

# Subsurface metagenomics, functional microbial diversity analysis and gene discovery in deep and hot petroleum reservoirs - Bi-annual report

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Stable isotope probing (SIP) is dependent on the presence of an active growing population of microorganisms that are capable of assimilating a suitably labelled substrate. Unfortunately, this was not the case for produced water from the Valhall oil field (down hole temperature 90-100°C). Water sampled from the well head was found to contain no observable cells, nor could any DNA be isolated. The large quantity of DNA that was previously isolated from Valhall production water and described in the original application, is explained by that water having actually been sampled from a primary oil separation tank (~40°C), this information only became apparent after the project had started. When the original DNA sample from the tank was compared on the basis of 16s rRNA sequencing to a later sample, it was found that there was no overlap between the microbial populations. The earlier sample was dominated by clostridia and epsilon proteobacteria, whereas a library from the second sample was dominated by sequences most strongly related to a pelobacter species. The lack of concordance between the samples taken from the produced water system, suggests it is unlikely that it represents an accurate proxy for conditions in the actual reservoir.

In the absence of any other ideas, I decided to take a closer look at *Anaerophaga thermohalophila*, an organism which has been identified in many mixed oil/water environments, including the Valhall produced water system. Initial genomic sequencing using the Illumina system by itself didn't provide a satisfactory genome assembly and at that time (August 2010) the Norwegian Sequencing Centre appeared to have a large backlog of work: so I decided to employ a commercial sequencing company, GATC-Biotech to repeat the sequencing using the 454 system. However, one year later and multiple machine runs later they still haven't delivered data of the required quality. When they do finally provide adequate results, the draft sequence of the *A. thermohalophila* type strain (accession no. AEWI00000000) will be updated, and the as yet private sequence from the Valhall isolate will be annotated to reflect the differences between it and the type strain.