

Combined application of μ EL, CLSM and MRI for the analysis of microbial biofilms

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Biofilms are microorganisms and their polymers associated with interfaces in environmental, technical and medical habitats. The function of biofilms in all of these systems is strongly related to their structure. The biofilm structure in turn is again determined by nutrient availability as well as hydrodynamic conditions. In order to examine both, structure as well as function of biofilms, a combination of different advanced techniques is needed, such as μ -electrodes (μ EL), confocal laser scanning microscopy (CLSM) and magnetic resonance imaging (MRI). In this study river biofilms cultivated in a rotating annular reactor were investigated. In a first step oxygen μ EL were used to measure gradients in an area of 25 mm² of the biofilm in a flow chamber at a mean flow velocity of 3 cm/s. The results showed a complete penetration of oxygen into the biofilm. The concentration boundary layer between bulk and biofilm showed irregularities at the biofilm surface on the order of +/- 100 μ m. In a second step the same biofilm area was investigated directly by multi-channel CLSM as well as after cryo-sectioning. Algae were detected by their autofluorescence, bacteria were stained with Syto fluorochromes, glycoconjugates of the extracellular polymeric substances (EPS) were detected using lectin-binding analysis. The biofilm had a mean thickness of 450 μ m. The very dense base layer on the substratum side consisted of chemoautotrophic (bacteria) and phototrophic (algae) micro-organisms. The less dense surface layer was thicker and mainly dominated by EPS glycoconjugates, some areas showed heterotrophic bacteria as well. The surface irregularities which were already detected with the microelectrode technique could be confirmed by CLSM results. In a third step the biofilm sample was examined by MRI in a flow through chamber. The three-dimensional biofilm structure was imaged using a series of fast RARE images with different echo times, thus enabling the acquisition of fast three-dimensional relaxation time maps. The flow pattern around the biofilm surface was measured in different two-dimensional planes. All MR images were recorded with a spatial resolution of 63 μ m, which is sufficient to resolve the irregularities of the biofilm surface observed with the other two techniques. The three-dimensional images reveal areas of different biofilm density, and individual filaments, which align with the flow field, of up to 1 mm length can be observed. In conclusion, only the combination of several advanced techniques specific for micro-gradients (μ EL), structural features (CLSM) and hydrodynamic conditions (MRI) allow a complete description of structure, dynamic and function of microbial biofilms. The summed up results of this first combined approach revealed details not available with previous methods.