3-D VISUALIZATION OF BIOFILMS IN POROUS MEDIA WITHOUT USING A CONSTRAST AGENT

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Keywords: X-ray tomography, biofilm, monitoring, porosity

Summary: In this study, the challenge is to characterize biofilm growth in a natural aquifer, identified as a potential future location for storing biogas. More precisely, we want to evaluate the impact of biofilm growth on the porosity by means of high-resolution X-ray computed tomography without using artificial contrast agents as these substances could affect the phenomenon of interest.

1. INTRODUCTION

The study of biofilm growth in porous media is of growing interest in various fields such as medicine, wastewater treatment, chemical engineering and hydrogeology, but also in the study of natural reservoirs used for geological storage or geothermal energy. Indeed, biofilm growth can reduce the porosity, which impacts the permeability as well as other properties of the medium. The study of biofilms at the pore scale (micrometric scale) can permit to better understand biofilm growth and to evaluate its impact on the physical properties of the medium.
The characterization of biofilms in a water-saturated porous medium has been attempted by means of X-ray computed tomography. This technique enables inner inspection of a sample in a non-destructive way and produces a high resolution and digital volume of the scanned sample. Differentiation between the biofilm and the liquid phase is however a difficult task due to the very close X-ray attenuation coefficients of water and biofilms as the latter merely consist of low-Z elements and contain a high proportion of water [1]. Classically, a contrast agent as for example a 1-chloro-naphthalene solution, a barium sulfate (BaSO4) suspension or silver-coated microspheres [2] is used to mark the liquid phase. The contrast agent is introduced a posteriori in the bio-film containing sample, the idea being that the contrast agent would not penetrate inside the biofilm and thus improve the contrast between water and biofilm. However, this process presents several drawbacks (substitution of nutrient medium by contrast solution or suspension by injection) and can produce artifacts in the data analysis (difficulties to obtain a complete substitution result in segmentation errors, forced displacement of biofilms, ...). Another way is to introduce $FeSO_4$ in the liquid phase during the biofilm growth. Indeed, some biofilms naturally exhibit high a content of inorganic matter which promotes the contrast between biofilm and water solution. Consequences of this natural contrast agent on the biofilm growth can however not be excluded.
In this study, the challenge is to characterize the biofilm growth in a natural reservoir and to evaluate its impact on the porosity. The goal is to determine a protocol to monitor the biofilm growth without contrast agents or FeSO4.

2. EXPERIMENTAL METHOD

The natural reservoir is composed by sand and is saturated by water containing the bacterial cells under conditions of pressure and temperature (80 bar and 38°C). To simulate this reservoir, micro capillary tubes of 2 millimeters outer diameter are filled with sand and saturated by synthetic fluid mimicking reservoir water. Next, they are set for incubation at 37°C and atmospheric pressure under H_2 (80,%) and CO_2 (20 %) atmosphere . During the incubation, the bacterial cells can be adsorbed to the surface of the sand grains in the form of a monolayer and/or of micro colonies which can reduce the porosity of the medium. To visualize and to characterize the biofilm growth

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in this porous medium, scans are taken of samples extracted after different incubation times.
The differentiation between biofilm and water containing bacterial cells is very difficult due to their very close X-ray attenuation coefficients. That's why we choose to test two acquisition configurations: the first one favoring absorption contrast and the second one favoring phase contrast which could permit to obtain a better visualization of interfaces between water and biofilms. The tomograph used to conduct the tomographic acquisition is a Zeiss Xradia Versa 510, which features a combination of optical and geometric magnification. The generator was operated at 40 kVp to optimize the absorption contrast between each phase and the voxel size is 2,5 microns.

3. RESULTS

The results show that the biofilm can be visualized without disturbance of the medium and its growth can be monitored. As shown on figure 1a, biofilms which naturally exhibit high content of inorganic matter can be clearly distinguished from water in the absorption contrast configuration and the decrease of porosity can be quantified. The contrast phase configuration enables characterizing biofilms without inorganic matter more easily than the absorption contrast configuration (figures 1b and 1c). The monitoring of biofilm growth is currently in progress and the results will permit to quantify the kinetics of biofilm growth, the location of the biofilm in the medium, the loss of porosity and its impact on the permeability of the medium.

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Acknowledgments

This work benefited from the financial support of the RINGS project and the authors would like to acknowledge the project partners. Total is kindly acknowledged for making available a Zeiss Xradia Versa 510 to DMEX, permitting to perform the tomographic acquisitions reported in this abstract.

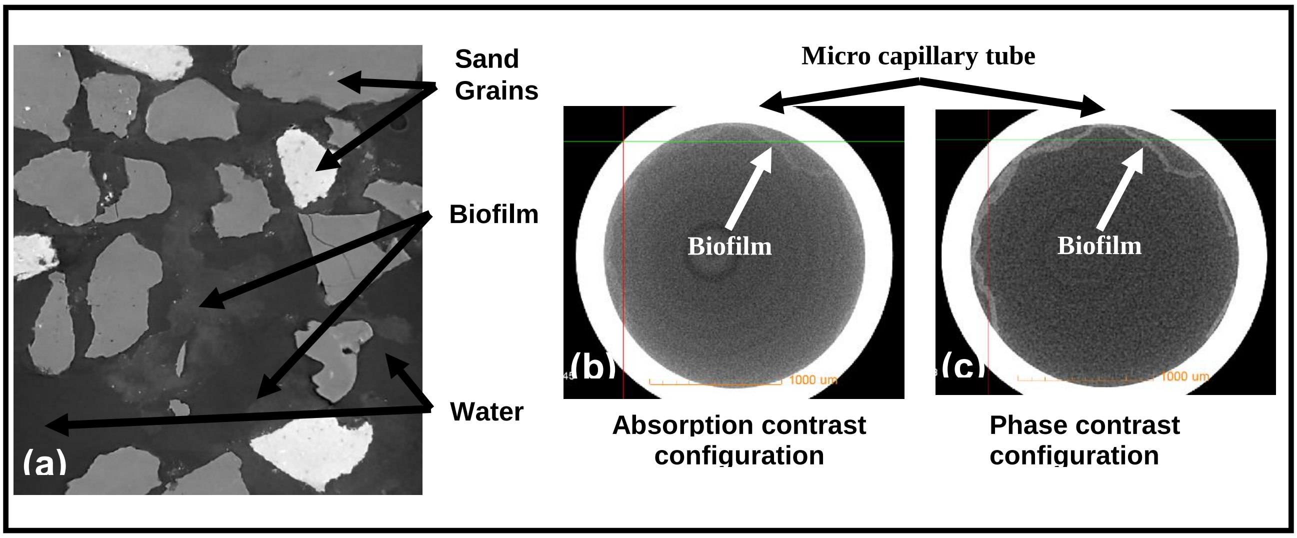

Figure 1: (a) Image (1.8mm x 2 mm) showing sand grains, biofilm containing inorganic matter and water. This image is obtained with the absorption contrast configuration. Biofilm with water in micro capillary tube (of 2 mm outer diameter) with (b) absorption contrast configuration and (c) phase contrast configuration. The voxel size for these images is 2.5 µm.