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Aerobic biodegradation process of petroleum and pathway of main compounds in water flooding well of Dagang oil field

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HIGHLIGHTS

- We investigate oil aerobic biodegradation by indigenous microbes in flooding well.
- The pathways of alkanes and aromatics were proposed.
- *n*-Alkane and partial PAHs were similar for aerobic and naturally biodegraded samples.
- Aerobic biodegradation caused change of biomarkers but some showed opposite tendency.
- This method represents a vital role in oil spill remediation.

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ABSTRACT

Aerobic biodegradation of crude oil and its pathways were investigated via *in vitro* culture and GC–MS analysis in water flooding wells of Dagang oil field. The *in vitro* aerobic culture lasted 90 days when 99.0% of *n*-alkanes and 43.03–99.9% of PAHs were degraded and the biomarkers and their ratios were changed. The spectra of components in the residual oil showed the similar biodegradation between aerobic process of 90 days and degradation in reservoir which may last for some millions years, and the potential of serious aerobic biodegradation of petroleum in reservoir. 24 Metabolites compounds were separated and identified from aerobic culture, including fatty acid, naphthenic acid, aromatic carboxylic acid, unsaturated acid, alcohols, ketones and aldehydes. The pathways of alkanes and aromatics were proposed, which suggests that oxidation of hydrocarbon to organic acid is an important process in the aerobic biodegradation of petroleum.

1. Introduction

Indigenous microorganisms inhabit aquatic as well as oil-bearing deep sub surface environments (Magot et al., 2000). Biodegradation of crude oil is a common process and takes place in many oil reservoirs all over the world (Prince et al., 2013). In undisturbed oil reservoirs, aerobic biodegradation is limited, due to lack of oxygen, and a few aerobic microbiota exist in meteoric formation water incursion that might contain oxygen. Therefore, anaerobic degradation, and particularly methanogenesis, might be the main crude oil biodegradation process in reservoirs (Aitken et al., 2004; Jones et al., 2008). However anaerobic and aerobic processes are synergistic and their microbiota lifecycles coexist in the oil reservoir (da Cruz et al., 2008), especially which are disturbed by human activity.

With the development of petroleum industry, secondary recovery processes started being used as enhanced oil recovery strategies. Among these processes, water flooding is widely applied due to its operational simplicity and low-cost (Shedid, 2006; Yadali Jamaloei et al., 2011). The injection water is recycled in the whole flooding process, by which oxygen and exogenous microorganisms can be taken into the reservoir, and may activate the aerobic degradation partially. Oil production will become more and more dens, viscous and heavy with the time of extraction (Head et al., 2003; Aitken et al., 2004; Jones et al., 2008). However, limited information has been reported on aerobic biodegradation in oil reservoir, and many research just suggested that biodegradation can cause oil souring and increased viscosity, sulfur and metal content

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(Ahsan et al., 1997; Lindstrom and Braddock, 2002; Head et al., 2003).

Water flooding is being used for enhanced oil recovery, in Gangxi zone of Dagang oil field, since 1972. A recent study (Jiménez et al., 2012) showed that the crude oil was highly degraded in this zone. An emerging method Microbial Enhanced Oil Recovery (MEOR) (Castorena-Cortés et al., 2012; Gudiña et al., 2012; Kobayashi et al., 2012; Pei et al., 2013), is trying to be used in flooding well for enhanced oil recovery. Understanding the aerobic biodegradation in oil reservoir may provide some suggestion on MEOR as well as the oil spill remediation.

The objectives of our study were to investigate the occurrence of aerobic biodegradation processes in this oil reservoir, and characterize the aerobic biodegradation pathways of hydrocarbons such as *n*-alkanes and PAHs (polycyclic aromatic hydrocarbon) by identifying the metabolites produced in the degradation process.

2. Methods

2.1. Study area and sampling

The oil and water samples of ten oil wells were collected from the Gangxi zone in the Dagang oil field. It is located in the west of BeiDagang structural belt and is an anticline structure complicated by faults. It comprises an area about 55 km^2, 11 km long and 3–5 km wide. It was found in 1965 and developed since 1970. The main production layer is the lower member of the neogene Minghuazhen formation and the neogene Guantao formation. Its fault block oil–gas reservoir area is small and poor in connectivity. The temperature of sampling reservoir ranges from 50 to 70 °C and the depth ranges from 950 to 1800 m.

The sampling campaign took place in May 2012. Water and petroleum samples were collected directly from the wellhead of production wells in sterile glass bottles. Two oil samples were used in this study: a non-biodegraded sample (O1) to be used in biodegradation experiments and a biodegraded sample (O2) for comparison. The bottles were completely filled with the samples and then hermetically sealed in order to prevent oxygen influx. All samples were stored at 4 °C temperature in the laboratory until further analysis or processing.

