

The effect of bactericide treatment on planktonic bacterial communities in water cooling systems⁺

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Abstract

Bactericides were applied to experimental open recirculating cooling-water systems at concentrations found to be effective under laboratory pure-culture conditions. Total aerobic plate counts and bacterial population structures were determined over a period of 48 h. In all cases the total aerobic count increased one day after bactericide addition, and decreased rapidly after ca. 36 to 40 h. Population shifts occurred during the course of all four treatments. In all cases different species became dominant concurring with fluctuations in the planktonic plate count, indicating stress reaction of the biofilm. The species diversity decreased after treatment with dichlorophen, thiocarbamate and methylenebis-thiocyanate, and increased upon treatment with humic acid. Species susceptible to bactericides in pure culture were found to be the dominant planktonic survivors. An example is *Pseudomonas stutzeri* which was the dominant survivor after treatment with thiocarbamate and with dichlorophen.

Introduction

Bacteria in aqueous surroundings attach preferably to surfaces (Hoppe, 1984), where they produce copious amounts of adhesive exopolymers (Characklis and Cooksey, 1983). This adhesion and subsequent metabolism leads to formation of biofilms (McCoy et al., 1981). Such bacterial biofilms promote fouling and corrosion of metal and other surfaces (Costerton, 1981; Ford and Mitchell, 1990; Hamilton, 1985; Iverson, 1987). Many industrial water systems are, therefore, treated with bactericides to reduce the bacterial load and degree of corrosion. Because attached bacteria are more resistant to bactericides than are free-floating (planktonic) ones (Heinzel, 1988; LeChevalier et al., 1988), the design of effective treatment programmes poses certain difficulties.

Attached bacteria cannot effectively be dispersed without killing most (Costerton, 1981; Costerton et al., 1986). Therefore the enumeration of bacteria in biofilms does not yield representative results. As a result, quantitative evaluation of bactericides has been performed mostly on planktonic samples (Eigener, 1988). As cells in biofilms and the planktonic communities are in continuous exchange (Costerton et al., 1986), the planktonic community does reflect on the sessile one. Death of cells in the planktonic phase would influence the equilibrium and shifts would occur in both the planktonic and the sessile populations. Investigation of the planktonic phase would, therefore, yield insights into the sessile one, and into the system as a whole.

Bactericides attack targets of cell function (Wainwright, 1988), placing the bacterium under stress. It is well recognised that communities under stress have a lower species diversity and select for fitter species (Atlas, 1984). As bactericides are selective in their action (Heinzel, 1988), application of any one could result in selection for resistant bacteria. Where a

bactericide is the stress factor, fitter species would be those resistant to or more tolerant of the specific bactericide. As diversity is inversely proportional to productivity, it would influence the degree of corrosivity of the biofilm. Although information on the selective activity (bactericide fingerprints) of a range of bactericides has been published (Brözel and Cloete, 1991a), the result of *in situ* application is unknown. Few studies have been reported regarding the reactions of aqueous bacterial populations to various bactericide treatments (Cloete et al., 1989b), and on the resulting species diversity in water-cooling systems.

We chose a range of bactericides from the reported series, based on their ability to kill a range of bacteria found dominant in South African cooling-water systems (Cloete et al., 1989a). A novel bactericidal product derived from oxidised coal and currently under evaluation in this laboratory was also used. The bactericides were applied to small open recirculating water-cooling units at concentrations found cost-effective under laboratory pure-culture conditions, in order to study their efficacies *in situ*.

Materials and methods

In situ application of bactericides

Experimental units

Three identical open recirculating water-cooling units with a volume of 100 l each, and operating at ca. 14 °C, were used in this study. The water was originally taken from the municipal water supply, but no make-up water was added during the 48-h study period. A well developed biofilm was visible in each system. None of the systems had previously been treated with bactericide, and had been operating over a period exceeding three months. This study was performed during the third quarter of 1989.

Bactericides evaluated

A range of bactericides was chosen from the series evaluated previously (Brözel and Cloete, 1991a). In that study the selective

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