International Biodeterioration & Biodegradation 63 (2009) 1023–1029

Contents lists available at ScienceDirect

International Biodeterioration & Biodegradation

journal homepage: www.elsevier.com/locate/ibiod

Microbial degradation of water miscible metal working fluids

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ARTICLE INFO

Article history:
Received 13 February 2009
Received in revised form
17 July 2009
Accepted 17 July 2009
Available online 14 August 2009

Keywords:
Metal working fluid
Biodegradation
Microbial community
Cutting fluid

ABSTRACT

Water miscible metal working fluids (MWF) are prone to contamination by bacteria and fungi. In the present work it was investigated which components of a model MWF emulsion were most readily degraded by microorganisms and which are relatively resistant to biodegradation. The microbial community colonising an MWF emulsion, during its lifetime under in-use conditions was analysed up to species level. Shifts in the composition of microbial community were related to chemical changes in the MWF emulsion over time.

1. Introduction

Water miscible metal working fluids (MWF) fulfill several functions in manufacturing technique. During machining processes as turning, milling, boring, and grinding MWF perform the cooling of work piece and tool, lubricate the process, and mediate the removal of the chipping. In spite of the tremendous benefit for achieving optimised machining of metal goods there are economic and ecological risks (Garbrecht et al., 2008). Microbial contamination and degradation of water miscible MWF are widespread problems leading to functional and hygienic concerns.

Water miscible MWF are prone to contamination by bacteria and fungi. Microbial degradation of the fluids causes a range of problems beginning with discolouration of the emulsion and evolution of malodour to the loss in quality of the work pieces and tool failure. Moreover high loads of bacterial or fungal contamination are a hygienic problem and pose a risk to the health of the workers.

More than 300 different substances are known to be used in MWF (Häusser et al., 1985). Single MWF can contain up to 60 different components. Water miscible, emulsifiable-oil concentrates that are used to form emulsions consist of 20–70% of an oil (mineral oil, polyglycol, natural or synthetic esters), emulsifiers as fatty alcohols or amino alcohols, corrosion inhibitors (fatty acids, amines, borates), and further compounds as extreme pressure additives, foaming inhibitors, and biocides. Often the exact chemical composition cannot be determined because substances of technical purity grade are used, which means a purity of 85–95% (Häusser et al., 1985; Greim, 2006).

Frequently, a loss in properties of metal working fluids is counteracted by adding biocides or presumably depleted additives to emulsions already affected by microbial degradation. But, these additions can never reconstitute the original state of the metal working fluid. Finally, MWF degraded by activity of microorganisms prematurely become waste that has to be disposed cost-intensively. For example, consumption of MWF concentrate was around 30,000 metric tons in Germany in 2004, alone. Based on the fact that prior to use the concentrate is diluted with water to form a 5% emulsion the total amount of MWF used in Germany was 600,000 metric tons. Coolant maintenance regimens to increase in-use longavity of MWF emulsions will therefore leads to significant cost savings.

Microbial contamination of MWF is not a new topic. First investigations of microbial colonisation of these technical fluids and of the effects of growth of bacteria and fungi on the properties of MWF were already made in the 1920s (Kenndall, 1920; McConnell, 1922). Lee and Chandler (1941) isolated bacteria and studied their growth in 'cutting compounds'.

There are different ways for microorganisms to get into the MWF emulsions. One source of contamination can be via the water used to dilute the concentrate. According to German drinking water regulation (2001) tap water is allowed to contain up to 100 viable bacterial cells per mL. The use of ion exchangers for desalination

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doi:10.1016/j.ibiod.2009.07.005

has no effect on reducing the microbial load of the water. Residues of used MWF, remaining even after cleaning in tubing or dead spaces, may lead to a rapid recontamination after the system has been refilled with new MWF emulsion. Microorganisms can enter MWF by the way of work pieces or personnel handling the machines. Moreover, fresh MWF emulsions can be easily infected by microorganisms attached to dust particles and aerosols.

Microbial colonisation cannot be prevented completely and permanently under working conditions, but the lifetime of MWF emulsions can be extended by establishing and operating a good maintenance system.

Microbial degradation of MWF does not occur as a simultaneous reduction of all components, but successively. Compounds easily degradable by microorganisms will be utilised first and more rapidly. Inevitably, this will influence or even disrupt distinct properties of an MWF emulsion leading to losses in performance and a decrease in work quality (Rakić and Rakić, 2002).

This work will focus on the microbiological aspects of degradation of MWF emulsions, on selective degradation of single components, microbial population analysis and shifts of microbial community composition within an MWF system.

2. Materials and methods

2.1. Measurement of aerobic degradation of MWF components

To study aerobic microbial degradation of MWF components different ingredients were mixed with mineral medium without any other carbon source in a sealed atmosphere of air in glass bottles. These bottles were inoculated with bacteria isolated from contaminated MWF emulsion. In regular intervals samples were taken from the sealed gas phase to determine CO_2 concentration by gas chromatography.