2.2. Establishment of aerobic biodegradation microcosms

The biodegradation experiment was conducted in 200 ml autoclaved Erlenmeyer flasks containing 100 ml of mineral medium and approximately 800 mg of crude oil. The mineral medium consists of NaCl (0.5 g), KCl (0.4 g), KH_2PO_4 (0.5 g), $CaCl_2$ (0.1 g), NH_4Cl (0.4 g), $MgCl_2$ (0.4 g) and 1 ml trace element solution in 1000 ml deionized water adjusted to pH 7.0. The trace element solution contains $FeCl_2{\cdot}4H_2O$ (1.5 g), ZnCl (70 mg), $MnCl_2{\cdot}4H_2O$ (100 mg), H_3BO_3 (6 mg), $CoCl_2{\cdot}6H_2O$ (190 mg), $CuCl_2{\cdot}2H_2O$ (2 mg), $NiCl_2{\cdot}6H_2O$ (24 mg), $Na_2MoOH{\cdot}2H_2O$ (36 mg) and HCl (25%, 10 ml) in 1000 ml deionized water.

Microcosms were inoculated with 10 ml of production water from the well of 1545.89–1791.02 m – depth and 55.8 °C, which was water-flooding since 2010. The non-inoculated controls with crude oil were used for comparing chemical changes of crude oil by flooding-water. Considering the oxygen was brought by injection water mainly, which temperature was 30–40 °C, all microcosms were incubated on a shaker at 150 rpm and 30 °C for 90 days (da Cruz et al., 2008). The amount of dissolved oxygen (DO) was determined at the beginning of the experiment, and at the 7th, 21th and 90th day. It ranged from 7.7–8.2 mg/l. Samples (3 of each kind) were sacrificed at 0, 7, 14, 21 and 90 days for gas-chromatography coupled to mass spectrometry (GC–MS) analysis of oil.

2.3. Gas-chromatography mass-spectrometry of oil

The oil was extracted from the microcosm with dichloromethane (CH_2Cl_2) (10 ml × 3 times). Organic extracts were combined, dehydrated through a 2 g anhydrous Na_2SO_4 column and concentrated under a gentle stream of nitrogen gas. The eluate was cleaned through 2 g of Al_2O_3 (5% w/w deactivated) column, eluting with hexane-dichloromethane (1:1 v/v), concentrated and exchanged to hexane (1.0 ml) by a gentle solvent evaporation under a stream of nitrogen gas. The extracts were analyzed by gas chromatography mass spectrometry als reported elsewhere (Alzaga et al., 2004; Wang et al., 2010).

GC–MS analysis was performed using a GCMS-QP2010 SE gas chromatograph–mass spectrometer (SHIMADZU), fitted with a capillary column (RESTEK, USA) RXI-5 ms (30 m × 0.25 mm i.d., 0.25 μm film). The carrier gas was He at 1.2 ml/min; the injection temperature was 280 °C; the temperature programme was: 60 °C (hold 1 min) to 150 °C at 15 °C/min and then to 320 °C (hold 10 min) at 6 °C/min.

2.4. Semi-quantitative analysis of oil biodegradation

The biodegradation process was monitored by following the changes in the chemical composition of the oil using the 17α(H),21β(H)-hopane (*m*/*z* 191) as an internal conservative molecular marker, due to its biological stability (Prince et al., 1994). The distributions of *n*-alkanes relative to hopane were determined by using the *m*/*z* 85 and 191 fragment ions, respectively. Linear alkylcyclohexanes, alkylbenzenes and alkyltoluenes were quantified by monitoring their characteristic ions (*m*/*z* 82, 92 and 106, respectively). Terpane (*m*/*z* 191) and sterane (*m*/*z* 217 and 218) were identified and semi-quantitatively determined by selective ion monitoring (SIM) chromatography in (Chakhmakhchev et al., 1996; Cheng et al., 2008; Hauser et al., 1999; Peters et al., 2005).

The distribution of aromatic compounds (biaromatic to tetraaromatic) was based on the molecular ion for each: BT, benzo(b)thiophene; B, biphenyl; N–N3, naphthalenes (*m*/*z* 128, 142, 156, 170); F–F3, fluorenes (*m*/*z* 166, 180, 194, 208); P–P3, phenanthrenes (*m*/*z* 178, 192, 206, 220); D–D3, dibenzothiophenes (*m*/*z* 184, 198, 212, 226); Py–Py3, fluoranthenes and pyrenes (*m*/*z* 202, 216, 230, 244); C–C3, chrysenes (*m*/*z* 228, 242, 256, 270). In addition, several molecular markers were used to calculate indexes for fingerprinting and weathering assessment: The different biomarker ratios and characteristic ions are specified in Table 1. Biomarker ratios were statically analyzed through analysis of variance (ANOVA). Standard statistical package, MINITAB (version 16) was employed for the analysis.

2.5. Qualitative analysis of metabolites

Hydrocarbon biodegradation metabolites were analyzed by gas-chromatography mass-spectrometry (GC–MS).

Metabolites were recovered by esterification after the extraction of oil, as follows: An aliquot of 80 ml culture was taken, adjusted to pH 10 with by adding an appropriate amount of 1 M sodium hydroxide solution, and filtrated to remove the insoluble impurities. The filtrate was dried at 105 °C and, after cooling room temperature, 4 ml alcoholic solution of sulfuric acid (10% v/v) was added for esterification. The solution was maintained at 80 °C for 1 h. Then the 10 ml of deionized water was added to stop the reaction and the product was extracted immediately by shaking (×3) with 15 ml of hexane. The extract was dehydrated passing it