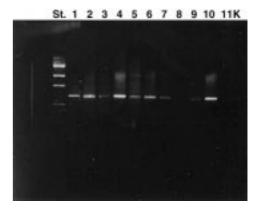
# Engineering (No.1 - 1998

# Detection of thermophilic bacteria in oil reservoirs

Oil reservoirs may be contaminated with bacteria, organisms known to cause corrosion, souring of oil wells or plugging of sediments reducing pressure during production. This contamination is usually associated with the presence of significant concentrations of thermophilic sulphate-reducing bacteria (thSRB) in the reservoir water, requiring temperatures above 60-70°C for optimal growth (in contrast to mesophilic bacteria which normally grow at temperatures below 40-50°). Heterotrophic thSRB have traditionally been detected in reservoir water by culture methods. Samples are cultivated in synthetic media containing simple organic components under anoxic conditions and high temperature. By using dilution series viable thSRB have been cultivated by Most-Probable Number counts (MPN). From these enrichment cultures bacterial strains can be isolated and characterised. In addition the polymerase chain reaction (PCR) amplification method can be utilised for detection of low concentrations of bacteria in the reservoirs. With the PCR method specific DNA sequences are repeatedly synthetized by repeated temperature profiles. After 30 temperature cycles a single DNA is theoretically synthetized to approximately 10<sup>9</sup> copies. The PCR method can be used for characterisation of bacteria by amplification of ribosomal DNA sequences defining specific groups of microorganisms phylogenetically. The PCR method is rapid and final results may be obtained within 2 days after start of sample processing. The detection limit in our hands has been close to 10 bacteria per sample. Figure 1 gives an example of how PCR products are visualised by electrophoretic methods. At SINTEF Applied Chemistry we have been working with detection and

quantification of bacteria causing problems in oil reservoirs. A PCR amplification system for detection of groups of bacteria in water samples from oil reservoirs was developed, i.e. methanogenic bacteria and various groups of SRB. Positive PCR products defining groups of SRB were detected as single bands by horizontal gel electrophoresis, as shown in Figure 1. Culture methods (MPN-counts) revealed significant concentrations of viable thSRB in samples with positive PCR. In conclusion the combined methods of PCR and MPN-counts can be used for diagnosing microbiological fouling problems of oil wells.



### Figure 1.

PCR products amplified from 30 ml produced water from various oil reservoirs (samples 1-11). The samples were amplified with a PCR system defining the SRB genera Desulfovibrio and Desulfobulbus. Estimated size of the PCR products is 520 base pair (BP). St is a DNA standard with bands of known no. of bp. 11K is a negative control.

### Contact persons:

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# **Methods used for Compost Stability Assessment**

Compost stability has interested operators of composting plants and compost users for decades. Knowledge of the rate of stabilisation and the stability of the products permits adjustments of the process and give possibilities for making comparison of process efficiencies among different systems. The environmental authorities make demands to compost stability, but do not suggest methods. To day simple methods measuring the self-heating potential in the compost is most common in use (Dewar test). The method is rather time-consuming, and the reliability of the results are debatable. The density and moisture content of the compost critically affect the results of the self-heating tests.

## **Electrochemical respiration test**

Electrochemical respiration measurements at SINTEF are carried out in a Sapromat respirometer (Voith). The results of a typical electrolytic respirometer test are presented in table 1. They show significant differences in the oxygen consumption rates between the sludge and the different compost samples. The highest rates were observed during the first 24 hours making it possible to distinguish between the different compost samples during a one day test.

Material and conditions	Ripening time	Dry matter (%)	рН	Sapromat BOD <sub>max</sub>	Sapromat BOD <sub>1</sub>	Sapromat BOD <sub>7</sub>
Sludge	-	25,8	6,6	9,6	7,6	3,53
Low stabilit	3-4 days	32,9	8,4	5,5	4,4	1,63
Middle stabilit	15 days	51,8	7,6	2,3	1,4	0,80
High stabilit	> 60 days	52,4	7,9	0,8	0,7	0,83
Bio compos	8 month	52,1	8,7	0,1	0,1	0,10

Table 1

Ripening time, dry matter, pH and oxygen consumption rates in sludge and compost samples measured in the electrolytic respiration test (Sapromat). The oxygen consumption rates are expressed in mg  $O_2$  gram dry matter <sup>-1</sup> hour <sup>-1</sup>

BOD<sub>max</sub>
BOD<sub>1</sub>
Maximum oxygen consumption rate
Oxygen consumption rate measured as an average during 24 hours.
BOD<sub>7</sub>
Oxygen consumption rate measured as an average during 168 hours



Sewage sludge and unstable compost produce strong and unpleasant smells.

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# Planning Underway for SINTEF's International Marine Environmental Modelling Seminar'99

The 1998 IMEMS in Lillehammer in early March was a resounding success, judging from the feedback we have received to date. Over 40 papers were presented to the 75 participants, representing 15 countries from around the world. These papers are now in review for publication in <u>Environmental Modelling and Software</u> (Elsevier) and <u>Spill Science and Technology Bulletin</u> (Pergamon).

