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Microbial community analysis of three hydrocarbon reservoir cores provides valuable insights for the assessment of reservoir souring potential

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ABSTRACT

Three hydrocarbon reservoir cores were obtained from a high temperature non-waterflooded offshore reservoir. All three cores were taken from a 56-m section of the same well. Under sterile conditions, DNA was recovered from the inner section of each core and the microbial community profiles were deduced by sequencing the 16S rRNA marker gene. Taxonomic analysis of the Operational Taxonomic Units (OTUs) recovered, identified a high proportion of members from the *Oxalobacteraceae* family (38.5%) followed by members from the *Pseudomonadaceae* and *Comamonadaceae* families (29.1% and 12.8% respectively). Representatives of all these families are known to degrade hydrocarbons as well as to use nitrate as a terminal electron acceptor under anaerobic conditions. Assuming these predominant microorganisms are indigenous to the reservoir and have not been introduced with the drilling fluids they might exhibit a relatively rapid response to nitrate injection for souring control. On the contrary, very few sulfate reducing bacteria (SRBs) were detected in these cores (<0.01%) suggesting unfavorable conditions to SRB growth. This however may well rapidly change upon seawater injections in the absence of nitrate addition.

This study sets the microbial profiling "baseline" for the prediction of souring through modelling as well as for any upcoming biomonitoring surveys.

1. Introduction

Subseafloor sediments and deep subsurface biosphere ecosystems host a large number of microbial cells (2.9×10^{29} cells) with a range of metabolic capabilities (D'Hondt et al., 2004; Kallmeyer et al., 2012). Although bacterial abundance decreases logarithmically with depth, active microbial communities have been detected down to 2500 m below the seafloor (Inagaki et al., 2015) and bacterial endospores have also been detected in deeply buried sediments (Lomstein et al., 2012). Temperature, which increases linearly along a gradient of 24°C–36 °C per kilometer of depth, is considered as one of the major limiting factors for life in subseafloor sediments. Temperatures close 100 °C, found theoretically around 3 km below the seafloor, normally denature proteins, membranes and nucleic acids, inhibiting most microbial life (Rothschild and Mancinelli, 2001). Furthermore, prolonged exposure to high temperature, also known as paleo-pasteurization, can limit microbial life in subsurface sediments (Adams et al., 2006). The availability of nutrients and degradable substrates is also a strong limiting factor for microbial growth and activity in subsurface environments (Head et al., 2003). However, within the large expanses of nutrient and substrate poor sediments of the deep biosphere, oil reservoirs might be considered oases. Indeed, oil reservoirs harbor a large variety of hydrocarbon and/or gas deposits which can be degraded and used as carbon sources by microorganisms (Head et al., 2003). Furthermore,

Abbreviations: MIC, microbially influenced corrosion; SRBs, sulfate reducing bacteria; NRBs, nitrate reducing bacteria.

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sulfur and nitrogen containing compounds in oil (e.g. benzothiophenes and benzocarbazoles) might provide nutrient sources for these activities (Oldenburg et al., 2006). Therefore, higher microbial activities have been predicted to occur in oil reservoirs versus other deep biosphere habitats especially at oil water transition zones at the base of the oil column (Bennett et al., 2013; Head et al., 2014). The deepest signatures of heavy oil biodegradation were observed in 4 km deep reservoirs (Jones et al., 2008) and drilling expeditions have detected bacteria in coal shale down to 2 km below the seafloor (Fry et al., 2009). However, despite these indicators for microbial life in deep oil reservoirs, the presence of indigenous microbial communities within oil reservoir remains controversial (Ollivier and Magot, 2005). Indeed, based on the logarithmic decrease of cell abundance, indigenous bacterial abundances should be very limited and drilling methods are suspected of contaminating recovered samples through exposure to additional microbes during drilling or core recovery.

