EI Research report

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

RESEARCH REPORT

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

1st edition

November 2009

Published by
ENERGY INSTITUTE, LONDON
The Energy Institute is a professional membership body incorporated by Royal Charter 2003
Registered charity number 1097899

The Energy Institute (EI) is the leading chartered professional membership body supporting individuals and organisations across the energy industry. With a combined membership of over 13 500 individuals and 300 companies in 100 countries, it provides an independent focal point for the energy community and a powerful voice to engage business and industry, government, academia and the public internationally.

As a Royal Charter organisation, the EI offers professional recognition and sustains personal career development through the accreditation and delivery of training courses, conferences and publications and networking opportunities. It also runs a highly valued technical work programme, comprising original independent research and investigations, and the provision of IP technical publications to provide the international industry with information and guidance on key current and future issues.

The EI promotes the safe, environmentally responsible and efficient supply and use of energy in all its forms and applications. In fulfilling this purpose the EI addresses the depth and breadth of energy and the energy system, from upstream and downstream hydrocarbons and other primary fuels and renewables, to power generation, transmission and distribution to sustainable development, demand side management and energy efficiency. Offering learning and networking opportunities to support career development, the EI provides a home to all those working in energy, and a scientific and technical reservoir of knowledge for industry.

This publication has been produced as a result of work carried out within the Technical Team of the Energy Institute (EI), funded by the EI's Technical Partners. The EI's Technical Work Programme provides industry with cost-effective, value-adding knowledge on key current and future issues affecting those operating in the energy sector, both in the UK and internationally.

For further information, please visit **http://www.energyinst.org**

The EI gratefully acknowledges the financial contributions towards the scientific and technical programme from the following companies

BG Group
BP Exploration Operating Co Ltd
BP Oil UK Ltd
Centrica
Chevron
ConocoPhillips Ltd
EDF Energy
ENI
E. ON UK
ExxonMobil International Ltd
Kuwait Petroleum International Ltd
Maersk Oil North Sea UK Limited
Murco Petroleum Ltd
Nexen
Saudi Aramco
Shell UK Oil Products Limited
Shell U.K. Exploration and Production Ltd
Statoil Hydro
Talisman Energy (UK) Ltd
Total E&P UK plc
Total UK Limited

ISBN 978 0 85293 546 0

Published by the Energy Institute

Further copies can be obtained from: Portland Customer Services, Commerce Way, Whitehall Industrial Estate, Colchester CO2 8HP, UK.
t: +44 (0)1206 796 351 e: **sales@portland-services.com**

Electronic access to EI and IP publications is available via our website, **www.energyinstpubs.org.uk**.
Documents can be purchased online as downloadable pdfs or on an annual subscription for single users and companies.
For more information, contact the EI Publications Team.
e: **pubs@energyinst.org**

CONTENTS

Contents cont. **Page**

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.

ACKNOWLEDGEMENTS

This project was commissioned by the Energy Institute's Microbiology Committee. The work was undertaken by Dr Heike Hoffmann, Dr Carol Devine and Dr Stephen Maxwell (Intertek Commercial Microbiology) and steered by members of the Microbiology Committee, who during the project cycle included:

Simon Ashton	ExxonMobil
Paula Carberry	QinetiQ
Simon Christopher	BP
Brian Crook	HSL
Carol Devine	Intertek Commercial Microbiology
Bob Eden	Rawwater Engineering Company Ltd
Graham Hill	ECHA Microbiology
Kerry Hoad	Energy Institute
Joan Kelley	CABI
Joachim Koenig	Consultant
Jan Larsen	Maersk Oil & Gas
Bart Lomans	Shell
Jenny Lyn	Energy Institute
Torben Lund Skovhus	Danish Technological Institute
Elaine McFarlane	Shell Global Solutions
Andrew Price	Oil Plus
Tony Rizk	Saudi Aramco
Jim Stott	Intertek - Capcis
Ian Vance	Centromere Ltd

The Institute wishes to record its appreciation of the work carried out by the authors and also its gratitude for the valuable contributions made by the steering panel during the course of the project.

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.