces the growth and influences the biofilm-forming capacity of microorganisms from field coolants, thereby efficiently protecting MWFs from biodeterioration. Overall, 164 bacterial strains classified into 40 species were collected from 69 machines in ten plants visited. Among them, *Comamonas testosteroni* was the most numerous species, and *Pseudomonas* was the most abundant genus. The isolation of *Mycobacterium immunogenum* was done from MWF for the first time in Poland. The number of bacteria in MWFs and size of microbial populations in biofilms were not affected by the presence of biocides in the industrial samples. In MWFs contaminated with microorganisms with the highest biofilm-forming capacity, several species of *Enterobacteriaceae* were identified. The proper management of MWF delivery systems is required to control biofilm development even when MWFs containing biocides are used. Moreover, good hygiene practices are needed to prevent the metal working machines from biofilms. This case study can help in appropriate monitoring the MWF distribution systems vulnerable to microbial colonization in metal industry.

Introduction

The presence of microorganisms in MWFs causes significant economic losses (Theaker and Thompson, 2010). The bacteria and fungi may grow in all water-miscible MWFs (Saha and Donofrio, 2012). Several types of contaminated MWF may lose their lubricant and anticorrosion properties and would therefore have to be replaced more frequently to maintain the proper quality of the finished products. The oil mist, a bioaerosol generated during machining and grinding may have adverse effect on health of employees (Simpson et al. 2003). Despite many studies on measures for prevention and reduction of microbial contamination of MWFs, the problem has not yet been solved. Biocides are usually incorporated into MWF formulation in order to reduce the number of microorganisms in MWFs. However, maintaining the optimal concentration of biocide in the coolant's circulation systems is sometimes problematic due to the evaporation of water from the coolant. When fresh water is replenished, the proprietary tests are not often conducted for quantifying the final biocide concentration. Moreover, biocides used in high concentrations may affect the health of workers and cause dermatitis following contact with MWFs (Vijay et al. 2009).

When the microbial contamination of MWFs is not properly controlled biofilms are formed and biomass becomes visible in sumps. Occasionally, they may clog the delivery systems of the coolant. The manufacturing process has to be shut down in such incidents, and the machine cleaned and disinfected before it can be refilled with fresh coolant.

The problem of biofilm formation in MWFs has not yet been studied in detail. Only a few papers published so far have described the propensity of microorganisms from MWFs to form biofilms (Mattsby-Baltzer et al. 1989; Falkinham 2009; Lucchesi et al. 2012; Saha et al. 2012; Trafny, 2013). In our opinion, there is a lack of data concerning field biofilms, i.e., the data on how, when and where

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these consortia are formed on machines in metal manufacturing plants.

The studies on biofilms in MWFs are hindered by a lack of relatively simple methodology for biofilm development and assessment. Growing biofilms on stainless-steel coupons for several days does not allow analysis to be performed for many field samples of MWFs simultaneously. Recently, two methods for assessing the size of a microbial population in a biofilm have been introduced to the MWFs research area. These two methods are an ATP bioluminescence assay (Kapoor and Yadav, 2010) and an MTT assay performed in microtiter plate format (Trafny et al. 2013). These methods work in a similar range of bacterial densities and allow monitoring the viability of microorganisms that develop biofilms in MWFs.

The biofilms in MWFs are usually composed of more than one species of microorganisms (Theaker and Thompson, 2010; Elias and Banin, 2012). *Pseudomonas* sp., *Alcaligenes* sp., *Comamonas testosteroni* and even enteric bacteria together with nontuberculous mycobacteria were isolated from contaminated MWFs (Gilbert et al. 2010; Kapoor and Yadav, 2012). Nontuberculous mycobacteria are not only naturally resistant against many antimicrobial agents because of the specific structure of their cell walls (Brennan and Nikaido, 1995), but also particularly easily create biofilms in water-containing environments (Mullis and Falkinham, 2013) and, therefore, are particularly hard to remove from MWF delivery systems and sumps.

The aims of this work were to determine whether the use of MWFs containing biocides reduces the growth of bacteria in the in-use coolants and influence the capability of the organisms to develop biofilms. The experiments were performed on microorganisms from the real MWF samples collected from ten metal industry factories, where no prior surveillance was undertaken to monitor the microbiological quality of coolants.