During the first years of oil reservoir exploitation, the microbial component of reservoirs has been largely ignored and uninvestigated by oil companies. However, offshore technologies such as seawater injection for secondary oil recovery and production enhancements have considerably changed the situation. Indeed, corrosion issues at oil producing facilities are frequently reported and microbial communities appear to be largely responsible for these significant damages, generally referred to as microbially influenced corrosion (MIC) (Little et al., 2007; Vigneron et al., 2016). Likewise, producing wells with injection water breakthrough frequently show signs of souring (hydrogen sulfide in the produced oil) due to microbial activities (Hubert and Voordouw, 2007). Numerous investigations have highlighted the role of sulfate reducing bacteria (SRBs) in these processes (Enning and Garrelfs, 2014) and the addition of nitrate to the injection water has been adopted as a common procedure to limit the growth of sulfate reducing microorganisms (Hubert and Voordouw, 2007). Nitrate has potentially three inhibitory effects on sulfate reducing bacteria: i) Many sulfate reducing bacteria are also nitrate reducers and will shift their metabolism to nitrate reduction which is energetically more favorable, leading to reduced sulfide generation. ii) Nitrite, the product of nitrate reduction is an enzymatic inhibitor of the enzyme involved in sulfate reduction. iii) Nitrate reducers may outcompete sulfate reducers for electron donors (Hubert and Voordouw, 2007). However, the success of this treatment appears to depend on different physicochemical parameters such as the downhole temperature or the retention time of injected water in the oil reservoir as well as the initial microbial community (Gittel et al., 2009; Liebensteiner et al., 2014; Shartau et al., 2010). Therefore, it is extremely advantageous to estimate the metabolic potential of the indigenous microbial community of an oil reservoir.

In this study, 16S rRNA gene analyses were used in conjunction with a comprehensive mineralogical and geochemical analysis of core samples collected from an undeveloped field in south east Asia as part of a souring potential assessment. The primary goal of this study was to investigate the presence of sulfate (and sulfur) reducing bacteria (SRBs) and nitrate reducing bacteria (NRBs). Further, the study attempts to evaluate the likelihood of reservoir souring due to the presence (or absence) of indigenous SRB. Strong SRB presence would suggest favorable conditions for their growth in a reservoir. In addition, the existence of indigenous NRBs was investigated which in the presence of nitrate as electron acceptor would be able to outperform the SRBs and prevent reservoir souring from happening. To address these questions, the microbial communities from three reservoir cores taken from the reservoir were analyzed.

2. Materials and methods

2.1. Sample description

The core samples described in this study were collected to assist with defining the baseline (*in-situ*) parameters of an undeveloped, exploratory field. Core materials used in this analysis are from a single test well drilled as part of the field's development plan, which included a reservoir souring potential assessment study. The field consists of mildly over-pressured shallow marine Miocene top-set sands. The average reservoir temperature is 95 °C. The test well was drilled and cored using SYN-TEQ oil-based drilling fluids (Baker Hughes, Houston, TX, USA). All core pieces originated from the same bore hole and the collection depths ranged between 2.8 and 2.9 km below the median sea level (Fig. 1). Upon subsampling, three pieces of reservoir core material were dipped in wax in the field for preservation before being sent for analysis. The waxed cores were transported at ambient temperature and reached the molecular biology lab within a month of their retrieval. Once in the lab, cores were stored at 5 °C and processed within 2 weeks of their arrival. DNA isolated from the cores was stored at −20 °C until needed for amplicon preparation. Best practices for sample preservation and prevention of contamination were followed as described previously (Tsesmetzis et al., 2016a).

2.2. CT scanning

Prior to genomic analysis the drill cores were subjected to computed tomography (CT) scanning in order to assess their integrity, drilling mud intrusion and homogeneity using Shell's in-house Siemens Volume IV medical CT-scanner. During this operation the samples remained preserved in the wax. The helical scanner has a resolution of sub-millimetre scale allowing the detection of any (natural or induced) cracks and large heterogeneities.

2.3. Petrographical and chemical analyses

X-Ray diffraction analysis (XRD) was executed on one sample (Table 1). A portion of each sample was gently crushed, mixed with distilled water plus a few drops of ammonia as a dispersant and placed in an ultrasonic bath for 30 min to release the maximum amount of clay into suspension. The clay suspension was then centrifuged to deposit the entire <2 μm fraction, which was filtered (mixed with a little distilled water to make a thick slurry) onto unglazed ceramic tiles. A further portion was ground to a powder in a McCrone micronising mill (using industrial methylated spirits to minimize structural grinding damage). The dried powder was side-packed into a powder diffraction holder. The sample was scanned on a Siemens PSD X-ray diffractometer using Ni-filtered CuKα radiation. The clay tile was scanned using a 0.02° step width, with 0.2 mm slits from 2 to 40° 2θ. The tile was scanned again after treating with glycol, after heating at 400 °C for 4 h, and after heating at 550 °C, also for 4 h. The whole rock powder sample was scanned with a 0.02° step width, and 0.2 mm slits, from 5 to 70° 2θ.

2.4. Core cutting

The sampled reservoir cores contained considerable amounts of hydrocarbons and very little biomass. Both of these factors can negatively impact the isolation of sufficient amounts of DNA for downstream processing (i.e. DNA amplification, DNA sequencing, etc.). Moreover, any external exposure of reservoir cores to their surroundings contaminates them with exogenous microbes which can significantly distort the picture of the *in-situ* reservoir microbial

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