

Shifts of the indigenous microbial communities from reservoir production water in crude oil- and asphaltene-degrading microcosms

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ABSTRACT

Microbial metabolism of crude oil is an important process in both microbial enhanced oil recovery and bioremediation. Recently, diverse microorganisms have been detected and isolated from oil reservoirs. In this work, crude oil- and asphaltene-degrading microcosms were constructed using two different reservoir production water samples, which had distinct bacterial communities. After two three-week enrichments culture, GC-MS and FT-IR analysis showed that crude oil and asphaltene were biodegraded. Microbial communities in microcosms using the same carbon source showed high similarity, which suggested similar processes of microbial succession in both crude oil- and asphaltene-degrading consortia. *Parvibaculum*, *Pseudomonas*, *Alcanivorax*, *Devosia*, *Hydrogenophaga* and *Dietzia* were found in all crude oil degrading microcosms and *Parvibaculum*, *Alcanivorax*, *Hyphomonas*, *Flavobacterium* and *Reyranella* were found in all asphaltene degrading microcosms, which might play important roles in crude oil or asphaltene degradation. The results indicated that reservoir production water might serve as a microbial species pool which contained the indigenous core microbiomes for crude oil degradation. This work provided new insights into the understanding of microbial diversity in reservoir production water and the potential role of reservoir production water as a microbial species pool for oil degrading microorganisms.

1. Introduction

Crude oil was the most consumed primary energy resource (Chow et al., 2003). An increased demand for crude oil has given a strong impetus to the development of enhanced oil recovery (EOR) technologies. Among these, microbial-enhanced oil recovery (MEOR), which was suggested as early as 1926 by Beckman (Donaldson et al., 1989), has been investigated over the past two decades because it is environmentally friendly, as MEOR products are all biodegradable and will not accumulate in the environment (Lazar et al., 2007). To use MEOR, microorganisms or nutrients are injected into oil wells to recover residual oil by microbial crude oil metabolism (Bachmann et al., 2014). Besides MEOR, microbial crude oil metabolism is also essential for bioremediation crude oil components.

Crude oil is a complex mixture of thousands of chemicals, which can be divided into four classes: saturates, aromatics, asphaltenes, and resins. Several bacterial strains and the key enzymes involved in saturate and aromatic degradation have been reported under aerobic or

anaerobic conditions (Mbadinga et al., 2011; Nie et al., 2014; Rojo, 2009; van Beilen and Funhoff, 2007; Widdel and Rabus, 2001). However, there are few reports on the biological degradation of asphaltenes. A few studies reported that asphaltene could be degraded by microorganisms (Leahy and Colwell, 1990; Pineda-Flores and Mesta-Howard, 2001), but the degrading ratio was low and the mechanisms were still unclear.

Oil reservoirs are an important component of the deep biosphere, where indigenous microbial communities have existed over millions of years (Li et al., 2017a). For many oil reservoirs, following the primary recovery of oil by the natural pressure within the reservoirs, the secondary recovery methods such as water injection and the tertiary recovery methods such as CO₂ injection are used to recover the residual oil. Chemical compounds, such as electron acceptors, donors, nutrients have been injected into the oil reservoirs. Water injection also increased the concentrations of oxygen in the shallow subsurface zones of oil reservoirs. These injected substances changed the microbial community composition, dominated by slow-growing anaerobes, to a higher

