

## El Research report

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An investigation of fluorescence *in situ* hybridisation (FISH) as a routine tool to monitor sulphate-reducing bacteria in oil field systems

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RESEARCH REPORT

AN INVESTIGATION OF FLUORESCENCE *IN SITU* HYBRIDISATION (FISH)  
AS A ROUTINE TOOL TO MONITOR SULPHATE-REDUCING BACTERIA IN OIL FIELD SYSTEMS

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## FOREWORD

This project was undertaken to determine whether fluorescence *in situ* hybridisation (FISH) is a suitable and practical method for use in the oil industry. It was suggested that these methods may improve upon the traditional culture-based viable sulphate-reducing bacteria (SRB) counts, and could be employed as a routine monitor for microbially influenced corrosion (MIC) and reservoir souring associated with their activity.

FISH allows the quantitative determination of the composition of complex microbial communities. Due to the challenges of identification and enumeration of many oil field microorganisms using traditional cultivation methods, several research groups and service companies already routinely apply FISH to oil field samples.

Part 1 of this report provides general information and guidance for non-microbiologists on the application of FISH. A detailed technical discussion is presented in section 2, supported by existing relevant scientific literature and data from laboratory trials. Part 3 provides an example of a general method for total cell enumeration using FISH.

It is envisaged that this report will be used by non-microbiologists to learn about the principles and application of FISH including the relative benefits of FISH as compared to traditional methods. Furthermore, it is hoped that the document will provide practitioners with detailed technical information on the methodologies.

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## SUMMARY

This project was undertaken to determine whether fluorescence *in situ* hybridisation (FISH) is a suitable and practical method for use in the oil industry. It is hoped that these methods may improve upon the traditional culture-based viable sulphate-reducing bacteria (SRB) counts, and could be employed as a routine monitor for microbially influenced corrosion (MIC) and reservoir souring associated with their activity. Significant works were undertaken to confirm that sampling, fixation, transportation, probe selection, analytical parameters and data interpretation could be optimised and standardised such that the widest range of oilfield samples could be investigated using one standard method.

The conclusion was that these methods were indeed suitable for use in the oil industry. An example of a standard method is provided detailing the procedure for the use of a non-specific general FISH probe for SRB (SRB385), and including a simple method for a direct total cell count (DAPI staining) with defined sample fixation and transportation criteria. More detailed information on the microbial population and its diversity can also be determined by employing genus- and species-specific FISH probes. However the use of these probes would mean modification of the enclosed exemplified procedure as conditions will be different for certain probes.

The document is presented in three parts:

- Part 1: General information and guidance for the non-microbiologist on the application of FISH.
- Part 2: Technical discussion, data from laboratory trials and literature studies to verify these methods.
- Part 3: An example of a general method for DAPI staining and FISH using the general SRB probe SRB385.