The mineral medium used consisted of 1.0 g K_2HPO_4, 1.0 g $NaNO_3$, 0.5 g $MgCl_2$, and 1.0 g $MgSO_4 \cdot 7H_2O$ in 1000 mL deionised water. The pH value of the medium was 8.5–9.0 to approximate the conditions usually found in MWF emulsions. Serum glass bottles were filled to 10% of the total volume with mineral medium which left 90% of the total volume to be filled with air. 100 mg of an MWF component were added as sole carbon source. Controls were prepared without any additional carbon source. The inoculum was a cell suspension of a mixed culture of bacteria isolated from contaminated MWF emulsion and cultivated on tryptic soy broth agar (DIFCO 0370-17-3). Cell counts in the test solution were at approximately 10^6/mL. The serum bottles were sealed airtight with butyl rubber plugs. The rubber plugs were fixed with aluminium caps. The test bottles were incubated at 25 °C in the dark.

All substances tested were technical grade as used in MWF formulations customarily.

The following components or component groups were tested for microbial degradability: mineral oil (naphtenic), fatty acids, fatty acid ethanol amide, monoethanol amine, alkyl amides, and fatty alcohol ethoxylates.

Samples were taken from the gas space of each test bottle in regular intervals. The gas samples were manually injected into a gas chromatograph (Shimadzu GC-17A) equipped with Porapak N column (Alltech, 1.5 m length) and a temperature conductivity detector. Temperature set-up was as follows: injector: 70 °C, column oven: 100 °C, detector: 120 °C. The carrier gas was helium. Flow rate was at 30 mL/min CO_2 was detected with a retention time of 0.75 min. Area counts of the detected CO_2 peaks were determined using Shimadzu Class VP vs. 4.3. CO_2 concentrations were calculated in relation to the N_2/O_2 peaks detected during the same measurement.

2.2. Microbiological tests accompanying a production process using a model MWF

A model MWF emulsion was used in a grinding machine during a production process over the course of up to 25 weeks. During this time no new MWF concentrate was added. Volume losses due to evaporation and abrasive slurry were adjusted by adding tap water. In this way the concentration of the emulsion was kept in a range between 4% and 6%.

2.2.1. Model MWF

In order to achieve a rapid colonisation of the MWF emulsion, a model MWF without biocides was composed by a partner from MWF producing industry on the basis of standard products. The number of components of the model MWF was limited to create a simplified system, without compromising the basic functions of an MWF emulsion. The MWF concentrate used for the tests contained naphtenic mineral oil, monoethanol amine, boric acid, fatty acid amide, iso-nonanic acid, oleyl-/cetyl alcohol ethoxylate 5EO, oleyl-/cetyl alcohol ethoxylate 2EO, alkyl aryl sulfonate, triethanol amine, siloxane defoaming agent, and palmitic acid in water. Emulsions were prepared with water containing approximately 5% of the mixture described above. The pH value of the emulsion was 9.2.

2.2.2. Sampling of the model MWF

In weekly intervals a sample of approximately 200 mL of the model MWF emulsion was taken from the tank of the grinding machine after the MWF emulsion was thoroughly mixed to get a homogeneous sample. Sub-samples were used to determine chemical and physical properties of the emulsion such as pH value, droplet size of the emulsion, nitrate and nitrite concentration, and concentration of the emulsion.

2.2.3. Enrichment of microorganisms colonising MWF emulsions

Samples taken weekly were analysed microbiologically in two ways. First, commercially available dip slides were used as recommended for their standard application. This method is widely spread and routinely applied by users of MWF to monitor the microbiological state of the emulsion. Secondly, the samples were diluted (10, 100 or 1000 times depending on their microbial load) using a weak detergent solution (0.001% TWEEN 80, 0.9% NaCl in water). 50 μL of each dilution were plated on different agar media. For enrichment of bacteria CASO agar (DIFCO 0370-17-3), Pseudomonas agar F (DIFCO 0448-17-1), and BR agar (Bunt and Rovira, 1955, modified) were used. To detect fungi Czapek Dox agar (DIFCO 0339-17-3) and malt extract agar (Merck 1.05391.050) were used. The inoculated agar media were incubated in an incubator at 25 °C for 3–7 days. After this time colony forming units (CFU) were counted and microbiological contamination was calculated as CFU/mL.

The most abundant colony forms were isolated and stored for use in further applications.

2.3. Molecular biological analysis of the bacterial community

2.3.1. DNA extraction

Samples of MWF emulsion were centrifuged at 4 °C and 16,000 × *g* for 15 min. The supernatant fluid was discarded. The pellet was resuspended in buffer solution (10 mM Tris, 1 mM EDTA, pH 8.0). The suspension was centrifuged (16,000 × *g*, 15 min). The resulting pellet was resuspended with 500 μL of a lysis buffer solution (20 mM Tris, 2 mM EDTA, 1.2% Triton-X-100 v/v, 10 mg/mL lysozyme, pH 8.0) and incubated at 37 °C for 30 min.

