## Characterization of biofilm formation on a humic material

Rodrigues A. L.<sup>1</sup>, Brito A. G.<sup>1</sup>, Janknecht P.<sup>2</sup>, Inácio J. <sup>3</sup>, Machado A. V.<sup>4</sup>, Nogueira R.<sup>1</sup>

The present work intends to study the biofilm formation in the presence of a commercial humic material as a carbon source. A model biofilm flow cell of poliacrylic material was developed to simulate flow conditions in a river. Two identical flow cells of this type were used to compare the effect of humic material on the growth of biofilm and suspended biomass. Ten removable coupons, consisting of a commercially available thermo polypropylene material designated as Matala<sup>TM</sup>, were located inside each flow cell for biofilm sampling. The coupons' specific surface area was 203.5 m2/m3. Both experiments were carried out with synthetic river water inoculated with a community of microorganisms collected in a Wastewater Treatment Plant. One experiment used this water alone, which had a background carbon concentration of  $1.26 \pm 0.84$  mg L-1. In the other one, humic material was added to obtain a carbon concentration of  $9.68 \pm 1.00$  mg L-1. In both experiments biofilm and suspended biomass growth were studied weekly, over ten weeks, taking into account four parameters: volatile suspended solids (VSS), protein content, culturable cells and total countable cells.

Culturable and total countable cell results indicate that the presence of humic material did not significantly enhance biofilm growth. VSS and protein analyses, however, showed that the biofilm growth in the presence of humic material was higher (Figure 1). These results suggest that adscrption of humic material onto the biofilm occurred, which was further confirmed by Fourier transform infrared spectroscopy analyses. All results indicated that biomass growth was stronger in biofilm than in suspension.

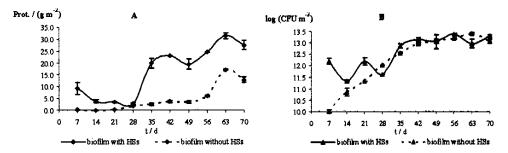


Figure 1 Biofilm growth with and without the presence of humic substances over a period of ten weeks. The protein and culturable cells per square meter are presented in panels A and B, respectively.

The biofilm's microbial community composition was analysed using the 16S rRNA approach. Biofilm formed in the presence of humic material contained bacteria belonging to the subclasses beta-Proteobacteria, Cupriavidus metallidurans and several species of the genus Ralstonia as well as gamma-Proteobacteria, represented by Escherichia coli. In the biofilm formed without humic material, the presence of beta subclass-Proteobacteria, represented by the species Variovorax paradoxus, and bacteria belonging to the group Bacteroidetes was detected. An important conclusion of the present work is that the presence of humic material did not significantly enhance the biofilm growth, but influenced the bacterial diversity in the biofilm.

Keywords: humic material; biofilm; flow cell; microbial community composition; FTIR.

<sup>&</sup>lt;sup>1</sup>IBB – Institute for Biotechnology and Bioengineering, Centre of Biological Engineering, University of Minho, Campus de Gualtar 4700-057 Braga, Portugal

<sup>&</sup>lt;sup>2</sup>Stadtwerke Düsseldorf AG-Wasserwirtschaft und Technik, Himmelgeister Landstrasse 1, 40589 Düsseldorf, Germany

<sup>&</sup>lt;sup>3</sup>Centro de Recursos Microbiológicos (CREM); Biotechnology Unit, Faculty of Sciences and Technology, New University of Lisbon, 2829-516 Caparica, Portugal

<sup>&</sup>lt;sup>4</sup>IPC - Instituto de Polímeros e Compósitos, University of Minho, 4800-058 Guimarães, Portugal