The reaction of bacterial cultures to oxidising water treatment biocides

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Introduction

Surfaces exposed to water are often colonised by bacteria which grow to form biofilms (Characklis, 1990). These bacterial biofilms cause biofouling of water systems, leading to a decrease in system efficiency, as well as to microbially influenced corrosion where metal surfaces are involved (Cloete et al., 1992). Many industrial water systems are treated with one, or a combination of biocides in order to eliminate or reduce biofouling. It has been established that bacteria resident in industrial water systems develop resistance to non-oxidising bactericides such as isothiazolone, dichlorophen, thiocarbamate and quaternary ammonium compounds (Brözel et al., 1993; Brözel and Cloete, 1993). Resistance develops over time and is not acquired but develops by adaptation during exposure to sub-inhibitory concentrations (Brözel and Cloete,1994). Resistant cells are able to grow in the presence of otherwise inhibitory concentrations of non-oxidising bactericides (Brözel et al., 1993).

The oxidising bactericides such as hypochlorous acid and 3bromo-l-chloro-5,5-dimethylhydantoin are used in various applications to control or prevent bacterial biofouling in industrial water systems. It is not known whether bacteria in such water systems become more resistant to oxidising bactericides during prolonged periods of treatment (Brözel and Cloete, 1993). Certain cases have been reported (Cloete et al., 1992) where treatment regimes did not yield satisfactory results after a given period, posing the question of possible resistance. Certain bacteria are known to adapt rapidly to hydrogen peroxide by the so-called oxidising stress response, becoming more resistant following exposure to a low level of the oxidant. These includePseudomonas fluorescens and P. putida (Katsuwon and Anderson, 1989), Escherichia coli (Storz et al., 1990) and Bacillus subtilis (Hartford and Dowds, 1992). If bacteria resident in industrial water systems possessed such an oxidising stress response, they too would become more resistant following exposure to initial low levels of

oxidant. This would render treatment regimes ineffective because these bacteria would continue to grow.

The aim of this study was to investigate whether bacteria found dominant in the planktonic phase of industrial water systems (Brözel and Cloete, 1992) develop increased tolerance to the oxidising bactericides hypochlorous acid and 3-bromo-l-chloro-5,5-dimethylhydantoin under planktonic conditions.

Materials and methods

Cultures and media used

Three bacterial isolates found to attain a dominant position in cooling-water communities after various bactericide treatment regimes, *Pseudomonas aeruginosa, P. stutzeri* and *Bacillus cereus,* were used (Brözel and Cloete, 1992). These were maintained on R2A agar slants (Reasoner and Geldreich, 1985) containing 1% glycerol, and subcultured monthly. R2A medium was made up as follows (per litre): 0.5 g peptone (Biolab); 0.5 g yeast extract (Biolab); 0.5 g casamino acids (Difco); 0.5 g glucose (BDH); 0.5 g soluble starch (BDH); 0.3 g Na pyruvate (Merck); 0.3 g K₂HPO₄ (Merck); and 0.05 g MgSO₄ (Saarchem). For solid R2A medium, 12 *g*-*l*^{*l*} agar (Biolab bacteriological grade) was added.

Bactericides evaluated

Hypochlorous acid was prepared fresh as an aqueous solution by dissolving $Ca(OCl)_2(Olin)$ in deionised water. 3-bromo-1-chloro-5,5-dimethylhydantoin (Aldrich) was also prepared fresh by dissolving 0.1 g in 1 *ml* ethanol (96 % m/v), and then adding sterile deionised water to 10 ml because it is difficult to dissolve in H₂O at this concentration.

Determination of the minimum inhibitory concentration

Bacterial strains were cultured in 100 *ml* R2A broth in 250 *ml* Erlenmeyer flasks under orbital shaking at 100 r-min-¹ for 24 h at 30°C. R2A was chosen because it has a similar level of suspended organic content as many industrial waters, i.e. 2.8 g-l-l as opposed

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Received 2 September 1994; accepted in revised form 1 December 1994.