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SHORT COMMUNICATION
Rapid bacterial counts in metal working fluids

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A rapid (10 s) automated fluorescent method to estimate viable bacteria in metal working fluids (MWF) was compared with dip-slide cultures. The BactiFluor method compared favorably with 107 MWF (r=0.99) and with 30 other metal processing fluids.
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Introduction

Bacterial growth and degradation of metal working fluids (MWF) is a well-known problem in metal working industries [3]. Bacterial enzymes can cause changes in viscosity thereby altering lubricity and cooling efficiency. Acids produced as a result of excessive or uncontrolled bacterial growth can significantly lower pH, causing corrosion. Finally, other bacterial by-products (e.g. endotoxins) can become aerosolized and present potential inhalation-related health effects [1,5–8].

To control bacterial contamination and subsequent adverse effects, routine bacterial counts are performed on MWF, and when appropriate, numbers are controlled through the addition of biocide. The most widely used method to determine bacterial concentration is culture on solid medium. This requires at least 48 h or longer to obtain meaningful results. Since the microbial disposition of the MWF often changes significantly by the time culture results are available [4], fouling may have already occurred. On the other hand, adding biocide empirically could result in high maintenance costs. For these reasons, it would seem beneficial to have a bacterial detection test that is more rapid than cultural methods and at least as accurate, and cost-effective.

Use of microbial vital stains has increased over the last several years and would appear to have utility in detecting bacteria [2]. However, preparing samples via filtration, staining, and microscopic counting is tedious. Therefore, it was the purpose of this investigation to determine whether fluorescent vital stain(s) could be used in combination with a fluorescence polarization instrument (PolarScan, Associates of Cape Cod, Inc., Falmouth, MA) to rapidly quantify viable bacteria in MWF.

Materials and methods

Fluorescent stain was obtain from Molecular Probes (Eugene, OR). A mixture of the SYTO 9 green fluorescent nucleic acid stain and the red fluorescent nucleic acid stain, propidium iodide, was used initially according to the manufacturer's suggestions for microscopy. It was decided that only viable bacteria were of interest and therefore, the use of SYTO 9 stain and propidium iodide stain was altered for optimal detection of viable bacteria. It should be noted that MWF that use green dyes, for example, fluorescein, as concentration indicators, are usually not readable with the BactiFluor system, since noise significantly exceeds the signal generated by the presence of viable bacteria. Also, MWF cloudiness is generally not problematic since only 10 μl of sample is used. To compare fluorescence intensity with bacterial growth, dip slides (MCE, Inc., Lake Placid, NY) of the type routinely used by the metal working industry were used. Standard curves for enumerating viable bacteria were developed initially using pure cultures and laboratory-prepared contaminated MWF. Field samples of MWF were transferred to a central laboratory facility where they were cultured using a dip slide and PolarScan tested within 15 min. This was done by adding 10 μl of MWF into 1 ml of 10 mM acetate buffer, pH 6.0, in a 10$\times$75 mm borosilicate glass tube which was mixed by vortexing it for a few seconds. This sample was used as a background blank after which 1 μl of the altered stain reagent (BactiFluor) was added to the tube and again mixed. The tube was reinserted into the PolarScan chamber and measured. After approximately 10 s, the estimated number of viable bacteria appeared on the PolarScan screen and results were saved to a text file for later retrieval and analysis. Dip slides were incubated at ambient temperature for 72 h and then graded according to manufacturer's instructions as $<10^3$, 10^3, 10^4, 10^5, 10^6, 10^7, or $>10^7$.

Results and discussion

To determine the relationship of fluorescence intensity to numbers of bacterial cells, a suspension of *Escherichia coli* was grown in nutrient broth and a standard plate count was performed. Serial 10-fold dilutions of the *E. coli* suspension were made and a 10-μl aliquot was placed into 1 ml of acetate buffer to obtain a background signal. For bacterial enumeration, 1 μl of BactiFluor was added and the fluorescence intensity was measured for each serial dilution. After plate counts were obtained (48 h), fluorescence intensity was plotted against the number of bacteria and a standard curve was generated (Figure 1).

