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APPLICATION OF MRI AND CLSM FOR THE ANALYSIS OF BIOFILM DETACHMENT

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ABSTRACT

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 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 confocal laser scanning microscopy (CLSM) and magnetic resonance imaging (MRI). In this study biofilms cultivated in tubular reactors were investigated with respect to biofilm detachment.

Detachment of biomass from biofilms is still a nearly unknown process which has to be investigated in more detail. MRI is a method which supplies information on the gross structure of biofilms, its surface and hydrodynamic conditions at the bulk/biofilm interface, whereas CLSM can be used to analyse the structure and composition of biofilms. For example, bacteria were stained with SYTO fluorochromes and glycoconjugates of the extracellulare polymeric substances (EPS) were detected using lectin-binding analysis. Both the structural data and the shear stress which can be calculated from the velocity field are key parameters for understanding biofilm detachment.

In the paper presented here, a fast quantitative MRI technique was used to investigate the detachment from a heterotrophic biofilm which was grown in a tube reactor. The investigated biofilms were cultivated in a test segment (Length 12 cm, diameter 7 mm) at a constant Reynolds number (2000 and 3000) and a substrate load of 1.5, 6 and 10 g Glucose / m² and day. For the MRI experiments, the test segments with the biofilm were connected to the flow loop and placed inside the NMR magnet. During the experiment different hydrodynamic conditions were adjusted for two minutes (Reynolds number Re: 3000, 4000, 5000, 6000, 7000, >9000). Flow velocity and relaxation time were then measured at laminar flow conditions.

The MR images show very impressively the increasing detachment of biomass from the biofilm surface with increasing shear forces. At the highest shear force, only a thin biofilm of about 100 to 200 µm thickness with a very homogeneous surface remained in the test tube.
The CLSM images for similar detachment experiments in biofilm tube reactors with a diameter of 26 mm show similar results. After application of high Reynolds numbers (>10000) a base biofilm, which can not be removed with the applied shear forces, remains on the surface.
The methods and the experimental results can be used to develop combined techniques of using biocides and increased shear forces by which unwanted biofilms may be removed.