

**RECOVERY OF MICROORGANISMS/INTACT MEMBRANE LIPIDS (IPLS)
FROM OIL/WATER MIXTURES—LAB EXPERIMENTS AND NATURAL WELL-
HEAD FLUIDS**

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Most of the world's remaining petroleum resource has been altered by biodegradation which has a negative impact on oil quality and production. Petroleum biodegradation proceeds in any oil reservoir that has a water leg and has not been heated to temperatures over 80°C. Microbial activity in deep sediments is known for over 75 years. However, relatively little is known about the microbial populations involved in subsurface hydrocarbon degradation. A better understanding of microorganisms resident and active in petroleum microcosms, their physiological properties and then geochemical roles will aid both oil exploration and production. Although the research in petroleum microcosms is strongly increasing in the last decade little success is reported until yet.

The main challenge is the difficulty of detecting microorganisms in natural oil/water mixtures which often occur as emulsions at the well-heads. Two reasons are behind this difficulty. Firstly, the most active biodegradation may have been peaked long ago and therefore the original petroleum microorganisms who degraded the oils are not present anymore; or secondly, the majority of microbial cells remain stuck to the oil phase after separation of the oil phase from the water phase whereas the water phase is most commonly investigated for microorganisms.

To investigate the latter case, we carried out experiments spiking oil/water mixtures with microbial cells and in comparison analyzed many natural well-head fluids from the Western Canada Sedimentary Basin (WCSB). We analyzed the IPLs of microorganisms, such as phospholipids and corresponding archaea-related tetraethers using liquid chromatography – mass spectrometry (LC-MS) techniques in combination with microbiological 16S rRNA genes analysis. While microbiology has been able to isolate microorganisms from deep sediments contamination is a major problem, especially in petroleum reservoirs where drilling mud contamination is common. Intact polar membrane lipids (IPLs) have better prospects in some settings as biomass chemotaxonomic indicators as they can accumulate in the oil column in the reservoir at natural abundance levels in petroleum systems. Even though

developments in LC-MS techniques allow us to now detect relatively low concentrations of biomass, the natural abundance of microorganisms has still to be much higher than using microbiological techniques but contamination issues are less severe when PCR amplification is not involved. The objective was to determine the recovery of microorganisms/IPLs in experimental oil/water mixtures and relate their occurrence to the diversity of microorganisms in natural fossil fuel reservoirs. Table 1 shows the conditions and results of lab experiments for estimating the recovery of microorganisms/IPLs using an oil field isolate *Desulfovibrio* sp. strain Lac6 [U46522 ("clone DVIB57")] (Voordouw et al., 1992). The methods can successfully recover organisms and their lipids. Using this method we discuss the distribution of lipids in several heavy oilfield waters.

Experiment	Water/oil ratio	conditions	All PG+PE [% recovery]	Single dominant PG+PE [% recovery]
Lac6	-	Culture; same cell number	100	100
Shaker	50/50	170 rpm; 4 h, 30°C	74.2	74.2
Shaker	70/30	170 rpm; 4 h, 30°C	83.4	83.4
Blender	50/50	5 min 20-22k rpm only oil +5 min 16-18k rpm incl. microorganisms; 50°C	58.6	58.7
Blender	70/30	5 min 20-22k rpm only oil +5 min 16-18k rpm incl. microorganisms; 50°C	61.6	61.6
Ultrasonic probe	70/30	Pulsemode 50%; approx.35 W; 8 min, 25°C	80.8	80.8

Table 1. Conditions and results of the lab experiments for estimating the recovery of microorganisms/IPLs using an oil field isolate *Desulfovibrio* sp. strain Lac6 [U46522 ("clone DVIB57")] (Voordouw et al., 1992).

REFERENCES

- Voordouw, G., Voordouw, J.K., Jack, T.R., Foght, J.M., Fedorak, P.M., and Westlake, D.W.S. (1992) Identification of distinct communities of sulfate-reducing bacteria in oil fields by reverse sample genome probing. *Applied Environmental Microbiology* **58**: 3542–3552.