



Comparative study of microbiological monitoring of water-miscible metalworking fluids



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ARTICLE INFO

Article history:

Received 29 August 2014

Received in revised form

22 November 2014

Accepted 23 November 2014

Available online 31 December 2014

Keywords:

Lubricant degradation

Maintenance

Cutting fluids

Bacteria

Microbiology

Enumeration

Adenosine triphosphate

Condition monitoring

ABSTRACT

Microbial activity is one of the primary causes of water-miscible metalworking fluid (MWF) deterioration in application. Although it is impractical to maintain MWF under microbe-free conditions, timely, accurate and easily interpreted microbial contamination condition can substantially reduce the risk of MWF biodeterioration. This paper compares different condition monitoring methods detecting the microbial contamination of MWF related to their speed and precision in laboratory and field samples. Two culture tests (plate count and dip-slides) and two adenosine triphosphate (ATP) test methods were compared. Additionally the matrix assisted laser desorption/ionization time of flight mass spectrometry (MALDI-ToF-MS) is introduced as a method for the identification of bacterial strains isolated from MWF. The results clearly indicate that MWF monitoring is in need of standardization concerning sampling, area of measurement, and analysis of determinable cell counts as well as species communities.

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Introduction

In manufacturing technology the majority of forming and machining processes are dependent on the use of metalworking fluids (MWF). The importance of MWF becomes apparent when you consider the fact that every year 580,000 metric tons of MWF have to be disposed in Germany alone ([German Federal Office of Economics and Export Control, 2012](#)). Globally, annual water-miscible MWF consumption is estimated at 40 million metric tons ([Cheng et al., 2006](#)). The importance of water-miscible MWF (for the remainder of this paper, the term *MWF* will refer to only water-miscible MWF) in metalworking was demonstrated by Ernst ([Ernst, 1951](#)). He observed: “directly or indirectly, machining affects every aspect of our civilization. Every product we use, wear or eat is related to metal cutting, either directly, in its own manufacture, or

indirectly, through the manufacture of the machine that makes it” ([Ernst, 1951](#)).

Machining processes exhibit a high energy turnover due to separating and forming. In application, MWF perform fundamental tasks such as removing the heat generated by the metal removal process, reducing friction in the contact zone between tool and work piece, and transporting metal chips, tailings and fines typically produced during metal removal process. In order to meet process-specific performance demands beyond these primary functions, MWF are formulated with a variety of mono and multi-functional additives. Examples of MWF functional properties provided by additives include: wetting ability (surface tension modification), compressive strength (extra-pressure – EP – lubricity), emulsifying ability (in water-miscible MWF), corrosion inhibition, anti-foaming performance. Moreover MWF formulations must be compatible with seals and machine tool coatings, be easy disposability, be mild to the skin and environmentally benign. Perhaps paradoxically, MWF are expected to be biostable while in use and biodegradable once disposed.

In order to perform these functions, modern MWF formulations often contain more than 15 different chemical components. More than 300 MWF performance additives are available to MWF

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compounds. Many MWF formulation components are technical grade petroleum derivatives. Increasingly, plant-based raw materials are also being used. The degree of purity of the raw materials ranges from 85% to 95%, so that admixtures of unknown origin and concentration must also be reckoned with (Häusser et al., 1985). Typically MWF are distributed as concentrates and are diluted with water to end-use concentrations of 2%–10% before use.

The high concentration of water (typically $\geq 90\%$ in end-use diluted), as well as the chemical composition of MWF create a good habitat for microorganisms – particularly bacteria and fungi. In the course of their application nearly all MWF components can be utilized by microorganisms as a source of nutrients and energy. Uncontrolled microbial contamination in MWF invariably leads to biodeterioration of both the fluid and MWF system. Common biodeterioration symptoms include: production of malodorous volatile organic chemicals, selective depletion of functional additives (and concomitant performance degradation), decreased tool-life, premature filter-indexing, MWF system fouling and shortened MWF performance life. These changes typically translate into reduced cutting-tool performance life and increased finished-part rejection rates (Passman, 1988; Koch, 2008). Moreover microbial activity influences the accumulation of uncharacterized MWF-component derivative chemicals and microbial metabolites the used MWF. As neutralizing amines are consumed, reserve alkalinity and pH drop. Weak organic acid metabolites (for example Krebs cycle intermediates) react with dissolved chloride, nitrate, nitrite and sulfate ions to form strong inorganic acids. These, in turn, cause demulsification of oil micelles characteristic of end-use diluted emulsifiable oil and semisynthetic MWF. Biodegradation of corrosion inhibitor additives induces corrosion on work pieces and machine tools. Microbial volatile organic compounds (MVOC) produce unpleasant odors and discolorations/stains can occur (Passman, 1988).