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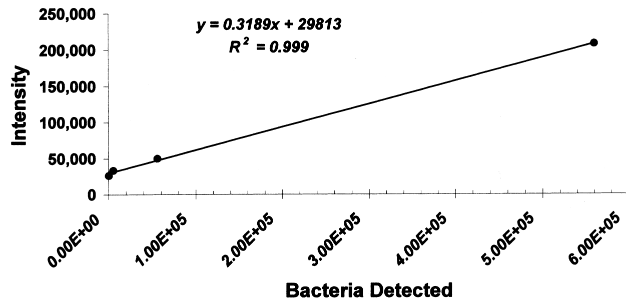

Figure 1 Relationship of fluorescence intensity to *E. coli* concentration.

It was apparent both from the reagent manufacturer's insert (BacLight, Molecular Probes) and from our experimentation that reagents had to be fine-tuned to accommodate a variety of environmental conditions. Since MWF represent a unique microbial environment, a MWF-unique equation was generated from the results of the fluorescence intensity and the dip slide counts. The results are shown in Figure 2. One hundred and seven MWF were measured. Of the 101 with fewer than 10,000 bacteria per milliliter by dip slide culture, 94 showed no growth, 5 had 10^3 bacteria/ml, and 2 had 10^4 bacteria/ml. Two additional MWF had 10^6 bacteria/ml and four had 10^7 bacteria/ml. Growth of 10^4 bacteria or less exhibited similar intensities; they were consolidated into a single group for constructing a standard curve for the MWF (r=0.9902). This was done because previous experiments revealed a sensitivity of approximately 10^3 bacteria. Consequently, MWF with less than 10^5 bacteria per milliliter may appear negative since 10 μl is used (10^5 per milliliter=10^3 per 10 μl). MWF samples with 10^4 bacteria or less per milliliter showed fluorescence variation, which may be explained by the fact that the concentration of biocide (if any) and the time of its addition were unknown. For example, it could take approximately 24 h of MWF circulation *in situ* for a sample to become BactiFluor negative. However, a sample taken within a few hours after the addition of biocide will usually not grow bacteria because the biocide is in contact with the sample during the growth phase on the dip slide. Consequently, it is recommended that BactiFluor be used before the addition of the biocide or with the knowledge of biocide addition. Indeed, BactiFluor was designed to provide real-time results to avoid needless use of biocide. Therefore, a negative BactiFluor result would suggest that biocide addition is unnecessary.

Use of BactiFluor was evaluated in other types of metal processing fluids; the results are summarized in Table 1. There was excellent agreement between the dip slides and the BactiFluor system for the three different types of fluids tested. One of the cleaning fluids that had 10^6 bacteria per milliliter was negative by BactiFluor. Because the chemical composition of these fluids was unknown, it is possible that some non-specific quenching occurred. While this is one explanation, it would seem unlikely that this was not observed for any of the other cleaning solutions. A more likely explanation may be the sampling method. Since only 10 μl is used for the BactiFluor test, it is possible that the MWF bottle was not sufficiently shaken or that some particulate matter interfered with obtaining a representative sample. Such a sampling error is almost non-existent when using a dip-slide since at least 1 ml is used. Nevertheless, the BactiFluor instant bacterial estimation system exhibited remarkable correlation with the dip-slide method with several categories of metal processing fluids.

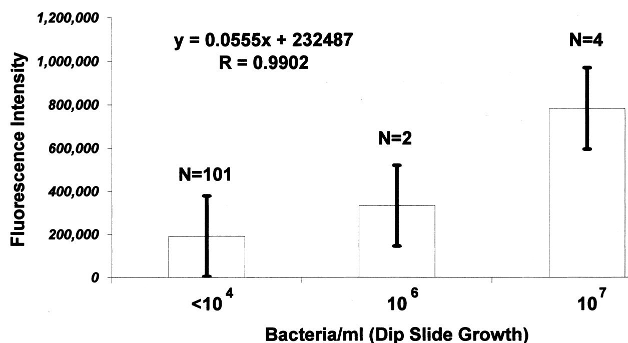

Figure 2 Relationship of fluorescence intensity to bacterial concentration in MWF.

Table 1 Bacterial dip slide counts versus BactiFluor fluorescent counts

Type of fluid	Dip slide growth (number tested)	Mean bacteria per milliliter (BactiFluor)
Aluminum rolling	$<10^4$ (1)	None detected
	10^6 (3)	5.23×10^6
	10^7 (3)	8.63×10^6
	$>10^7$ (1)	1.72×10^7
Cleaning fluids	No growth (11)	None detected
	10^4 (1)	4.23×10^6
	10^6 (1)	None detected
Rust preventives	No growth (7)	None detected
	$>10^6$ (2)	1.96×10^6

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