After enzymatic lysis and addition of glass beads the mixture was treated in a mixer mill (Retsch MM301) adjusted to 30 Hz for 3 min. Subsequently 50 μL of a solution containing Proteinase K (600 U/mL)

were added. The preparation was incubated at 60 °C on a shaker (300 rpm) for 60 min 500 μL of a mixture of phenol, chloroform, and isoamyl alcohol (25:24:1) were added. The preparation was vigorously mixed and centrifuged at 4 °C and 14,000 × *g* for 15 min. The aqueous phase was transferred to a fresh container and the extraction was repeated. The resulting aqueous phase was mixed with an equal volume of chloroform/isoamyl alcohol (24:1). The mixture was centrifuged (14,000 × *g*, 15 min). The aqueous phase was isolated. 750 μL ethanol and 30 μL 3 M sodium acetate solution were added to precipitate the DNA. The mixture was incubated at −20 °C for 1 h, at least, before it was centrifuged (4 °C, 16,000 × *g*, 15 min). The supernatant was discarded. The pellet was washed with 80% ethanol and centrifuged again. After discarding the supernatant of this centrifugation step and drying the pellet it was dissolved in buffer solution (10 mM Tris, pH 7.5). DNA concentration was determined spectrophotometrically.

The procedure described above was also applied to isolate DNA from organisms grown in pure culture.

2.3.2. Polymerase chain reaction (PCR)

For this study the bacterial primers BA101f (TGGCGGACGGGT GAGTAA) and BA518r (CGTATTACCGCGGCTGCTGG) were used to amplify a part of the 16S rRNA genes (Zhu et al., 2003). Forward primer BA101f was used with a GC clamp attached to the 5′-end (CGCCCGCCGCGCCCCGCGCCCGTCCCGCCGCCCCCGCCCG) for DGGE (Muyzer et al., 1995). PCR was performed using an Eppendorf Mastercycler gradient. A 50 μl PCR assay contained 50–100 ng DNA, 500 nM of each primer, and 20 μL of Eppendorf MasterMix 2.5× (final concentration: 1.25 U Tag DNA polymerase, 50 mM KCl, 30 mM Tris HCl, 1.5 mM Mg^{2+}, 0.1% Igepal-CA630 and 200 μM of each dNTP).

After 4 min of initial denaturation at 94 °C, a touchdown PCR was performed (Don et al., 1991). Cycle-denaturation at 94 °C for 45 s, annealing 45 s, and primer elongation at 72 °C for 45 s. The starting annealing temperature of 57 °C was decreased by 0.5 °C per cycle to 50 °C. At this annealing temperature 16 additional cycles followed and then a final extension was carried out at 50 °C for 5 min.

2.3.3. DGGE analysis

The DGGE analysis was performed on the BioRad DCode (Universal Mutation Detection System) at a constant voltage of 180 V at 58 °C in 0.5× TAE buffer solution (20 mM Tris base, 10 mM acetic acid, 0.5 mM EDTA, pH 8.0) for 210 min 20 μL PCR product were loaded on a 6.5% polyacrylamide gel, followed by a 6.5% polyacrylamide gel with denaturing gradients ranging from 20 to 45%. The denaturant combination contains 7 M urea and 40% (v/v) deionised formamide representing 100% denaturant. After electrophoreses DNA bands were stained with ethidium bromide in a aqueous bath containing 0.5 mg/L ethidium bromide for 15 min. The stained DNA bands were documented by photography using a transilluminator (Biometra, Tl-1). The single bands were extracted and stored at 4 °C in 30 μL buffer solution (10 mM Tris base, 1 mM EDTA, pH 8.0), overnight. 3 μL of the resulting eluate were used as PCR template for reamplification. PCR was carried out under the same conditions as described above. The forward primer was used without a GC clamp. The purification of the PCR products was carried out using the Qiagen MinElute PCR Purification Kit. The reverse primer BA518r was used as sequencing primer. The sequencing was done by *AGOWA genomics*, Berlin, Germany. The sequence analysis of the PCR products and identification of the bacterial strains were done by BLAST search of the NCBI sequence database [http://blast.ncbi.nlm.nih.gov] followed by importing and alignment to local 16S rRNA database in the software package ARB (Ludwig et al., 2004).

3. Results

3.1. Aerobic degradation of MWF components

As can be seen from Fig. 1 carbon dioxide was released by aerobic degradation from different components or component groups at different rates or intensities. Mineral oil was not degraded aerobically by the bacteria used as inoculum, as well as a range of other substances of minor importance (data not shown in this work). High rates of microbial degradation could be shown for fatty acid amides as well as for fatty acids (data for the latter not shown). Fatty alcohol ethoxylates were also readily degraded by the bacteria used in the tests. Some microbial utilisation could be shown for fatty acid ethanol amide.

Degradation of monoethanol amine (MEA) by four strains of bacteria isolated from the MWF emulsion was studied in more detail because of the importance of MEA for pH stability of the emulsion. It can be seen from Fig. 2 that three of the four *Pseudomonas* strains were able to use MEA as the sole carbon source after differing times of adaptation. The most rapid utilisation of MEA occurred when the four organisms were used as a mixed inoculum.

