

# FISH AND CALCOFLUOR STAINING TECHNIQUES TO DETECT *IN SITU* FILAMENTOUS FUNGAL BIOFILMS IN WATER

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Filamentous fungi (ff) are a ubiquitous and diverse group of eukaryotic organisms. However, fungal involvement in biofilms has not been demonstrated unambiguously. They may be responsible for the production of tastes, odours and mycotoxins in water [1] and so rapid detection is important. Detection of ff by conventional methods is complex, indirect and time consuming: results can be obtained only after 3-5 days because of slow growth. In order to study the presence of ff in water biofilms two methods for direct detection were used: (a) the FISH technique which employed the EUK516 probe, 5'-ACCAGACTTGCCCTCC-3' labelled with the red Cy3 dye at the 5' terminal; (b) Calcofluor White M2R fluorescent dye which stained the fungal cells walls blue. *Penicillium brevicompactum* was used in pure culture to establish the methods and then real water biofilm samples in PVC-C and cast iron coupons were studied.

The FISH method demonstrated the filamentous structures we assume were fungi after approximately 5 hours. In contrast, the Calcofluor method revealed ff in less than one hour, and so it is possible for a rapid assessment of the presence of fungi in biofilms. When the two methods were combined additional information was possible to extract such as combining the images of empty filaments (blue) with intact protoplasm (red).

In conclusion, FISH and Calcofluor staining provide rapid, direct and unambiguous information on the involvement of ff in biofilms which form in water.

## Acknowledgements:

This work was undertaken as part of the research project supported by the European Union within the Fifth Framework Programme, "Energy, environment and sustainable development programme", n° EVK1-2002-00108. There hereby follows a disclaimer stating that the authors are solely responsible for the work. It does not represent the opinion of the Community and the Community is not responsible for any use that might be made of data appearing herein. R.R.M. Paterson was supported by the grant FRH/BPD/14923/2004 from Fundação para a Ciência e Tecnologia (FCT), Portugal.

## Reference:

[1] Paterson, R.R.M., Lima, N. (2005) Fungal contamination of drinking water. Water Encyclopedia: Water quality control. John Wiley & Sons, Inc. DOI: 10.1002/047147844X.wq1516.