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CONFOCAL LASER SCANNING MICROSCOPY AND FLUID DYNAMIC GAUGING AS A TOOL FOR DETERMINATION OF BIOFILM STABILITY

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ABSTRACT

Confocal laser scanning microscopy (CLSM) has become an indispensable technique for studying the in situ structure as well as the cellular and polymeric composition of microbial biofilms. The 3-dimensional data sets are amenable to digital image analysis for visualisation and quantification of volumetric and structural parameters. Fluid dynamic gauging (FDG) has been developed as a tool to study organic and crystalline fouling on surfaces. By knowing the exact geometry of the experimental setup in combination with computational fluid dynamics the shear stress at the surface of the substratum can be calculated. Thus FDG may also be employed in order to calculate the adhesiveness as well as the forces for detachment of microbial films from surfaces.

In this study heterotrophic biofilms were cultivated in a rotating disk reactor from activated sludge. Biofilms developed under different substrate conditions and different shear stress were then used for FDG experiments. The biofilms were exposed to two different shear conditions (6.2 and 7.3 *Pa*). Before and after FDG biofilms were examined by CLSM in the 2-channel mode for measuring bacterial and glycoconjugate biomass. From the results the following conclusions were made: 1) under high shear stress and/or low substrate load, biofilms develop into thinner films and films having a higher density; 2) based on the observations, the biofilms maybe best described according to the model of Characklis and Wilderer (1989); 3) the biofilms consist of a stabile and homogeneous base biofilm and a more heterogenic surface biofilm; 4) by applying a shear stress of 7.3 *Pa*, it is not possible to completely remove the biofilm; 5) the more homogeneous the biofilm the higher is its stability; 6) a high proportion of extracellular polymeric substances (EPS) glycoconjugates in the polymer matrix supports the stability of the biofilm; 7) furthermore multi valent ions (Fe^{2+}) increase the stability of biofilms.

It is suggested that the combination of CLSM and FDG are useful tools for determination of biofilm stability and the forces necessary for biofilm detachment. In addition, CLSM provides detailed information about biofilm constituents and consequently allows structure-function studies related to biofilm stability. Both techniques together may be employed as a measure for organic fouling and biofouling control in technical systems.