3.2. Chemical changes of the model MWF in use

The pH value of the model MWF emulsion was initially at 9.2. During the course of the experiment the pH value gradually decreased to 7.3. At the start the emulsion had a concentration of 5.2%

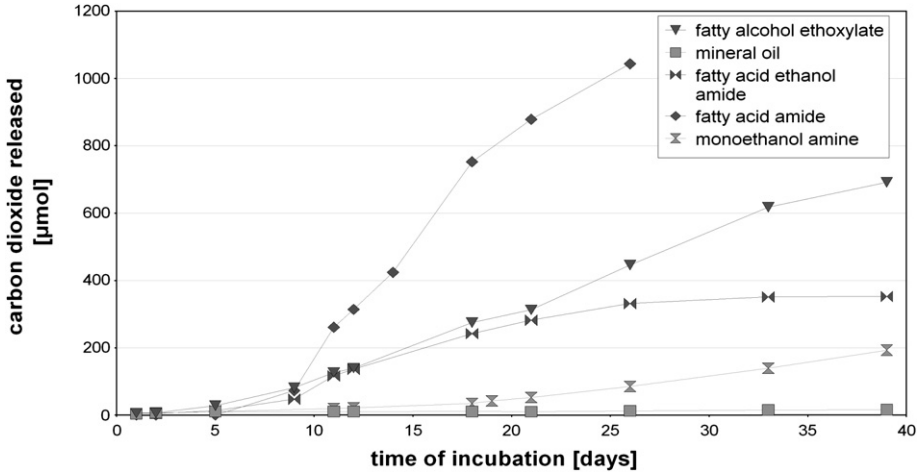

Fig. 1. Time course of evolution of CO_2 from different MWF components by a mixed culture of bacteria isolated from a contaminated MWF emulsion.

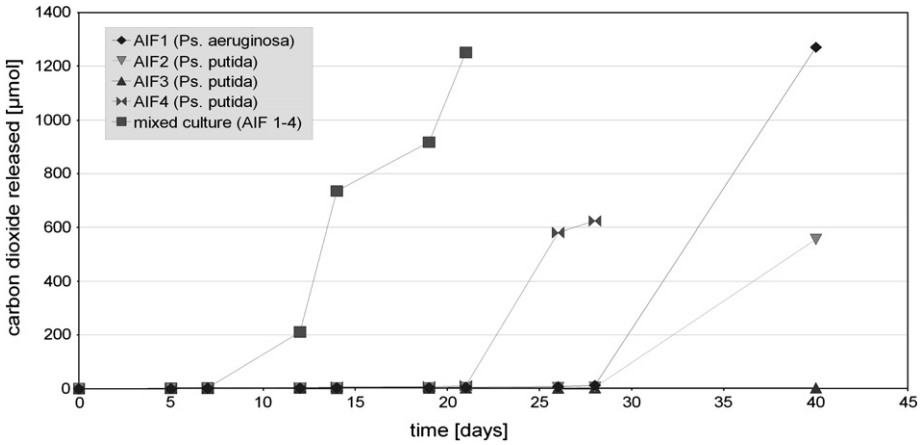

Fig. 2. Monoethanol amine utilisation by four strains of bacteria isolated from contaminated MWF emulsion.

as measured by refractometry. An interesting observation was made concerning nitrate and nitrite concentration. From the start nitrate concentration in the emulsion increased. After nine weeks maximum values of 16 mg/L and more were reached. After six weeks nitrite became detectable. Starting at week 13 nitrate and nitrite concentrations decreased to be no longer detectable until week 26 (Fig. 3).

During the experiment the particle size of the emulsion and the distribution of particle sizes were also monitored. As can be seen from Fig. 4 the median size of the emulsion particles remained stable for the first ten weeks of the experiment. After this time median particle size increased slightly. After week 18 median particle size of the emulsion increased rapidly. The median particle size distribution was shifted to larger particles (inset Fig. 4).

3.3. Microbial culture counts

After a short phase of a few days during which bacterial and fungal growth was below the limit of detection bacteria and fungi could be enriched from the MWF emulsion. Culture counts for bacteria increased to reach approximately 10^6 CFU/mL. In general bacterial culture counts stayed between 10^6 CFU/mL and 10^7 CFU/mL until the end of the experiment. Fungi started to occur in the MWF emulsion parallel to bacteria but reached only culture counts up to approximately 10^4 CFU/mL.

After week 2 and throughout the first 20 weeks fungi were below detection limits in two of the weekly samples, at week 10 and week 16. After week 19 fungi were not detectable anymore until the last week of the experiment (Fig. 5). Morphological studies and molecular biological analysis identified the fungi enriched from the MWF emulsion to belong to the species *Fusarium oxysporum*.

Concurrently, microbial colonisation of the MWF emulsion was determined using commercially available dip slides. It appeared that this method was not as reliable as the determination of cell counts using the plate count method. The dip slide method was less sensitive in some cases. Cell counts estimated using dip slides were frequently one to two orders of magnitude below the values calculated using the plate count method. Fig. 6 shows an example for this observation. The samples used to create these data were collected from the MWF emulsion of a machine tool.

