Optimization of Biofilm Structure by Means of an Evolutionary Platform

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Abstract

Biofilms are the dominating form of microbial life on Earth. Their structure influences the bulk liquid flow around and *vice versa* commonly termed fluid-structure interaction. We suppose that there is an 'optimal' biofilm structure, which is the result of the parameters dynamically acting in the particular cultivation system. To systematically investigate the influence of the cultivation conditions on the biofilm structure an evolutionary robotic platform will be used allowing to manipulate several parameters. Since the biofilm structure is in focus of our research, the representative visualization is a necessity. Imaging is thus performed by means of optical coherence tomography. The gained knowledge will merge in an improved understanding of the fluid-structure interaction in biofilm systems and should further allow to control the structural evolution of biofilms with respect to their desired purpose.

Introduction

Microbial life on Earth is dominated by biofilms. Biofilms form in aquatic environments in nature and the technosphere. Suspended microorganisms such as bacteria, fungi and algae attach at some point irreversibly to surfaces and start to form a biofilm by reproduction and most important by the production of extracellular polymeric substances (EPS). Those EPS are of microbial origin build the backbone of the biofilm (Characklis et al., 1989). This matrix has sometimes named 'the housing' and is composed of polysaccharides, proteins, nucleic acids, lipids and co-polymers (Neu and Marshall, 1990; Flemming et al., 2007). It can of course be understood as the housing, because it provides a protective growth environment for the microorganisms embedded within. Biofilms are thus able to withstand harsh environmental conditions such as extremely high or low pH value, temperatures greater than 50 °C and intense electromagnetic radiation to name just a few. Furthermore, The formation of biofilms does influence and change mass transfer processes at interfaces. Water and wastewater treatments employ several biofilm technologies to mineralize substrates and convert nutrients. The performance of these treatments is closely related to the microorganisms which are present within the biofilms as well as to the biofilm structure developed. The fluid-structure interaction in these systems affects the mass transport and mass transfer processes and thus the overall system performance. To date the relation of biofilm structure and its function is not fully understand. By visualizing the biofilm structure at different scales the distribution of biomass, inorganic enclosures, cavities and biofilm constituents can be revealed *in situ* and three-dimensionally. By acquisition of concentration profiles (i.e., dissolved oxygen) directly within biofilms information of the metabolic activity can be assessed. Combining those information allows a more precise description of the fluid-structure interaction of the investigated biofilm system. However, this knowledge is very seldom used to 'design' the biofilm structure or control the evolution of the structural development.

Within our research we thus try to apply the knowledge gained in the past in a kind of bottom-up approach to design the biofilm structure to optimize mass transport and in conclusion mass transfer processes. Therefore, a microbial fuel cell has been selected as model biofilm system with easy to determine performance.

Biofilm Structure Visualization by Means of Optical Coherence Tomography

To understand the impact of the biofilm structure on the fluid-structure interaction in detail, it is important to visualize the biomass completely. A clear differentiation of biomass and cavities which could allow for advective mass transport is essential. Furthermore, the biofilm structure needs to be described on the mesoscale (mm-range; Morgenroth and Milferstedt (2009)) to evaluate the overall structure rather than local structural variations and properties.

Optical coherence tomography (OCT) has its origin in ophthalmology. It is an interferometric method which allows a depth-resolved detection of refection signals within translucent tissues (Fujimoto, 2003). OCT has been introduced in biofilm research about a decade ago (Xi et al., 2004, 2006; Haisch and Niessner, 2007). By acquisition of consecutive depth-profiles (A-scan) an optical section in xz-direction (B-scan) through the biofilm is achieved. A

set of adjacent B-scans builds the volumetric dataset (C-scan). Compared to a microscopic imaging modality such as confocal laser scanning microscopy widely used in biofilm research, OCT requires no staining and allows fast, non-invasive and *in situ* imaging of the mesoscopic biofilm structure at high speed and at high resolution (Wagner et al., 2010).

Imaging was performed using a GANYMEDE spectral domain OCT system (Thorlabs GmbH, Germany). It allows to visualize biofilm volumes up to a size of $10 \times 10 \times 2.14\,mm^3$ at a lateral resolution (x and y) of maximal $8\,\mu m$ and an axial resolution of $2.1\,\mu m$. Such scans are acquired within 1 min or faster. The biofilm was scanned directly inside the microfluidic flow cell used for cultivation.

A reconstruction of a C-scan of biofilm grown in a flow cell is shown in Figure 1. The flow channel is almost completely filled. Biomass aggregates are located close to the walls as well as in the center of the channel. Open spaces between the aggregates are also visible. It can be supposed that supply with nutrients and substrate is enhanced in the center due to the faster flow of the bulk liquid whereas the shear stress is decreasing towards the wall of the flow channel. In addition to the biomass distribution across the flow channel OCT provides insights into the internal structure of these aggregates.

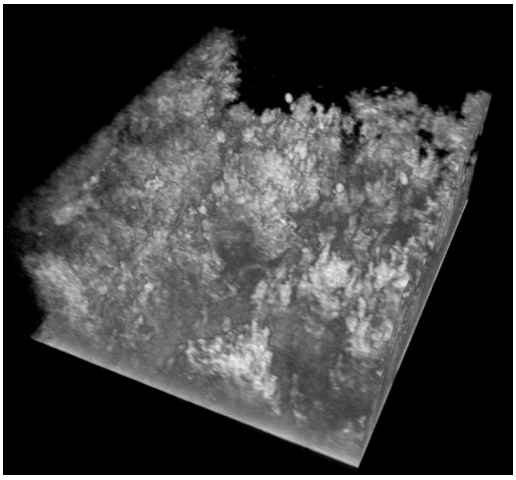

Figure 1: Three-dimensional reconstruction of an OCT C-scan showing biofilm grown in a flow cell. The xy-plane has an area of approx. $1.7 \times 2\,mm^2$ whereas the height is approx. $0.8\,mm$.

Evolution of Biofilm Structure

Volumetric porosity and mean biofilm thickness are structural parameters which can be used to quantify the biofilm structure. Changes are captures as well, which could be correlated to (i) the cultivation conditions and (ii) to the development state of the biofilm. With respect to growth in an MFC the electrical output (voltage and current) should be related.

Here we would like to mention that this work is in progress. Nevertheless, it is assumed that there exists an 'optimal' biofilm structure. This structure does not develop by chance. It is rather the result of all parameters influencing the biofilm growth and decay: flow channel geometry and shape, hydraulic retention time, concentration gradients, biomass distribution and structure as well as flow characteristics. Probably, this list is incomplete. To estimate the triggering parameters systematic experiments are necessary. These experiments, however, should not be performed by humans. Thus, an evolutionary robotic platform will be set up which in a first step allows for the repetition of experiments under defined conditions. The platform will further acquire the biofilm structure by means of OCT. Subsequent image analysis will extract structural characteristics which are then correlated to the cultivation conditions. This part can be understood as a kind of training. In a second step the functionality of the robotic platform is extended to precisely manipulate the biofilm structure to maintain and optimize the performance of the MFC in a controlled environment. The manipulation mechanism can for example include liquid handling (locally resolved deposition of nutrients or toxins, respectively), flow alteration, (re)inoculation (e.g., cell printing) and controlled detachment events.

The gained knowledge should merge in an improved understanding of the fluid-structure interaction in biofilm systems. Moreover, it will allow to control the structural evolution of biofilms with respect to their desired purpose (i.e., wastewater treatment or production of pharmaceutical compounds). This should somehow follow an *if...then...else* scheme instead of experimental expertise, which has been gained decades ago.

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