[P5]

CHARACTERIZATION OF BIOFILMS FORMED IN HUMIC SUBSTANCES BY FLUORESCENCE *IN SITU* HYBRIDIZATION (FISH)

A. L. Rodrigues*, A. G. Brito*, P. Janknecht**, R. Nogueira*[1]

**IBB – Institute for Biotechnology and Bioengineering, Centre of Biological Engineering, University of Minho, Campus de Gualtar 4700-057 Braga, Portugal*
***Stadtwerke Düsseldorf AG - Wasserwerke, Himmelgeister Landstrasse 1, 40589 Düsseldorf, Germany*
Corresponding author. Tel + 351 253 605 409. E-mail:regina@deb.uminho.pt

Rivers are the major source of freshwater for the drinking water sector. Dissolved organic carbon in rivers results mainly from humic substances (HSs). The presence of HSs in water treatment plants is undesirable because they increase coagulant and disinfectant demands, and in the presence of chlorine may provoke the formation of dangerous disinfection by-products (DBPs) such as the carcinogenic trihalomethanes (THMs). Therefore it is crucial to improve environmental technologies increasing water quality prior to treatment. Biofilms are major sites of carbon cycling in streams and rivers. However, the composition of bacterial communities growing on HSs is poorly documented in literature. Thus, it is important to improve the knowledge about the biofilms' structure and microbial composition to understand their contribution in the self-purification of surface water.

The present work assessed the microbial composition of biofilms formed in the presence and absence of HSs as a carbon source using fluorescence in situ hybridization (FISH) with rRNA-oligonucleotide probes. Two biofilm flowcells were operated in parallel during ten weeks; one with synthetic river water, displaying a background carbon concentration of 1.26 ± 0.84 mg L^{-1}, the other with added HSs and an overall carbon concentration of 9.68 ± 1.00 mg L^{-1}. The microbial composition of the biofilms was assessed using FISH with rRNA-targeted oligonucleotide probes labelled with the indocarbocyanine dye Cy3. First the samples were hybridized with the EUB338 probe designed to target almost all bacteria. Then, within this domain, the alpha, beta and gamma-subclasses of Proteobacteria were labeled with the respective group specific probes ALF1b, BET42a and GAM42a. Also, the Cytophaga-Flavobacterium cluster of Bacteroidetes was labeled with probes CF319a and CF319b. Unlabeled probes were used in equal amounts with probes BET42a and GAM42a to obtain optimal stringency conditions. Non-specific binding was checked with the NON338 probe (negative control). FISH was performed using the hybridization and washing buffers. Following FISH, samples were stained with DAPI (2 mg L^{-1}). Total bacterial cells and probe-specific targeted cells were visualized using an epifluorescent microscope (Olympus BX51) equipped with optimal filter sets for DAPI and for Cy3, respectively. Probe specific binding was determined semiquantatively.

The percentage of DAPI-stained bacterial cells within the domain Bacteria (EUB338 probe) was around 80 % in both biofilms. FISH analysis of the biofilm formed in the presence of HSs indicated that beta-Proteobacteria accounted for the highest percentage of the hybridized bacteria (50 %), followed by alpha-Proteobacteria (20 %) and gamma-Proteobacteria (10 %). Regarding the biofilm formed without HSs, the representative bacterial groups were alpha-Proteobacteria (30 %), beta-Proteobacteria (30 %) and Cytophaga-Flavobacterium cluster of Bacteroidetes (15 %). Bacteria targeted with probe NON338 accounted for less than 0.5 % of the DAPI counts. The present study indicates that the microbial community of the biofilm formed with HSs is dominated by beta-

Proteobacteria while in the biofilm formed without HSs both alpha-Proteobacteria and beta-Proteobacteria accounted for the same amount.