Biofilms – Formation and prevention

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Abstract

Flocculation and sedimentation in the activated sludge process have direct influence on the quality of the effluent water from wastewater treatment plants. Confocal laser scanning microscopy (CLSM) of green fluorescent protein (GFP)-marked cells showed their position in the floc matrix in situ. Hydrophobic cells attached not only on the surface but also within flocs, whereas hydrophilic cells did not. Polymer addition is common practice in wastewater treatment and was shown to increase adhesion of planctonic cells to flocs. Results also point out the problem with surfactants as pollutants since they decrease the water quality of the effluent by destabilizing flocs and decreasing bacterial adhesion to flocs.

N-acetyl-L-cysteine (NAC) is commonly used in medical treatment of patients with cystic fibrosis. Our results suggest that NAC also may be an interesting candidate for use as an agent to reduce and prevent biofilm formation on abiotic surfaces. The mode of action of NAC was found to be chemical, as well as biological, in the case of bacterial adhesion to surfaces. Quartz Crystal Microbalance with Dissipation monitoring (QCM-D) was shown to be a powerful and sensitive technique to investigate the effect of NAC on initial adhesion processes of bacteria. Surfaces coated with NAC increased the wettability of the surface and had affect on the viscoelastic properties of the interaction between the adhered bacteria and the surface. Altogether, NAC-coating on surfaces often has a god anti-adhesive effect and could both decrease initial adhesion and increase the detachment of adhered cells and spores. The kinetics of frequency and dissipation shifts was bacteria, life-cycle stage of bacteria and surface dependent. NAC present in media inhibited growth of various bacteria and changed the texture of the formed biofilm due to inhibition of extra cellular polysaccharide (EPS) production. The fact that NAC inhibited the formation of spores is of importance for the use of NAC, as an anti-biofilm substance since spores is known to be resistant to different kind of chemical substances and to be more adhesive.

NAC act on several regulatory pathways in the *Bacillus subtilis* cell since the substance has effects on protein expression involved in metabolism, cellular processes and signalling as well as information processing. We suggest that NAC regulated proteins involved in both initial adhesion, micro colony formation, EPS production and spore formation, possibly as a quorum sensing molecule, which have consequences for the cells ability to develop into a mature biofilm.

Keywords: adhesion; green fluorescent protein; wastewater; N-Acetyl-L-cysteine; extra cellular polysaccharide; wettability; quartz crystal microbalance; confocal laser scanning microscopy

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