Preventing Biofilm Formation Using Surfactants

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Microbial adhesion to abiotic surfaces and consequent biofilm formation has been documented in many environments. In many technical processes, the presence of microorganisms is acceptable as long as they remain planktonic. Hence 'disinfection' could be facilitated if attachment of microorganisms to a surface could be prevented. One strategy to prevent the formation of biofilms is to disinfect surfaces regularly, before biofilm formation starts. One of the most important means to prevent biofilm formation concerns surface-preconditioning using surfactants. Indeed, it has been reported that surface preconditioning with surfactants has been employed to successfully prevent bacterial adhesion. Other studies have also revealed the potential of chemical surfactants and biosurfactants to control bacterial adhesion and biofilms. In this review we will describe the current knowledge on the mechanisms involved in biofilm control and surface pre-conditioning by surfactants (natural and chemically synthesized, traditional and new formulations) and their role in biofilm prevention and control.

Biofilm Control Using Surfactants

The deposition of microorganisms on solid surfaces, and subsequent biofilm formation, are phenomena that happen naturally but are also part of the microorganisms' strategy to protect themselves from external toxic factors. Bacterial adhesion to surfaces and biofilm formation are well recognised phenomena in almost all of the industrial areas that deal with flowing water systems. Furthermore, bacterial adhesion and biofilm growth have become an increasing concern in food processing systems, public health and medical arenas. These systems have a wide range of characteristics that favours biofilm formation. Prominent among those problems associated with biofilms is their recalcitrance to even the harshest of prevention and control procedures (Costerton and Lashen 1984). Moreover, biofilms may act as continuous sources of pathogens that can contaminate surfaces and products. Accordingly, there has been a great deal of research to understand biofilm development and to identify improved strategies for biofilm control.

At present, an increase in the frequency of cleaning and disinfection pro-

grammes and the use of increased doses of chemical products, with marked antimicrobial properties, are used to reduce biofouling. In the past few years, Industry and Medicine have moved progressively towards the use of more biodegradable and less toxic compounds, such as surfactants. Surfactant is an abbreviation for surface active agent, which literally means active at a surface, and is characterized by its tendency to absorb at surfaces and interfaces (Jönsson *et al.* 1998). They are normally added to increase the washing effect of the sanitation practices because of their ability to alter the surface properties. Furthermore, they lower the surface and interfacial tensions of aqueous fluids, which comprise the ability to wet surfaces, penetrate soil and solubilise fatty materials (Christofi and Ivshina 2002; Glover *et al.* 1999; McDonnell and Russell 1999). Surfactants are comprised of two molecules with two different structural elements: a hydrophobic hydrocarbon (water repellent) group; and a hydrophilic polar (water attracting) group. They are generally characterized by properties such as the critical micelle concentration (CMC), the hydrophile-lipophile balance (HLB), chemical structure and charge, as well as by source (chemical or biological) (Van Hamme *et al.* 2006). Depending on the charge of the hydrophilic structural element, surfactants are classified as anionic, cationic, non-ionic and amphoteric or zwitterionic (Paulus 1993; Rossmoore 1995; Jönsson *et al.* 1998).

In addition to their detergent properties, surfactants also present noticeable bacteriostatic and biocidal properties thus they can act as multi-target agents against bacterial cells (Simões *et al.* 2006). In fact, surfactants may exert toxic effects by causing membrane disruption leading to cellular lysis, by increasing membrane permeability causing metabolite leakage and by altering physical membrane structure or by disrupting protein conformation thus interfering with important membrane functions such as energy generation and transport (van Hamme *et al.* 2006). For instance, cationic surfactants or quaternary ammonium compounds (QAC's) are employed both as disinfectants for manual processing lines, surfaces in the food industry and in medicine (Mereghetti *et al.* 2000; Massi *et al.* 2003). This is due to their excellent hard-surface cleaning, deodorization and antimicrobial properties (McDonnell and Russell 1999). The antimicrobial properties of QAC's depend on their structure and size, but especially on the length of the long-chain alkyl group. QAC's bearing the C_{14} alkyl group exhibit maximum antimicrobial activity. The efficacy of QAC's increases with temperature and pH (Paulus 1993). They are considered a relatively safe broad-spectrum biocide, but the use of these compounds in some fields has been limited by the discovery of microbial resistance against some QAC's. Anionic surfactants, such as SDS, exhibit some antimicrobial activity only in acid media (pH 2-3) or their undissociated state, but they have strong detergent properties (Hugo and Russell 1982; Rossmore 1995). They present themselves as alkali or amine salts of long-chain fatty acids or alkane sulphonates. In aqueous solution they dissociate to a large anion, responsible

for the strong detergent properties, and a small cation (Paulus 1993). Their antimicrobial effect is restricted mainly to Gram-positive bacteria, the active site being the cell membrane. Acid formulations of anionic surfactants are used as disinfectants in the dairy, beverage and food processing industries as well as homes (Hugo and Russell 1982; Paulus 1993).

