



## **Analysis of hyper-baric biofilms on engineering surfaces formed in the Deep Sea**

A. Meier (1), N.M. Tsaloglou (2), D. Connelly (2), B. Keevil (3), and M. Mowlem (2)

(1) University of Southampton, School of Ocean & Earth Sciences, Southampton, United Kingdom (a.meier@noc.soton.ac.uk), (2) National Oceanography Centre, Southampton, United Kingdom, (3) University of Southampton, Centre for Biological Sciences, Southampton, United Kingdom

Long-term monitoring of the environment is essential to our understanding of global processes, such as global warming, and their impact. As biofilm formation occurs after only short deployment periods in the marine environment, it is a major problem in long-term operation of environmental sensors. This makes the development of anti-fouling strategies for in situ sensors critical to their function. The effects on sensors can range from measurement drift, which can be compensated, to blockage of channels and material degradation, rendering them inoperative. In general, the longer the deployment period the more severe the effects of the biofouling become.

Until now, biofilm research has focused mainly on the eutrophic and euphotic zones of the oceans. Hyper-baric biofilms are poorly understood due to difficulties in experimental setup and the assumption that biofouling in these oligotrophic regions could be regarded as insignificant. Our study shows significant biofilm formation occurs in the deep sea. We deployed a variety of materials, typically used in engineering structures, on a 4500 metre deep mooring during a cruise to the Cayman Trough, for 10 days. The materials were clear plain glass, poly-methyl methacrylate (PMMA), Delrin<sup>TM</sup>, and copper, a known antifouling agent.

The biofilms were studied by fluorescence microscopy and molecular analysis. For microscopy the nucleic acid stain, SYTO<sup>®</sup>9, was used and surface coverage was quantified by using a custom MATLAB<sup>TM</sup> program. Further molecular analyses, including UV Vis spectrometric quantification of DNA, nucleic acid amplification using Polymerase Chain Reaction (PCR), and Denaturing Gradient Gel Electrophoresis (DGGE), were utilised for the analysis of the microbial community composition of these biofilms. Six 16S/18S universal primer sets representative for the three kingdoms, Archea, Bacteria, and Eukarya were used for the PCR and DGGE.

Preliminary results from fluorescence microscopy showed that the biofilm coverage on copper and PMMA was a third of that on Delrin<sup>TM</sup> and less than half the amount seen on glass surfaces. PCR showed that the microorganisms in these biofilms were predominantly Archea. DGGE conditions were optimised for the separation of PCR products from the three kingdoms. Sequencing data is still being processed. These results show that mitigation strategies are essential for any kind of long-term deployments of remote sensors even in the deep sea. Such strategies could consist for example of chlorine production through the electrolysis of seawater, back-flushing sensor channels with various chemicals, thin films of nickel/copper/zinc alloys in various ratios as surface treatments, quorum-sensing, furanone-treatment and micro-structuring of surfaces.