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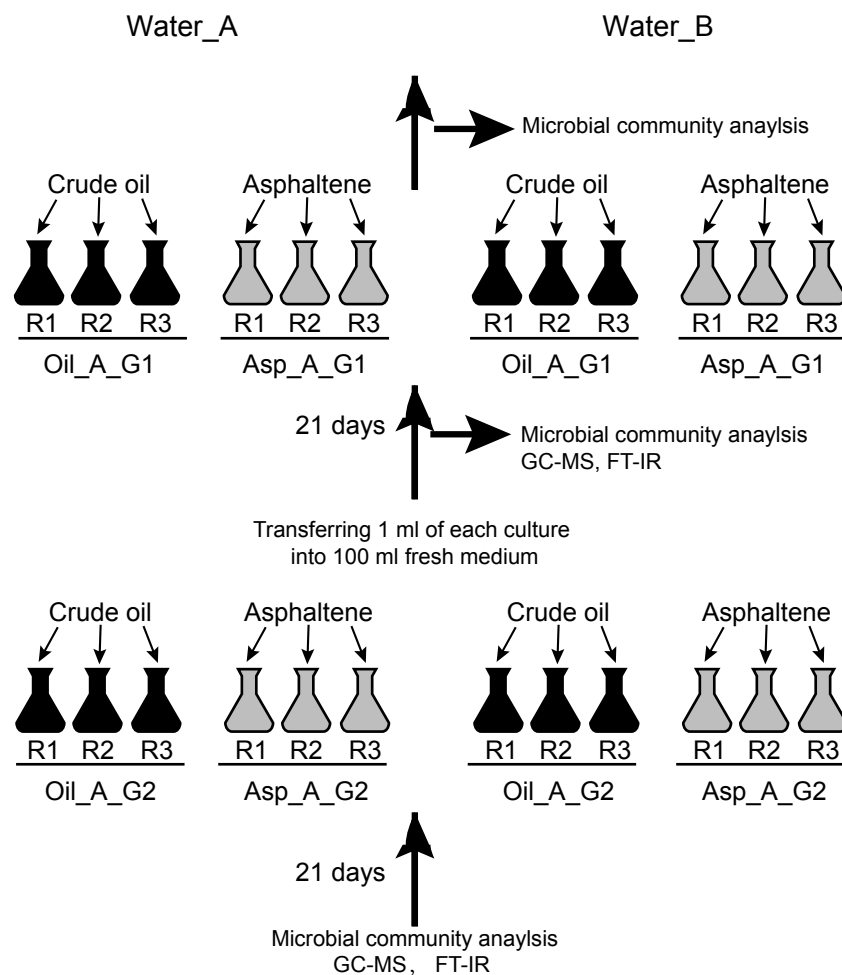


Fig. 1. Schematic procedure for microcosm construction.

abundance of faster-growing microorganisms (Vigneron et al., 2017). It is interesting that the chemicals and exogenous microorganisms in the injection water can change the composition of microbial communities in the reservoir. After a long period of water flooding, a new stable community, distinct from the one in the injection water, is formed. For example, comparative analysis of microbial communities in reservoir production water and in injection water showed enrichment of *Gammaproteobacteria* in reservoir production water (Cai et al., 2015; Ren et al., 2011; Tang et al., 2012). A high abundance of putative mesophilic aerobic hydrocarbon-degrading bacteria, including *Pseudomonas*, *Bacillus*, and *Acinetobacter* genera, has been found in oil reservoirs worldwide (Dahle et al., 2008; Gittel et al., 2009; Yamane et al., 2008). The effect of injection water on microbial community composition in reservoir production water also depends on the *in situ* temperature of the oil reservoirs (Wang et al., 2012). The higher the temperature of the reservoir was, the less the effects of the microorganisms in the injection water on the microbial community in the reservoir production water were (Zhang et al., 2012). It might be due to the poor adaptability of exogenous microorganisms in oil reservoirs. For example, the exogenous microorganisms *Enterobacter*, which could enhance oil recovery in the core flooding experiment (Sarafzadeh et al., 2014), were barely able to survive in the field trial of MEOR even with nutrients injected (Zhang et al., 2010). These studies suggested a relatively static microbial community in the oil reservoir after long-term exploitation.

Extreme environmental conditions in oil reservoirs, such as high concentrations of hydrocarbons, and sometimes high temperature and salinity (Lewin et al., 2013), provide strong selective stress on the composition of microbial communities. As a result, microorganisms

surviving in oil reservoirs are thought to have the ability to adapt to environments with high concentrations of crude oil. Metagenomic analyses showed diverse metabolic genes in reservoir production water, such as a high abundance of hydrocarbon degradation-related genes (Cai et al., 2015; Nie et al., 2016), supporting the role of reservoir production water and crude oil as a putative microbial pool of crude oil degraders, which were dormant in the reservoirs and could respond to favourable conditions. There are some interesting questions about microorganisms in oil reservoirs, e.g. whether the rare species (Jousset et al., 2017) in the oil reservoirs can be activated under certain conditions. Recent studies showed that the electron acceptors and temperature had strong influences on the microbial community composition of reservoir production water under anaerobic conditions (Li et al., 2012, 2017b). However, how the microbial communities from different reservoir production water change under the same conditions is still unclear.

In this work, to investigate the role of reservoir production water as a microbial species pool for crude oil degraders and the change of microbial communities during oil degradation, we constructed crude oil-degrading and asphaltene-degrading microcosms using reservoir production water from two oil wells. The profiles of the microbial communities from the reservoir production water and the enrichment cultures were compared. Results from this work revealed that diverse hydrocarbon degraders were activated during enrichment, although some were not detected in reservoir production water used in the present method.

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