NANOPARTICLES AS ANTIBACTERIAL FILLERS IN MACHINING PROCESS FLUIDS

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Abstract

Process fluids are important as they help create appropriate conditions for machine cutting by improving heat transfer, thereby extending the life of the cutting tool and increasing surface quality of the product. It is essential, however, that one understands the process fluid characteristics (cooling, lubricating and/or cleaning effect, health issues, stability, pureness) and its appropriateness (environmental safety, operating life) prior to use. Increasingly, nanoparticles are being added to process fluids to increase their antibacterial properties, particularly as regards bacterial contamination. In this paper, the antibacterial properties of process fluid nanoparticles are evaluated, along with their availability and characteristics. While antibacterial effects on test bacteria were often difficult to determine due to process fluid and nanoparticle composition, results indicated that nanoparticles reduced bacterial respiratory rates by around 90%. The addition of nanoparticles had no discernable effect on the tribological characteristics of the process fluids.

Keywords:

Nanoparticles; antibacterial effects; process fluids; machining.

1. INTRODUCTION

Process fluids are important in that they create appropriate conditions for effective machine cutting by improving heat transfer away from the cutting surface, thereby extending the life of the cutting tool and improving product surface quality. Nowadays, manufacturers of process fluids use formulations that are highly resistant to bacterial formation. Improved resistance can be achieved both by balancing the ratio of mineral oil, emulsifying agents, amines, esters, corrosion inhibitors, etc., and by adding bactericides. The main 'foods' for bacteria include emulsifiers, sulfonates, fatty alcohols, corrosion inhibitors and other organic components such as dust and sawdust. An appreciable amount of these organic materials is ultimately converted into carbon dioxide (CO_2) through biodegradation, a process that has already been applied to the disposal of used cutting fluids [1]. Biocidal ingredients, therefore, are important components of process fluids as such additives protect the fluids against biological attack by bacteria or fungi, thereby protecting the user against spores and endotoxins. Such biocidal ingredients, however, are frequently classified as significantly hazardous as they can produce highly unpleasant odors and cause allergic reactions and/or induce respiratory and skin problems in people exposed to the fluid. In addition, these products can have a huge ecotoxicological impact on the environment.

Recently, addition of metal oxides (e.g. Titanium dioxide [TiO_2], Silicon dioxide [SiO_2] and diamond [C]) has been seen as a promising alternative to conventional antimicrobial techniques, and especially when used in the form of nano-sized particles. The photocatalytic oxidation and reduction reactions of such metal oxides have been extensively studied, with TiO_2 now the most commonly used metal oxide due to its chemical stability, high photocatalytic efficiency, lack of toxic side effects and low cost [2, 3].

2. MATERIALS AND METHODS

Composition of machine process fluids – Measurement of process fluid chemical composition was undertaken at the Mass Spectrometry laboratory at the Institute of Chemical Technology Central

Laboratories. Qualitative analysis of fluids was performed using static headspace gas chromatography-mass spectrometry at elevated temperatures.

Three samples were analyzed: a process fluid composed mainly of oxygenated organic compounds comprising glycols and higher alcohols (PFM-2; four substances determined; pH 9.58) and two samples composed of various amines and one oxygen molecule (PFM-1 and PFM-3; around 20 substances determined; pH 9.04 and pH 8.95, respectively).

Composition and purity of nanoparticles used as antibacterial fillers – Pure TiO_2 was supplied by VWR Chemicals, USA (EC number 215-280-1); SiO_2 by Lach-Ner s.r.o, Czech Republic (CAS 7631-86-9/1). Diamond powder was supplied by ROZOL, s.r.o., Czech Republic (grain size 0.25/0 μm).