Koch (2008) has recently demonstrated the direct relationship between increased MWF bioburden, decreased MWF additive concentration (e.g. monoethanolamine (MEA)) and increased tool wear-rates.

The interaction of technical quality and the microbial load of water-miscible MWF has only recently become a focal point of scientific works (Passman, 1988; Brinksmeier et al., 2009; Koch et al., 2009; Rabenstein et al., 2009). Against the background of energy and resource efficient production the produced part's quality, reduced tool-wear and a long lifetime should be fundamental aims of MWF utilization. This can only be achieved if the technical properties of the MWF remain constant and within the specification range throughout its service life. For this purpose, an exact monitoring and analysis of the microbial load is invaluable. Passman has reviewed the adverse effects of uncontrolled microbial contamination in MWF (Passman, 2006). In the following sections, five different procedures for the detection of microbial contamination in MWF, which are common on the market, are compared. The results of the comparative examinations are concerned with two different systems of detecting microbial load by measuring ATP content, the estimation of culturable microbial population density with dip-slides and plate counts, and matrix assisted laser desorption/ionization time of flight mass spectrometry (MALDI-ToF-MS).

Materials and methods

Comparative studies

Comparative studies were conducted in order to demonstrate the capabilities and limitations of the detection systems – for example: chemical interferences with the ingredients of the MWF.

Two ATP testing methods and common dip-slides (DS) were employed. Standard plate counting (SPC) was used as a reference method.

Two MWF were used in the laboratory evaluation and the field examinations:

I) An emulsifiable MWF (E-MWF), it was a microbicide-free formulation that contained boron and amines. The E-MWF was designed for research projects (Rhenus Lub GmbH & Co KG, Mönchengladbach, Germany). The formulation was very close to commercial emulsifiable MWF and available for all partners within the research projects. The E-MWF was used in the laboratory evaluation (s. 2.2) as fresh and used emulsion. The used emulsion was collected of a milling and drilling machine tool, with a stand-alone recirculating MWF system. The nominal capacity was 800 L and included the tool, MWF reservoir, piping and pumps. This machine tool served also as the sample site for the field experiments described in 2.3.

In addition a separate 200 L tank in which no machining processes were performed was charged with the E-MWF (Reference-system). The E-MWF emulsion was made up with tap water and recirculated. To accelerate the microbial development chips and swarf of different steel grade were deposited in the Reference-system. In this case the microbial consortia was able to develop without the chemical and thermal stresses typical for the operating effects in a machine tool. The sample of the Reference-system was used for the laboratory evaluation (2.2) and was collected after an operating state of 29 weeks.

II) A semisynthetic MWF (S-MWF). The S-MWF was a commercial MWF generally used in grinding operations (Acmos GmbH, Bremen, Germany). Made up with tap water it was a translucent fluid. It included a formaldehyde-condensate microbicide (α,α',α'' -Trimethyl-1,3,5-triazin-1,3,5-(2H,4H,6H)-triethanol, CAS 25254-50-6). The S-MWF was used as fresh samples in the laboratory evaluation (s. 2.2) and the used samples were collected of a grinding machine with a stand-alone recirculating MWF system (s. 2.3). The volume of the MWF system was about 1.500 L.

Laboratory evaluation

Preliminary laboratory evaluation of the monitoring systems was performed in four microcosms with a volume of 250 mL each. Samples of these four fluids were analyzed by DS, SPC, ASTM E2694 and ASTM D7463. The microcosms 2–4 were inoculated in the laboratory with a bacterial pure culture. The pure culture had previously been isolated from a contaminated MWF and characterized as *Pseudomonas pseudoalcaligenes* or *Pseudomonas oleovorans* by MALDI-ToF-MS. The culture was grown in a Tryptic Soy liquid culture over-night on a shaker. The cell density was determined by microscopic direct count prior to inoculation and adjusted to $\text{Log } 6 \text{ mL}^{-1}$ in each sample. For microcosms 2, 3 & 4, samples for comparative culture and ATP tests were collected and tested approximately 30 min after the challenge inocula were added.

- 1) The first microcosm (Reference) was a used E-MWF. The sample was taken from the Reference-system, it had a service life of 29 weeks. Prior to the test procedure the indigenous microbial population in the used E-MWF was monitored by SPC method and had a cell density of $\text{Log } 5.0 \text{ CFU mL}^{-1}$.
- 2) The second microcosm (PuCu TW) was filter sterilized tap water with an inoculum of the pure culture characterized as *P. pseudoalcaligenes* or *P. oleovorans*.
- 3) A fresh E-MWF emulsion made up with filter sterilized tap water with an inoculum of a pure culture characterized as *P. pseudoalcaligenes* or *P. oleovorans* served as the third microcosm (PuCu E).

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