As surfactants are potentially toxic to specific microorganisms, it is not surprising that recently their specificity has been exploited and they are being used as antimicrobial agents and anti-adhesive agents for safe use without disturbing the environment (MacDonald *et al.*, 2000; Simões *et al.*, 2006). However, investigations on the impact of surfactants on microbial activity have generally been limited to the most common surfactants, therefore more studies are needed with new chemical surfactants, such as gemini and fluorosurfactants (Massi *et al.* 2003a; b), and with those produced by microorganisms – biosurfactants (Rodrigues *et al.* 2006; Van Hamme *et al.* 2006; Nitschke and Costa 2007). Splendiani *et al.* (2006) have showed that Teepol effectively reduces the *Burkholderia* spp. biofilm accumulation on the membrane walls of a laboratory-scale extractive membrane bioreactor. They suggested that this reduction was caused by changes in the attachment capability of the bacteria since the surfactant affected the development of the flagella. Some disinfectant formulations (particularly those based on aldehydes and peracetic acid - PAA) fix bacteria to the surface (Loukli *et al.*, 2004), which is obviously an undesirable trait for disinfection. Thus, when choosing disinfection products, both antimicrobial activity and non-fixing ability should be considered. This non-fixing capability, according to Loukli *et al.* (2006), can be achieved with the presence of surfactants, such as QAC's, since they found that PAA formulations, stabilized with surfactants, did not fix *Escherichia coli* biofilm to glass. Thus, they concluded that the presence of surfactant components in a stabilized peracetic acid may be effective for biofilm removal.

The cationic surfactant cetyltrimethylammonium bromide (CTAB) was investigated for its ability to control mature *Pseudomonas fluorescens* biofilms formed under laminar and turbulent flow in flow cells reactors (Simões *et al.* 2005a). The authors found that CTAB by itself did not cause the detachment of biofilms but it reduced the respiratory activity of the biofilm cells. Total respiratory inactivation was not achieved and, in almost all the cases studied, it was observed that biofilm respiratory activity recovered over time. However, the same authors, in another study (Simões *et al.* 2005b) reported that the synergistic action of CTAB and the application of high shear stress to mature biofilms, formed in a rotating device, increase its detachment. The physical stability of the biofilms was assessed using a rotating device, where the effect of the surfactant on the biofilm stability was evaluated through the variation of the mass remaining on the surface. More recently, Simões *et al.* (2007) also studied the effect of the anionic surfactant sodium dodecyl sulfate (SDS) on

Pseudomonas fluorescens biofilms. The data suggest that SDS exerts a considerable antimicrobial action against *P. fluorescens* in planktonic and sessile form, as it promoted significant respiratory inactivation, being similar the susceptibility of turbulent and laminar flow-generated biofilms to SDS application. However, SDS also had only limited antifouling efficacy, since it did not significantly reduce the amount of biofilm adhered to the metal surfaces (removal smaller than 15 %) and further it allow the rapid respiratory recovery of turbulent biofilms. Conversely to CTAB, SDS increased biofilm mechanical stability at low concentrations, which represents an additional factor of biofilm recalcitrance to sanitation. In a previous study, Cloete and Jacobs (2001) also noticed considerable difficulties in removing a mature biofilm from stainless steel surfaces using non-ionic and anionic surfactants. Both types of surfactants removed pre-attached *P. aeruginosa* bacteria entrapped in the biofilm, but total removal of the biofilm did not occur. From the data referred to above, it can be concluded that surfactants, when used against mature biofilms, have good disinfectant characteristics but poor ability to remove well established biofilms. In these cases, the use of a surfactant by itself is not sufficient for controlling biofilms.

Biofilm Response to Biocide/Surfactant Residues

One strategy to prevent the formation of biofilms is to disinfect surfaces regularly, before biofilm formation starts (Meyer 2003). However, if the biofilm sanitation practices are not effective, microorganisms and residues can remain in the equipment contributing to the re-growth of biofilms persistent to the sanitation products (Gilbert and McBain 2003). In recent years, there has been a growing concern of bacterial adaptation and resistance to biocides and surfactants. In order to overcome this drawback, it is crucial to comprehend all the parameters that can contribute to the prevalence of biocide and surfactant resistance. One of the factors that can contribute to that understanding is to establish whether pre-contact of bacteria with a chemical can contribute to their reduced susceptibility to that product. Thomas *et al.* (2005) reported that exposure of *P. aeruginosa* to dry residues of chlorhexidine formulations did not result in the strains becoming less sensitive to either these biocides or antibiotics. Conversely, a study by Loughlin *et al.* (2002) showed that two *P. aeruginosa* strains increased in resistance to benzalkonium chloride (BKC) when exposed to sub-MIC concentrations of the agent. Moreover, those strains also showed cross-resistance to other QAC's, but not with clinically relevant antibiotics.