The particles supplied were crushed using a "nano-grinder" CryoMill (Retsch, Germany). The particles were placed in a 50 ml chrome-steel bottle and were ground (pregrinding with a single 20 mm chrome-steel ball; grinding with three 10 mm chrome-steel balls; grinding with two 8 mm chrome-steel balls; grinding with one 10 mm chrome-steel ball) at a frequency of 30 Hz. After crushing, the size of particles was determined using scanning electron microscopy (Carl Zeiss Ultra Plus, Germany) of samples deposited as a thin layer in gold (Fig. 1). The diamond nanoparticles (nanoDP) were of 30 to 300 nm, nanoTiO_2 from 40 to 500 nm and nanoSiO_2 from 30 to 500 nm. Over time, larger units of about 50 μm were formed due to aggregation and sedimentation.

These nanoparticles were then measured with no further modification using a Nicolet DXR Raman microscope. Both nanoDP and nanoTiO_2 showed no discernable difference from the declared composition (small impurities in nanoDP were caused by crystal lattice defects). NanoSiO_2 contained impurities of opal (modification of SiO_2, probably contamination) and anatase (modification of TiO_2). The maximum total organic carbon concentration in the nanopowders was 20 mg/l.

Microorganisms – Escherichia coli microorganisms were isolated from a pure culture supplied by the Czech Collection of Microorganisms at the Faculty of Science of Masaryk University, Czech Republic.

Evaluation of microorganism respiratory rate – Substrate utilization by microorganisms was estimated from their respiratory rate. CO_2 release and O_2 consumption were measured in hermetically sealed 50 ml flasks containing 20 ml of media (Micro-Oxymax; Columbus Instruments, USA) cultivated at 22 ± 2 °C under aerobic conditions and shaken continuously for five days.

Assessment of microorganism condition – Each sample was evaluated using a ZEISS Axio Imager.M2 fluorescence microscope fitted with an AxioCamICc1 camera and a Colibri.2 fluorescent lamp. The LIVE/DEAD BacLightTM kit for monitoring and evaluating viability of bacterial populations was used to evaluate cell membrane integrity.

Determination of bacterial cultivation – Bacterial cultivations were determined according to standard methodology EN ISO 6222 (757821) of the Czech Office for Standards, Metrology and Testing. Samples were cultivated at 37 °C for 72 hours and the results presented as number of colony forming units (CFU/ml).

Measurement of pH and specific electrical conductivity – pH and electrical conductivity were measured using a SenTix 41-3 pH electrode and a TetraCon 325 conductivity probe connected to a WTW Multi 350i measuring device (Xylem, USA).

Determination of chemical oxygen demand (COD) – The sum of all organic substances in the sample was determined through the dichromate method using single-purpose cuvette tests (Hach-Lange, Germany).

Genotoxicity test – In this test we measure cytostasis, or the inhibition of cell growth and multiplication. A 5% (v/v in H_2O) solution of each process fluid was diluted in complete culture medium in order to obtain final concentrations of 0.01%, 0.005% and 0.0005%. A non-treated cell control, a positive control (H_2O_2-treated cells) and a solvent control (ddH_2O-treated cells) were also included in all experimental sets. The genotoxicity tests were conducted at the Lodz Regional Science and Technology Park Ltd., Poland.

Experiment design – Each batch-test consisted of 18 ml of process fluid (or control sample) + 2 ml saline solution with *E. Coli* (initial absorbance 0.93 at 600 nm; measured using a DR 6000 UV-VIS spectrophotometer; Hach-Lange, Germany) + 1 g/l of nanoparticle. The temperature was maintained within a range of 22 ± 2 °C.

For each control sample (control measurement), a basal salts medium comprising 0.043 g KH_2PO_4, 0.033 g K_2HPO_4, 0.178 g $(NH_4)_2SO_4$, 0.085 g $MgCl_2.H_2O$ and 0.25 ml of trace element solution (1 g/l $MnCl_2.4H_2O$, 0.196 g/l $CaCl_2$, 0.6 g/l $FeSO_4.7H_2O$, 2 g/l $Na_2MoO_4.2H_2O$) dissolved in 250 ml distilled water (with glucose [$C_6H_{12}O_6$] as the sole carbon-source) was used instead of the process fluid.