Materials and methods

Metal producing plants and metalworking fluids

Coolants and swabs samples were collected at ten plants of metal industry (No. 01–10). These plants were located in six provinces of Poland, in cities of all sizes. They were both medium and large engineering companies, which manufactured various metal components. The managers of these enterprises showed understanding for the need to monitor the quality of coolants. However, none of the companies conducted the surveillance of the microorganisms' quantity in the coolants. The exact dates of the last replacement of MWF in each lathe were not available. Thirteen commercial MWFs were used in these factories. Their major characteristics are shown in Table 1. According to the received declarations of persons in charge, additional biocides had not been added to the coolants in the plants visited. The coolants were exchanged relatively rarely; in most machines MWFs were used for at least 6–9 months.

Coolant samples and swabs

This study was performed from November 2011 to November 2012. Three types of samples were taken: i) the coolant directly applied to the work piece while the machine was running (MWF_{DIR}); ii) the coolant and slime from the reservoir (sump) of the machine (MWF_{RES}); iii) swabs from the machines, i.e., from the bed of lathe in manually operated machines or the walls and other metal parts in CNC (Computer Numerical Control) machining centers. In total, 196 samples of coolants and swabs were collected from 69 different metal processing machines. The samples were obtained from four band sawing machines (6.2%), 12 grinders (18.5%), 16 CNC machines (24.6%), 24 turning lathes (37%), seven routers (10.7%), one radial drill, and a wire-cut EDM (Electric Discharge Machining) machine. The samples (50 ml) of coolants were collected in sterile containers. The machine surfaces were swabbed with a foam spatula (Copan Diagnostics Inc., Corona, United States). The foam was then placed in sterile, sealed container (FL Medical srl, Torreglia, Italy) with 50 ml of phosphate buffered saline (PBS, pH 7.4). Samples of MWFS and swabs were transported to the laboratory in portable refrigerators (Campingaz, Italy) and kept in the incubator at a temperature of 22 °C until the next day morning. Then, the microbial identification and biofilm assays were performed. Foam swabs were transferred to a Stomacher bag (Seward Ltd., UK), squeezed by hand for one minute, and then the foam swabs were drained and removed with sterile forceps (Lewandowski et al. 2010). The suspensions obtained were further serially diluted (10-fold) and plated on the solid media.

Bacteria in coolants and swabs

Qualitative analysis

The coolant samples and swabs were spread on the following solid media: Columbia Agar with 5% sheep blood (bioMerieux), Cetrimide Agar Base (Beckton Dickinson), and Loewenstein-Jensen medium without salt ions, prepared in-house from fresh chicken eggs (Atlas, 1997). The media were incubated at a temperature of 30 °C in aerobic conditions for three to five days (Loewenstein-Jensen medium for at least 21 days). Pure colonies of bacteria were isolated, and Gram staining was done according to standard procedure. When Gram-positive bacteria were present in the sample, an additional staining according to Ziehl-Neelsen was performed

Table 1
Characteristics of metalworking fluids used in the metal producing plants.

Plant	Trade name	Manufacturer	Oil type	Biocide
01	Shell Dromus BX	Shell Metalworking Europe (Germany)	Mineral	No
02	Mobil Kutwell 42	Exxonmobil Lubricants and Specialties Europe (Belgium)	Mineral	3,3′-Methylene-bis-[5-methyloxazolidine]
03	Emulgol 42 GR	Orlen Oil (Poland)	Mineral	No
04	Ecocool 68 CF2	Fuchs Lubricants (USA)	Mineral	No
05	Cimstar 4800	Cimcool Industrial Products (The Netherlands)	Semi-synthetic	3,3′-Methylene-bis-[5-methyloxazolidine]
06	Syntilo R Plus	BP Europa SE (Branch in Poland)	Mineral	No
	Blasocut 2000 Universal	Blaser Swisslube AG (Switzerland)	Mineral	No
07	Hysol R	BP Europa SE (Branch in Poland)	Mineral	N,N-Methylenebismorpholine
08	Emulgol ES 12	Orlen Oil (Poland)	Mineral	N,N-Methylenebismorpholine
	Almaredge 51FF	BP Europa SE (Branch in Poland)	Mineral	No
09	Ultracut 370 Plus	Rocol House (England)	Semi-synthetic	No
10	Cimstar 560	Cimcool Industrial Products (The Netherlands)	Mineral	3,3′-Methylene-bis-[5-methyloxazolidine]
	Unicool WO	Orlen Oil (Poland)	Minera	N,N-Methylenebismorpholine