3.4. Bacterial population

The bands 3, 4, and 6 could be isolated from the MWF emulsion from the tenth week of experiment to the end at 24 weeks. All three bands represented species of the genus *Pseudomonas* (Fig. 7, Table 1). Band 2 (representing another *Pseudomonas* species) occurred in week 14 of the experiment and was detected in all samples thereafter. Form weeks 14–18 the bacterial species composition within the MWF emulsion seemed to be stable. After week 20 new bands could be detected representing additional *Pseudomonas* species (bands 1 and 7) and bacteria of the genera *Comamonas* (bands 9 and 10) and *Achromobacter* (band 11).

The most abundant colony forms of bacteria enriched from the MWF emulsion on agar media were isolated and identified by PCR

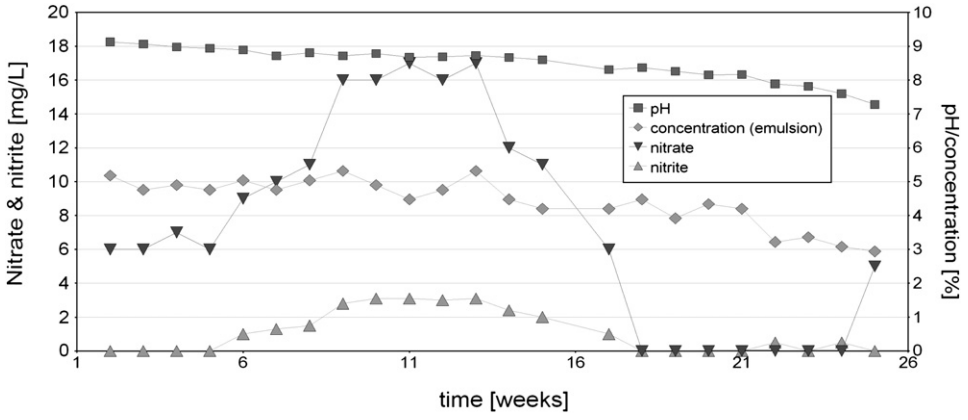

Fig. 3. Chemical changes of the model MWF during use and exposure to microbial contamination.

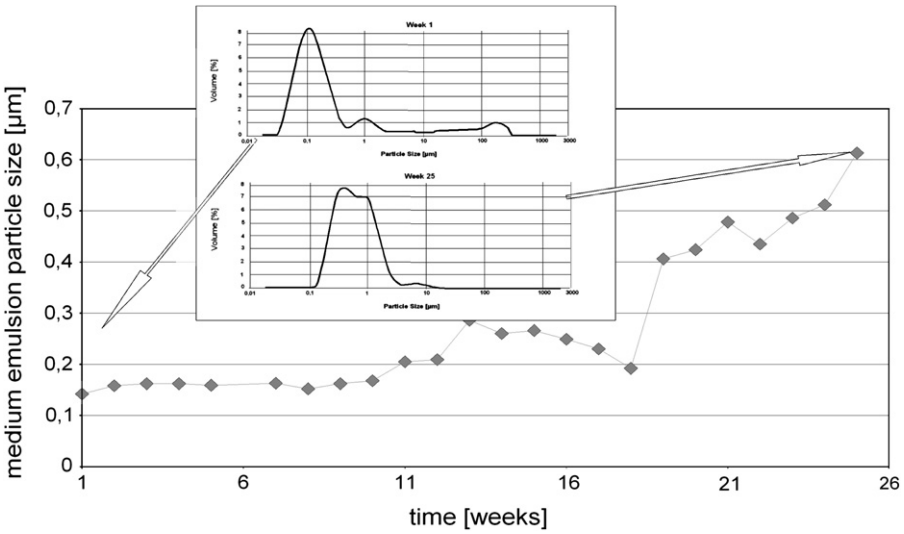

Fig. 4. Change of medium emulsion particle size during the time course of the experiment; the inset shows the particle size distribution at week 1, upper diagram, and week 26, lower diagram (data by courtesy of Dr. A. Fluri, Blaser AG).

and sequence analysis (2.3.3). They belonged to species of the genus *Pseudomonas*, namely *Pseudomonas aeruginosa* (1 strain) and *Pseudomonas putida* (3 strains).

4. Discussion

As can be concluded from the studies of microbial utilisation of single compounds MWF emulsions based on mineral oils are not degraded uniformly as a whole by microorganisms. In fact, some compounds are more susceptible to a rapid microbial degradation than others. The bacteria isolated from a contaminated mineral oil based MWF emulsion and used as the test inoculum degraded fatty alcohol ethoxylates, alkyl amides, and fatty acids most rapidly. Fatty alcohol ethoxylates are used as emulsifiers; alkyl amides and fatty acids are added as corrosion inhibitors and lubricating agent, respectively (Greim, 2006). The degradation of these functional compounds will therefore have an effect on the stability of the emulsion and influence the performance of the metal working fluid. Though the chemical composition of MWF became more sophisticated during the last 60 years the general principle of their composition remained as has the susceptibility to microbial colonisation.