3. RESULTS

3.1. Evaluation of microorganism respiratory rate

Higher respiratory rates for an observed fluid (no nanoparticles; pH 9.0) were observed for PFM-3 and PFM-1 (note, however, that respiration rates were mostly at detection limits). Sample pH was seen to affect growth in *E. coli* bacterial populations, with process fluids adjusted to pH7 having a higher respiration rate and higher proportion of living microorganisms.

Over the 90 hours of the experiment, the fluid without nanoparticles consumed approximately 0.05 mg O_2, with an average rate of oxygen consumption of around 0.00056 mg O_2/h (about 99.8% lower than the control measurements).

Respiratory rates (Table 1) were higher than expected due to impurities present in the nanopowders (either present in the mixture as purchased or included during the milling process). As a result, bacterial respiration was supported by these nanoparticles, rather than inhibited. Only in one sample (PFM-3 + TiO_2) was antibacterial activity clearly demonstrated (respiration rate zero).

Table 1 Comparison of total O_2 consumption (%)in process fluids after five days (with respect to a control measurement).

Samples	No nanoparticles	PFM + nanoDP	PFM + nanoSiO_2	PFM + nanoTiO_2
PFM-1	100	1400	1120	1300
PFM-2	100	0950	1250	6350
PFM-3	100	1370	2353	0006

3.2. Microscopic assessment of microorganism condition

Cell fluorescence microscopy (Table 2) indicated that bacteria were able to adapt to the process fluid environment (without nanoparticles), the only exception being process fluid PFM-1 where the number of living cells was lower after the first week of measurement. Bacterial viability was supported by the nanoparticles; with exception of PFM-3 (viability was close to zero).

Table 2 Comparison of the proportion of living cells (%) in process fluids after five days (with respect to a control measurement).

Samples	No nanoparticles		PFM + nanoDP		PFM + nanoSiO_2		PFM + nanoTiO_2	
	day 1	day 5	day 1	day 5	day 1	day 5	day 1	day 5
PFM-1	100	088	100	272	100	180	100	242
PFM-2	100	150	100	127	100	320	100	900
PFM-3	100	184	100	239	100	639	100	006

3.3. Determination of bacterial cultivation, pH, specific electrical conductivity and COD

CFU evaluation (Table 3) indicated that very few viable bacteria were able to survive in the process fluid over the first week of the experiment. The best process fluids in terms of antibacterial effect were PFM-1 and PFM-3, with a few colonies appearing in sample PFM-2 even after five days. Overall, with the exception of sample PFM-1 + nanoTiO_2, CFU evaluation indicated good overall antibacterial effect.

pH decreased slightly (values falling from around pH 9 to around 8.48; Table 4) throughout the experiment, though there was no obvious impact on the samples themselves.

Specific electrical conductivity oscillated only slightly (within measuring error) with values of around 3 mS/cm.

Similarly, COD oscillated just slightly (within measuring error) with mean values of around 80 000 mg/l. Overall, there was no significant decrease in COD over the experiment, suggesting that bacteria did not degrade the process fluid to any observable degree.

Table 3 Comparison of CFU (%) in process fluids after five days of the experiment (with respect to a control measurement).

Samples	No nanoparticles		PFM + nanoDP		PFM + nanoSiO_2		PFM + nanoTiO_2	
	day 1	day 5	day 1	day 5	day 1	day 5	day 1	day 5
PFM-1	100	002	100	002	100	001	100	029
PFM-2	100	050	100	001	100	002	100	006
PFM-3	100	001	100	004	100	003	100	002

Table 4 Comparison of process fluids pH after five day of the experiment.

Samples	No nanoparticles		PFM + nanoDP		PFM + nanoSiO_2		PFM + nanoTiO_2	
	day 1	day 5	day 1	day 5	day 1	day 5	day 1	day 5
PFM-1	9.04	8.95	9.09	8.95	9.06	8.94	9.05	8.96
PFM-2	9.58	9.03	9.65	9.32	9.49	9.38	9.54	9.40
PFM-3	8.95	8.69	9.00	8.69	8.85	8.73	8.88	8.74

3.4. Genotoxicity Test

Cytostasis in micronucleus tests should not exceed 50 to 60 %. Our results indicate that the process fluids significantly inhibited cell proliferation. All values exceeded cytostasis (Table 5).