We wish to thank our sponsors, Norsk Agip, Statoil, and Norsk Hydro for their financial assistance, which contributed significantly to the success of the arrangement.

Our congratulations to the winners of the sports competitions, with special applause for the Japanese, Italians and Dutch for beating the Norwegians at their own games!

Group A	Winner	Country	
Rump-Racer	Takashi Kojima	Japan	
	Guido Crispi	Italy	
Sled-Racer	Lena Ringstad-Olsen	Norway	
Group B	Winner	Country	
Rump-Racer	Steinar Sanni	Norway	
Itump-Itacei	Job Barretta	Netherlands	
Sled-Racer	Ivar Singsaas	Norway	

**O** STATOIL

Plans are now being made for IMEMS '99, which will again be held in Lillehammer. We invite you all to join us in 1999, especially the winners of the 1998 sporting events, who must be prepared to defend their titles!

Check our web site: <u>www.sintef.no/units/chem/</u> <u>environment/</u> for the latest information, or contact the seminar secretariat <u>may.ditlevsen@chem.sintef.no</u>.

Looking forward to next year!

Mark Reed, Chairman



# Two new members have recently joined the department, Janne Lise Myrhaug and Alf G. Melbye.

Janne Lise Myrhaug holds an MSc in chemistry from the Norwegian University of Science and Technology in Trondheim. She has just finished her master. The master was a joint project between Statoil research centre and the Norwegian University of Science and Technology in Trondheim . She was working with decahydronaphthalene as a source identification of oil contamination in fish. Her main fields of competence are:

- Source identification of oil contamination;
- Collecting and preparing fish species for experiments;
- Development of methods for exposing fish to offshore chemicals;
- Extraction and clean up of fish-samples;
- Analysis and behaviour of offshore chemicals;
- General water chemistry;

Janne will be a key-person in on-going characterisation of oil and offshore chemicals and their behaviour under different sea and weather conditions. Alf G. Melbye also holds an MSc in chemistry from the University of Trondheim. He has a total of 8 years of experience in research and consultancy, working mainly with environmental chemistry in connection with the petroleum industry. He has been working as an assistant lecturer at The University of Trondheim, has a four year period as a research scientist at Statoil Research Centre in Trondheim and was recently employed as a senior environmental chemist by Det Norske Veritas. His main fields of competence are:

- Analytical organic chemistry (sample preparations, analysis, and development of laboratory methods);
- Source identification of oil contamination;
- · Field sampling and experiments;
- Environmental impact assessments;
- End-disposal of offshore structures;
- Life cycle assessments;
- Project management;

Alf will be a key-person in on-going research projects dealing with chemical characterisation (including dissolution, determination of degradation and adsorption) of oil components in the sea, and development of laboratory methods for environmental analysis of oil components and offshore chemicals

### **Environmental Engineering**

# Workshop on oil slick thickness colour code.

In cooperation with the Norwegian Pollution Control Authority (SFT), SINTEF Applied Chemistry, Environmental Engineering arranged a workshop at the SFT location in Horten, March 2nd, 1998. Delegates from 7 Bonn Agreement Countries (Denmark, Sweden, Finland, Germany, the Netherlands, UK and Norway) participated. The aim was to present and discuss the recent findings from available literature documentation and laboratory experiments on colour-oil film thickness correlations carried out at SINTEF. This is a part of an ongoing project funded by 5 Bonn Agreement countries.

Visual observation of oil slicks using surveillance aircraft is the most common method for oil spill volume estimation. While experienced observers can achieve positive identification of an illegal discharge, more precise volume estimation based on the <u>colour</u> and <u>area</u> of spills has been identified as a complex task.

While basic colour and thickness correlation, i.e. the colour code, may be a good indicator for <u>thin</u> oil films there is a potential for a significant

improvement to the overall understanding of this correlation, hence improving the accuracy of oil volume estimation.

The current colour code used by most European countries is based on exercises and research conducted more than 10 years ago. Since illegal spills are easily identified visually, the main purpose of the colour code is to classify an oil spill as "combatable" or "noncombatable". Volume estimation is also important for response commanders when assessing need for recovery equipment and tank storage capacity of recovered oil.

Oil film thickness varies over a very wide range - sub-micron up to several millimetres. A small difference in colour reflects a significant difference in film thickness. Hence, colour perception - together with precise area calculation - is important for volume estimation.

In addition to a literature survey, SINTEF has performed an extensive and systematic laboratory test program to validate and calibrate present colour code



Environmental Engineering N-7034 Trondheim, Norway Phone: + 47 73 59 30 00 Fax: + 47 73 59 70 51(aut.) may.ditlevsen@chem.sintef.no manuals based on visual observations, in order to improve the quantification of oil slicks with thin oil films. An experimental procedure has been established for photographing oil films in small static laboratory tray systems, with focus on the apparent colour of the oil film. The test program included experimental series with ten oil types with different physical and chemical properties, and seven different oil film thicknesses in the range of 0.5-200µm.

The next phase in this project will be further investigation / verification of laboratory findings by field experiments.

Depending on the documentation obtained, the project may end up with a proposal for an amendment of the existing colour code.

### For further information contact:

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