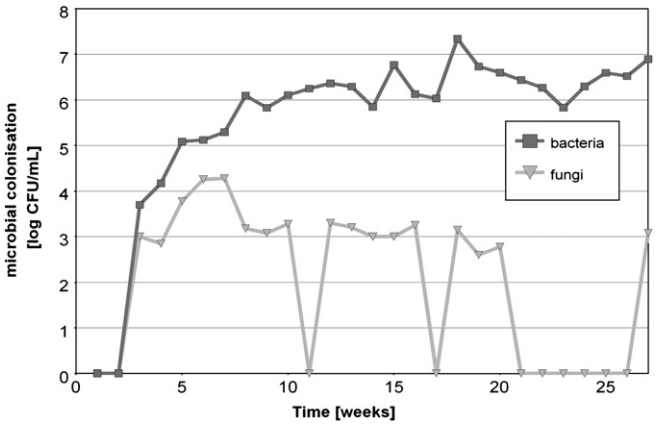

Fig. 5. Development of microbial contamination in the model MWF used in a grinding machine.

Lee and Chandler (1941) already described the microbial contamination of a mineral oil based MWF emulsion by bacteria. They assumed on the basis of their results that those organisms utilised mainly the naphtenic acids that served as an emulsifying agent in the system they studied.

The removal of an emulsifying agent by microbial degradation will alter the emulsion. In the present study it could be shown that the medium droplet size in the emulsion increased. This increase could be attributed to the formation of larger droplets in the time course of the experiment. A complete disruption of the emulsion may be counteracted by the microorganisms themselves. Bacteria of the genus *Pseudomonas* are known to form extracellular emulsifying agents especially in a hydrocarbon-rich environment (MacElwee et al., 1990). Indeed, most of the bacteria detected in the MWF emulsion during the present study belonged to this genus.

While the microbially induced reduction of emulsifying agents influences the emulsion properties directly and may be detected by observation of oil films floating on the emulsion surface the loss of functional compounds such as corrosion inhibitors may not be noted until the work pieces machined and stored by developing corrosion. Koch et al. (2004) describe a case in which after the occurrence of corrosion on work pieces corrosion inhibitor was added to the emulsion of the affected MWF system. The treatment led to a rapid increase in total cell counts in the system. As a consequence the emulsion had to be completely replaced.

Monoethanol amine (MEA) and triethanol amine function as neutralisation agents to stabilise the pH value in the MWF formulation. Whereas triethanol amine was not degraded as sole carbon source by the microorganisms isolated from contaminated MWF emulsion (data not shown), microbial MEA utilisation could be shown in the present study. Utilisation of MEA was increased when then organisms grew in mixed culture. Since the tap water, used to prepare the MWF emulsion, only contained up to 5 mg nitrate/L the nitrate detected during the experiment had to be released from another source. Hawthorne et al. (2005) studied biodegradation of MEA in contaminated industrial soils. The authors showed a rapid degradation of MEA in soils (see also Ndegwa et al., 2004). The substance was quantitatively conversed to ammonia which was then oxidised to nitrite and nitrate. It is very likely that similar reactions took place in the MWF emulsion. When nitrate and nitrite concentrations decreased below detection limit, the analysis of the

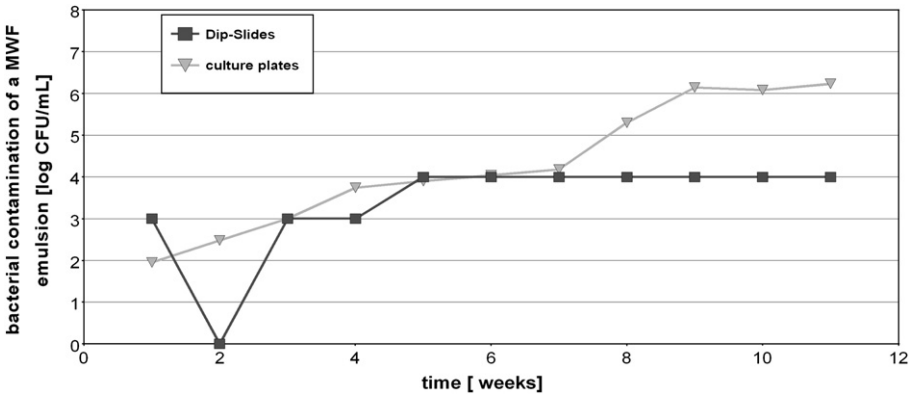

Fig. 6. Comparison of bacterial cell counts in the MWF emulsion of a machine tool during a period of 11 weeks.

bacterial community showed the appearance of bacteria closely related to *Comamonas testosteroni* and a *P. putida*. Some strains of the genus *Comamonas* are even able to reduce nitrate under aerobic conditions (Paturea et al., 1994). *P. putida* has also been shown to perform aerobic nitrate reduction (Carter et al., 1995).