In sample PFM-3 (0.0005% clean solution), cells displayed appropriate morphology and were clearly viable as green calcein was retained within the cytoplasm. In sample PFM-3 (0.01% clean solution), some cells were lost or showed a weaker green fluorescent signal around the nuclei. In PFM-3 (0.025% clean solution), most cells were lost and green fluorescent calcein was observed only around the nuclei. The nuclei were also smaller due to chromatin condensation in cells undergoing apoptosis. Only PFM-3 at the highest dilution level did not exceed the 60% upper limit for cytostasis in micronucleus tests (Table 5).

Table 5 Comparison of cytostasis (%) in process fluids.

Samples	Dilution (C-0.01)	Dilution (C-0.005)	Dilution (C-0.0005)
PFM-1	82.69	101.2	91.03
PFM-2	99.07	96.97	93.93
PFM-3	90.06	53.19	6.142

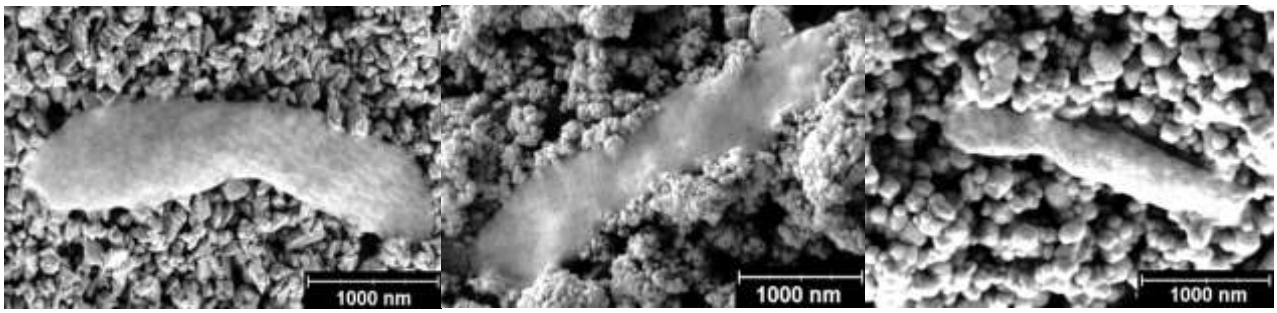

Fig. 1 Scanning electron microscope images of *E. Coli* bacteria in solutions containing nanoparticles (left to right: nano-DP, nano-SiO_2 and nano-TiO_2).

A range of mechanisms have been proposed to explain photocatalytic disinfection [4, 5]; the precise mechanism, however, remains unclear. While reactive oxygen species attacking the cell's surface has been proposed as the initial step in microbial inactivation [6], cell inactivation has been related to inhibition of cell respiration, decomposition of the lipopolysaccharide layer, decomposition of the outer membrane and structural disarrangement of the cytoplasmic membrane due to lipid peroxidation [6]. According to [7, 8, and 9], photocatalytic particles suppress cell growth and cell division, resulting in a 2x reduction in cell wall thickness. Our results indicate that bacteria degrade machine process oils very moderately (small change of COD values) and, aside from specific formulations of nanoTiO_2, presence of nanoparticles (SiO_2 and nanodiamond) had no significant effect on the degradation process, neither antibacterial effect.

4. CONCLUSION

Both microscopic and respirometry assessments indicate that bacteria are able to adapt to a range of process fluid environments. Nanoparticle antibacterial activity was only demonstrated in samples containing TiO_2; impurities in other nanopowders formulations appearing to support bacterial activity rather than suppress it. All process fluids showed significant inhibition of cell proliferation, with just one sample (composed of various amines and one oxygen molecule) applied at the highest dilution not exceeding the upper limit of the recommended range for cytostasis in micronucleus tests.

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