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Effects of oxygen injection on oil biodegradation and biodiversity of reservoir microorganisms in Dagang oil field, China

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ABSTRACT

Effects of oxygen inoculation were investigated on oil biodegradation and biodiversity of reservoir microorganism in Dagang oil field. The biodegrading was evaluated and analyzed or estimated by using in-vitro enrichment, denaturing gradient gel electrophoresis, real time PCR and GC–MS. The bacterial communities of production water containing reservoir microorganism were 30% lower than groundwater and injection water despite that all of them have rich biodiversity. After thirty days of oxygen treatment the *n*-alkanes degradation were 92.5% in injected water while 44.3% in groundwater and 92.4% in production water. The selected PAHs biodegradation rate in production water was lower as compared to the injection water but significantly higher than groundwater. The total bacterial biomass gradually increases after the oxygen injection and then decreases. The results suggested that aerobic condition could accelerate petroleum biodegradation and changes the microbial community structure in oil reservoir, which might be beneficial to MEOR.

Introduction

Petroleum is a major source of energy for the dynamic development of the world. The world demand for oil has been intensifying steadily over the past 20 years and reached more than 80 million barrels per day (Hasan et al., 2010). Only one-thirds of the oil in the underground natural storage can be extracted through primary and secondary production due to initial reservoir pressure and water flooding respectively (Li et al., 2002; Brown, 2010; Gudiña et al., 2012). Microbial enhanced oil recovery (MEOR) is a technique which utilizes microorganisms and their metabolic products to improve the recovery of entrapped crude oil from reservoir after an injection of nutrients and cultivated-exogenous microorganisms. But the application of an exogenous microorganisms in MEOR faces some technical difficulties such as the plugging of wells by injected bacteria themselves, the by-products of metabolism and corrosion problems (Brown, 2010). Although many solutions using spores, ultramicrobacteria or a lysogenic strain of bacteria instead of vegetative cells have been proposed, these problems still exist. Moreover, injected bacteria hardly penetrate into the oil-bearing sections (Kobayashi et al., 2012).

In order to solve the technical difficulties of using exogenous microorganisms, activating indigenous microorganisms to enhance oil recovery (AIMEOR) has been proposed this is a potential strategy in oil production (Belyaev et al., 2004; She et al., 2011). The presence of microorganisms in crude oil reservoirs has been known for more than 80 years and indigenous bacteria has been demonstrated existence in water flooding oil recovery stage (Soudmandasli et al., 2007; Dahle et al., 2008). Ren et al. (2011) have highlighted the great diversity of indigenous microorganisms. AIMEOR has better advantages as compared to injected exogenous microorganisms because indigenous microorganisms can adapt to the environment of the reservoir more easily than exogenous

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microorganisms and this technique does not require additional injection equipment.

However, there is a great controversy regarding either aerobic or anaerobic microorganisms involving in the degradation of petroleum in reservoirs. Some in favor of biodegradation is an aerobic process with meteoric water provided oxygen, while some scholars believe that bacteria metabolize petroleum hydrocarbons anaerobically (Aitken et al., 2004; Jones et al., 2007). On the basis of research carried out on bacteria in oil reservoir, hypothesis has been proposed that aerobic and anaerobic degradation of petroleum hydrocarbons occurs alternately in reservoir (Xu et al., 2004; Grabowski et al., 2005; da Cruz et al., 2008). Nazina et al. (2008) reported a slight enhancement in oil recovery as a result of the activation of aerobic processes through oxygen (O_2) or hydrogen peroxide (H_2O_2) and mineral salts supplements. This is another view of some scholars suggested injecting oxygen into the oil reservoirs to activate indigenous microorganisms. However, very limited information explained how the oxygen activates the indigenous reservoir microorganisms biodiversity to degrade oil and enhance the oil recovery.

Ren et al. (2011) have compared the microbial communities of injection water and production water and showed that each production well was composed of a unique microbial community. da Cruz et al. (2008) attempted to characterize aerobic degradation by indigenous bacteria from Pampo Sul Field and concluded that it will take millions of years to completely degrade the petroleum in the reservoir. To our knowledge, there is not much work on comparing the biodegradation of petroleum by injection water with that of production water. In addition, the change of reservoir microbial community during the petroleum degradation process has rarely been studied. The primary objective of this work is to characterize the biodiversity of microorganisms in groundwater, injection water and production water and investigate the aerobic biodegradation of petroleum when oxygen added. The change in the biodiversity of reservoir microorganisms during the aerobic degradation of petroleum is also assessed.