Lee and Chandler (1941) described that the bacteria they isolated from contaminated MWF were not able to grow on alkanes as the sole carbon source. These findings correspond with the results of the present study in which the microorganisms growing in MWF emulsion were not able to utilise mineral oil as the sole carbon source. This is remarkable because mineral oil sometimes amounts to up to 70% of the MWF concentrate and although the property of microbial hydrocarbon degradation is widespread in the environment (Leahy and Colwell, 1990).

Before addressing the bacterial community in MWF the differences in the results for bacterial cell counts have to be discussed, which were gained by enrichment cultures and dip slides, respectively. Dip slides are a convenient and easy way for the assessment of the microbial contamination of MWF emulsions by personnel microbiologically not trained. Dip slides are evaluated by comparing the microbial growth on the surface of the nutrient strip with reference pictures which rather leads to a subjective estimation than a reliable quantification (Warfolomeow, 1998). Cell counts higher than 10^6 CFU/mL cannot be resolved satisfactorily (Schweisfurt and Weirich, 1989).

The microbial flora of the MWF emulsion studied in this work was restricted to a relatively small range of species and strains although the tank holding the emulsion for use in a grinding machine was open and certainly exposed to airborne spores and dust with attached organisms. Still, molecular biological studies revealed the existence of a core population of bacteria which was stable over the entire length of the experiment after they had established themselves. These findings indicate that mineral oil based MWF emulsions represent a special environment with properties favouring colonisation by a defined and restricted group of microorganisms.

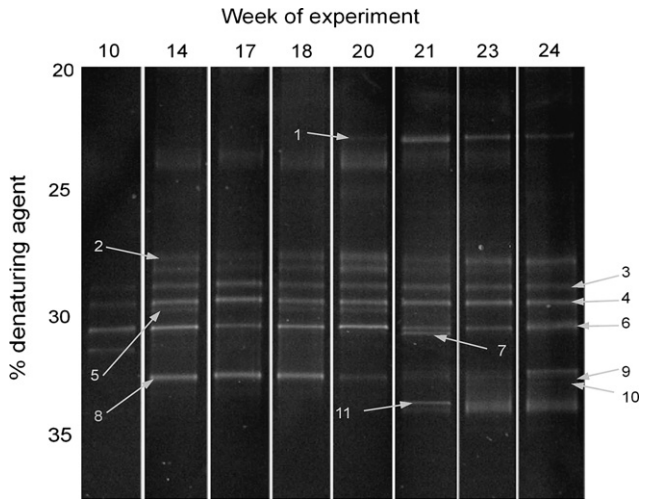

Fig. 7. DGGE analysis of the microbial population in the MWF emulsion used in a grinding machine over several weeks; identification of the individual DNA bands can be viewed in Table 1.

Table 1
Identification of bacteria detected in an MWF emulsion by DGGE analysis (Fig. 7).

Band No.	Phylogenic affiliation	Similarity [%]	Closest relative as determined by BLAST analysis	Accession No.
1	Gammaproteobacteria; Pseudomonadales; Pseudomonadaceae	98	*Pseudomonas* sp. MOLA121	AM990895
2	Gammaproteobacteria; Pseudomonadales; Pseudomonadaceae	99	*Pseudomonas fluorescens*	FJ147200
3	Gammaproteobacteria; Pseudomonadales; Pseudomonadaceae	99	*Pseudomonas mendocina*	AB461491
4	Gammaproteobacteria; Pseudomonadales; Pseudomonadaceae	99	*Pseudomonas pseudoalcaligenes* strain KS-1	EU815635
5	Gammaproteobacteria; Pseudomonadales; Pseudomonadaceae	99	*Pseudomonas pseudoalcaligenes* strain KS-1	EU815635
6	Gammaproteobacteria; Pseudomonadales; Pseudomonadaceae	99	*Pseudomonas pseudoalcaligenes* strain KS-1	EU815635
7	Gammaproteobacteria; Pseudomonadales; Pseudomonadaceae	99	*Pseudomonas* sp. YRR07	EU373434
8	Alphaproteobacteria; Caulobacterales; Caulobacteraceae	99	*Brevundimonas diminuta* strain ATCC 19146	EU497053
9	Betaproteobacteria; Burkholderiales; Comamonadaceae	99	*Comamonas testosteroni* isolate Cs1–5	AY367038
10	Betaproteobacteria; Burkholderiales; Comamonadaceae	99	*Comamonas testosteroni* strain H18	EU887829
11	Betaproteobacteria; Burkholderiales; Alcaligenaceae	99	*Achromobacter xylosoxidans* strain ybc-11	EU652103

Acknowledgement

This work was funded by IGF (Industrielle Gemeinschaftsforschung) and financed by the German Federal Ministry of Economics and Technology. We like to thank Dr. Hans Jürgen Schlindwein of RhenusLub GmbH, Mönchengladbach, Germany for kindly designing and providing the MWF used for the experiments carried out in this work, and Dr. Alexandra Fluri of Baser Swisslube AG, Hasle-Rüegsau, Switzerland for the analyses of the emulsion particle size. Likewise the authors thank the members of the committee accompanying the project for their advisory and technical assistance.

