Surfactants and the attachment of *Pseudomonas aeruginosa* to 3CR12 stainless steel and glass

TE Cloete* and L Jacobs
Department of Microbiology and Plant Pathology, University of Pretoria, Pretoria 0002, South Africa

Abstract

Five non-ionic and three anionic surfactants were evaluated using 4'6-diamidino-2-phenylidole (DAPI) staining, scanning electron microscopy (SEM) and spectrophotometry for their efficacy in preventing adhesion and removing *Ps. aeruginosa* attached to 3CR12 stainless steel coupons and glass. All the surfactants tested gave more than 90% inhibition of adhesion to the surfaces tested with no significant difference between the effectivity of the different anionic surfactants ($p > 0.18$) nor between the effectivity of the non-ionic surfactants ($p > 0.16$). The non-ionic and anionic surfactants resulted in more than 80% and 63% removal of attached *Ps. aeruginosa* cells, respectively. The non-ionic surfactants were significantly more effective in removing attached bacteria, than the anionic surfactants ($p < 0.001$). The prevention of attachment of *Ps. aeruginosa* cells to a glass surface, using the surfactants, was also monitored spectrophotometrically. There was no significant difference ($p = 0.437$) when comparing the DAPI - staining technique with spectrophotometric evaluations.

Introduction

Adhesion to surfaces is a common and well-known characteristic of micro-organisms in oligotrophic habitats (Zobell, 1943). This adhesion and subsequent metabolism lead to the formation of biofilms (McCoy et al., 1981). Bacterial biofilms promote increased biomass deposition (Whitekettle, 1991), resulting in fluid flow resistance, loss of heat exchange and microbially induced corrosion in industrial water cooling systems (Marshall, 1992).

Industries control unwanted biofilms, with varying degrees of success, by using biocides (Marshall, 1992; Cloete et al., 1998). The use of biocides, especially chlorine, in water reticulation and heat-exchange systems is effective only if the biofilm is removed manually. Chlorination of a mature biofilm is usually unsuccessful because the biocide only reacts with the outer portion of the biofilm, leaving a healthy and substantial bacterial community on the surface that rapidly regrows (Marshall, 1992). Bacteria within biofilms develop increasing resistance to non-oxidising biocides on repeated dosing (Cloete et al., 1992). Brözel and Cloete (1992) found that non-oxidising biocides also induced cross-resistance to other non-oxidising biocides.

More recently, surface-active compounds (surfactants) have been employed to prevent bacterial adhesion to surfaces. Currently there is no evidence that surfactants will have any mutagenic effects on bacteria, or that micro-organisms could become resistant to the action of surfactants, as in the case of biocides (Russel, 1990; Brözel and Cloete, 1992). Unfortunately, little published information is available on the effectivity of different biodispersants (surfactants) against bacterial attachment (Lutey, 1995). According to Paul and Jeffrey (1985), dilute surfactants completely inhibited the attachment of estuarine and marine bacteria. Surfactants result in uniform wetting of the surface to be treated and have an additional cleaning effect (Cloete et al., 1992; Lutey, 1995). Whitekettle (1991) found a correlation between the ability of a surface-active compound to lower surface tension and its ability to prevent microbial adhesion. White and Russel (1992) classified surfactants according to the ionic nature of the hydrophilic group viz. anionic, cationic, non-ionic and zwitterionic.

The aim of this study was to use DAPI staining, scanning electron microscopy (SEM) and spectrophotometry to monitor the adhesion of *Ps. aeruginosa* to stainless steel and glass surfaces and to use these methods to monitor the removal of a mature biofilm from a stainless steel surface using non-ionic and anionic surfactants (biodispersants).

Materials and methods

Organism used

Pseudomonas aeruginosa isolated from a cooling water system was used for all the experiments (Brözel and Cloete, 1992).

Surfactants used

Non-ionic and anionic surfactants were obtained from South African suppliers (Table 1). The dosing concentration of the surfactants was 20 $mg \cdot l^{-1}$ according to manufacturer instructions.

Experimental procedures

A continuous flow - through system (Jacobs et al., 1996) and a modified Pedersen device (McCoy et al., 1981) were used to determine the prevention of adhesion and biofilm removal of *Ps. aeruginosa* on a stainless steel surface and on glass.

A wild strain of *Pseudomonas aeruginosa* isolated from a cooling water system and identified in a previous study was used (Brözel and Cloete, 1992). A modified Pedersen device (McCoy et al., 1981) and a flow-through tube were connected in series with a peristaltic pump, which in turn was connected to a 4 l reservoir. *Pseudomonas aeruginosa* was cultured in 200 ml R2A broth (Reasoner and Geldreich, 1985) for 24 h at room temperature. Of this culture 20 ml were used to inoculate the reservoir containing 4 l R2A medium. The flow rate through the system was 1.8 $ml \cdot h^{-1}$.

* To whom all correspondence should be addressed.
☎(012) 420-3265; fax (012) 420-3266; e-mail: tecloete@postino.up.ac.za
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