Another important factor that must be considered for biofilm control is the effect of residues that remain on the surfaces after the use of an antimicrobial agent. According to Gibson *et al.* (1999) and Gilbert and McBain (2001), the pre-contact of surfaces with biocides and/or surfactants can give rise to the adsorption of some residues onto those surfaces that can affect biofilm development in two ways: i) modification of the biofilm formation capacity

since surfactant residues may act against cellular metabolism, favouring or impairing the adhesion forces that maintain the biofilm mechanical stability and /or ii) development of biofilms with less cohesive feature that can lead to biomass detachment. Some authors (Bower and Daeschel 1999) referred that the adsorption of a bioactive compound onto a clean food-contact surface could prove to be a useful strategy for inhibiting the initial adhesion of bacteria and thus biofilm formation. Machado *et al.* (2006) also showed that the preconditioning of surfaces with a traditional QAC (CTAB) and a new series of fluorinated QAC's, for 30 min, changed *P. fluorescens* and *S. sciuri* biofilms response to those antimicrobial products, particularly in terms of respiratory activity. In fact, the application of the QAC's to the biofilms formed on the conditioned surfaces increased the amount of the adhered biomass, independently of the kind of biofilm-forming bacteria, but reduced the respiratory activity of those biofilms. Conversely, Pereira *et al.* (2006), in another study, reported that biofilm formation was not reduced on silicone or stainless steel surfaces preconditioned with BKC (a cationic surfactant). Based on this work, it could be concluded that the adsorption of BKC to steel and silicone coupons may favour biofilm physiology which could augment its resistance to sanitation. However, it can be speculated that the BKC residues adsorbed onto surfaces may be at a far lower concentration than that initially applied and/or the time for surface preconditioning was not enough to allow the adsorption of a significant amount of BKC residues. These remarks highlight the need for further investigations into the effect of residues on biofilm growth and their role in the problem of antimicrobial resistance.

Biofilm Control with Biosurfactnts

Biosurfactants are a structurally diverse group of surface active molecules (high and low molecular weight) synthesized by microorganisms. Rhamnolipids from *Pseudomonas aeruginosa*, surfactin from *Bacillus subtillis*, emulsan from *Acinetobacter calcoaceticus*, are some examples of microbial-derived surfactants (Nitschke and Costa 2007). Some of them have antimicrobial activity against bacteria fungi, algae and viruses (Singh and Cameotra 2004) and hence may be considered as potential alternatives to chemical surfactants and other antimicrobial agents (Van Hamme *et al.* 2006, Rodrigues *et al.* 2006). Additionally to the antimicrobial activity, biosurfactants also possess anti-adhesive properties that make them attractive agents to impair the adhesion of bacteria to an inanimate surface and/ or to improve bacteria and biofilm detachment. Bioconditioning of surfaces with microbial surfactants (i.e. the previous adhesion of biosurfactants to solid surfaces) have been suggested as a new and effective tool to reduce bacterial adhesion and thus biofilm formation. For instance, Meylheuc *et al.* (2001) demonstrated that the adsorption of a biosurfactant (obtained from *Pseudomonas fluorescens*) to stainless steel surfaces reduced significantly the adhesion of *L. monocytogenes* on that metal surface. In more recent work, the same authors reported the previous adsorp-

tion of the same biosurfactant onto stainless steel coupons also improved the bactericidal effects of disinfectants (Meylheuc *et al.* 2006). In another study, Rodrigues *et al.* (2006) revealed that silicone rubber preconditioned with rhamnolipids reduced the number of adhered cells of *S. salivarius*, *S. aureus*, *S. epidermidis* and *C. tropicalis* and the perfusion of the biosurfactant to the adhered cells produced a very high detachment of the referred microorganisms. Another potential antimicrobial strategy to improve the action of antibiotics and biocides may be related to the use of biosurfactants in disrupting biofilms and reducing bacterial adhesion. With the disruption of the biofilms, the microorganisms embedded in those communities would be exposed to more of the toxic action of the antimicrobial agents, increasing thus their efficacy.

The promising data gathered in this area emphasises the need for further research focusing on biosurfactants and in their applications as possible substitutes to chemical surfactants. Nevertheless, there appears to be great potential for the use of biosurfactants in industry and medicine in the prevention of bacterial adhesion and biofilm control.

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