References

Bunt, J.S., Rovira, A.D., 1955. The effect of temperature and heat treatment on soil metabolism. Journal of Soil Science 6, 129–136.

Carter, J.P., Richardson, D.J., Spiro, S., 1995. Isolation and characterisation of a strain of *Pseudomonas putida* that can express a periplasmic nitrate reductase. Archives of Microbiology 163 (3), 159–166.

Don, R., Cox, P., Wainwright, B., Baker, K., Mattick, J., 1991. 'Touchdown' PCR to circumvent spurious priming during gene amplification. Nucleic Acids Research 19 (14), 4008.

Garbrecht, M., Heinzel, C., Eckebrecht, J., Koch, T., Brinksmeier, E., 2008. Relevance of cutting fluids in manufacturing processes. In: Lubrication Management and Technology. Fundacion Tekniker, Eibar Full Scientific Proceedings Lubmat Conference, 04.06–06.06.2008, San Sebastian.

Greim, H., 2006. Gesundheitsschädliche Arbeitsstoffe, Toxikologisch-arbeitsmedizinische Begründungen von MAK-Werten. 41. Lfg. Deutsche Forschungsgemeinschaft, WILEY-VCH, Weinheim.

Häusser, M., Dicke, F., Ippen, H., 1985. Kühlschmiermittel-Bestandteile und ihre gesundheitliche Wirkung. Zentralblatt für Arbeitsmedizin 35 (6), 176–181.

Hawthorne, S.B., Kubatova, A., Gallagher, J.R., Sorensen, J.A., Miller, D.J., 2005. Persistence and biodegradation of monoethanolamine and 2-propanolamine at an abandoned industrial site. Environmental Science & Technology 39 (10), 3639–3645.

Kenndall, 1920. Cause of skin sores and boils among metal workers. Houghton Research Staff 16.

Koch, T., De Paula Dias, A., Rabenstein, A., Walter, A., 2004. Mikrobiologie der Kühlschmierstoffe – Einfluss auf die Leistung und Standzeit. In: Bartz, W.J. (Ed.), Tribology and Lubrication Engineering, 14th International Colloquium Tribology. Technische Akademie Esslingen, Ostfildern.

Leahy, J.G., Colwell, R.R., 1990. Microbial degradation of hydrocarbons in the environment. Microbiology and Molecular Biology Reviews 54 (3), 305–315.

Lee, M., Chandler, A.S., 1941. A study of the nature, growth and control of bacteria in cutting compounds. Journal of Bacteriology 41 (3), 373–386.

Ludwig, W., Strunk, O., Westram, R., Richter, L., Meier, H., YadhukumarBuchner, A., Lai, T., Steppi, S., Jobb, G., Förster, W., Brettske, I., Gerber, S., Ginhart, A.W., Gross, O., Grumann, S., Hermann, S., Jost, R., König, A., Liss, T., Lüssmann, R., May, M., Nonhoff, B., Reichel, B., Strehlow, R., Stamatakis, A., Stuckmann, N., Vilbig, A., Lenke, M., Ludwig, T., Bode, A., Schleifer, K.H., 2004. ARB: a software environment for sequence data. Nucleic Acids Research 32, 1363–1371.

McConnell, W.J., 1922. Dermatosis following the use of cutting oils and lubricating compounds. U.S. Public Health Reports 37, 1773–1794.

MacElwee, C.G., Lee, H., Trevors, J.T., 1990. Production of extracellular emulsifying agent by *Pseudomonas aeruginosa* UG 1. Journal of Industrial Microbiology 5, 25–32.

Muyzer, G., Teske, A., Wirsen, C.O., Jannasch, H.W., 1995. Phylogenetic relationships of *Thiomicrospira* species and their identification in deep-sea hydrothermal vent samples by denaturing gradient gel electrophoresis of 16S rDNA fragments. Archives of Microbiology 164, 165–172.

Ndegwa, A.W., Wong, R.C.K., Chu, A., Bentley, L.R., Lunn, S.R.D., 2004. Degradation of monoethanolamine in soil. Journal of Environmental Engineering and Science 3 (2), 137–145.

Paturea, D., Davison, J., Bernet, N., Moletta, R., 1994. Denitrification under various aeration conditions in *Comamonas* sp., strain SGLY2. FEMS Microbiology Ecology 14 (1), 71–78.

Rakić, R., Rakić, Z., 2002. The influence of metal working fluids on machine toll failures. Wear 252, 438–444.

Schweisfurt, R., Weirich, G., 1989. Mikrobiologie der Kühlschmierstoffe. Tribologie 36 (4), 172–176.

Warfolomeow, I., 1998. Keimbelastung wassergemischter Kühlschmierstoffe. Die BG 110 (5), 274–281.

Zhu, X.Y., Lubeck, J., Kilbane, J.J., 2003. Characterization of microbial communities in gas industry pipelines. Applied and Environmental Microbiology 69 (9), 5354–5363.