Material and methods

Site description and sampling

The Dagang oil fields lies in the Huanghua depression of the Bohai Bay Basin and composes of an area approximately 24 km^2 in Cang County (Hebei Province, China) which is located about 200 km southeast of Beijing. The oil-bearing area has 16 oil fields developed nearly 1.1 billion tons of oil and covers 640 km^2 (Vincent et al., 2009). The Dagang oil reservoir temperature ranges from 35 to 80 °C and depths range from 1000 to 2500 m (Vincent et al., 2009; Jiménez et al., 2012). The oil field has been exploited for about 40 years and water-flooding technique has been applied for about 30 years (Nazina et al., 2007). One water injection well provides water to several production wells. Injection water with dissolved O_2 (0.8–2.6 mg L^{-1}) is separated from the co-produced formation waters and used for reinjection (Nazina et al., 2007).

All the samples used in this study were collected from Dagang oil field on 26th May 2012. The water and crude oil samples were directly collected from the sampling valves located on well heads of the wells and collected in triplicate using 2 L sterilized Schott bottles. In the filling of sampling bottles, air influx was prevented and exposure of samples to O_2 was minimized. The collected samples were stored at 4 °C until further analysis. Groundwater unexposed to oil contamination was collected from a village located nearest to the oil field. The production water was sampled from oil-bearing formations at a depth of 1500–1700 m in the reservoir. The sampled oil field has been dealt with water flooding treatment since 2010. The temperature of the production water and the injection water was 30–50 °C.

Establishment of aerobic biodegradation microcosm

All chemicals of Analytical Grade were obtained from various commercial sources. A basal salt medium (BSM) contained the following composition (g L^{-1}): 3.0 g of $(NH_4)_2SO_4$, 0.5 g of KH_2PO_4, 0.5 g of Na_2HPO_4, and 0.3 g of $MgSO_4 \cdot H_2O$, with 1.0 mL of a trace mineral solution [(g L^{-1}): $FeCl_3 \cdot 6H_2O$, 0.30; $FeSO_4 \cdot 7H_2O$, 0.30; $MnSO_4 \cdot H_2O$, 0.15; $ZnSO_4 \cdot 7H_2O$, 0.24; and $MoCl_2$, 0.20].

Crude oil and BSM were sterilized at 121 °C for 30 min before being used. To provide reservoir microorganisms, the groundwater, injection water and production water were used for inoculation. They were inoculated separately into sterilized 250-mL Erlenmeyer flasks containing BSM (20 mL water sample per 80 mL of BSM) supplemented with 1.0% (v/v) petroleum as the sole source of carbon and energy. Control experiments were prepared in 100 mL BSM containing 1.0% (v/v) petroleum without inoculating water. All the flasks were sealed with Parafilm® and incubated on a rotary shaker at 160 rpm at 30 °C for 30 days. Degraded oil and water samples were taken from incubation flasks at 5, 10, 15, 20, 25, and 30 days for gas chromatography-mass spectrometric (GC–MS) and biodiversity analysis, respectively. Three replicates from each treatment and control were studied.

Extraction method and gas-chromatography mass-spectrometry of oil

Residual oil of each sample from the biodegradation experiments was extracted with 20 mL dichloromethane for three times. The extracts were combined, dehydrated through an anhydrous Na_2SO_4 (2 g) column, and then reduced to 1–2 mL by a stream of nitrogen gas. The extracted oil was submitted to an Al_2O_3 (2 g, 5% w/w deactivated) column and eluted with a mixture solvent of dichloromethane-hexane (1:1 v/v). The extract was collected for GC–MS analysis.

GC–MS analysis was performed with a Shimadzu GC–MS-QP2010 SE (Tokyo, Japan) and a capillary column (RESTEK, USA) RXI-5 ms (30 m × 0.25 mm i.d., 0.25 μm film). The GC conditions were 1.0 μL injection volume, interface temperature of 280 °C and injector temperature of 300 °C. The column temperature was initially set at 60 °C for 1 min, raised to 150 °C at 15 °C min^{-1}, then raised to 320 °C at 6.0 °C min^{-1}, and finally held at 320 °C for 10 min. Helium gas was used as the carrier at 1.2 mL min^{-1} with a split ratio of 3:2. The sector mass analyzer was set to scan from 50 to 450 amu. Quantitative analyses of each essential oil component were carried out by peak area normalization measurement based on the average values of three injections of each sample.

DNA extraction and denaturing gradient gel electrophoresis analysis

The microorganism was harvested from 60 mL of water samples taken from flasks inoculated with production water and 200 mL of water samples taken from groundwater, injection water and production water using 0.45 μm cellulose nitrate filters (Sartorius AG W-3400, Göttingen, Germany) under sterilized conditions. Before microbial collection, the oil was separated from water by sterile separating funnels to avoid the membrane contamination. Total bacterial DNA was extracted with Soil DNA Isolation kit (MoBiol, USA). The extracted DNA were routinely analyzed by 1.0% (w/v) agarose gel electrophoresis in 1× Tris–acetate–EDTA (TAE) buffer stained with ethidium bromide to check its purity and relative molecular mass.