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Review article

Effect of biosurfactant on biodegradation of heteroatom compounds in heavy oil

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ABSTRACT

The ability of biodegradation on heavy components in heavy oil is still a controversial issue, and the effect of biosurfactants on resins is unclear. Four strains and biosurfactant were used to investigate the degradation ability of resins using gas chromatography-mass spectrometry (GC–MS) and negative-ion electrospray ionization Fourier transform ion cyclotron resonance mass spectrometry (ESI FT-ICR MS) by a stimulated aerobic biodegradation experiment. Results showed that the biosurfactant improved biodegradation rates for saturates, aromatics, resins, and asphaltenes by 115–231%, 130–168%, 116–161%, and 135–217%, respectively. The A/C value (the sum of relative abundance of the acyclic O2 species divided by the sum of relative abundance of the mono, di- and tri-cyclic acids) indicated that biosurfactants could accelerate the biodegradation process. Results also showed that the utilization preference of O-containing and N-containing compounds varies from different strains by analysing their distribution. A reduction in the relative abundance of N1O1 class was detected in all samples containing microorganisms only. A significant decrease in $C_{27}H_{48}O$ and $C_{28}H_{50}O$ was observed in all samples with the biosurfactant. Carbazoles, benzocarbazoles and dibenzocarbazaoles could be effectively used by the strain J2 with biosurfactant. The relative abundance of O1 class increased when treated with microorganisms only. However, it decreased in the presence of biosurfactants. Therefore, biosurfactant could alter the preference that microorganism utilized heteroatom compounds.

1. Introduction

Spills, leaks, and other releases of heavy oil result in serious ecological problems. Heavy oil residues in the surface and underground environments are of special concern. Therefore, the development of environmentally friendly technologies to degrade these organic compounds has gained increasing attention. Compared with traditional physico-chemical treatments, biodegradation has more advantages. Rizi et al. [1] reported that indigenous bacteria are more efficient for the biodegradation of oil pollutants. Many literatures have proved that microbial biodegradation serves potentially significant applications [2,3]. However, an important factor about the effectiveness of microbial biodegradation is microorganisms couldn't absolutely utilize hydrocarbons [2,4].

One of the pathways for microorganisms to degrade petroleum hydrocarbons is to produce biosurfactants (BS). Biosurfactant-producing bacteria commonly exist in surface and background environments among biodegrading-bacteria. By reducing surface and interfacial tension, biosurfactants can increase the solubility, mobility, bioavailability, and subsequent biodegradation of petroleum hydrocarbons [5,6]. Biosurfactants are amphipathic surface-active molecules, and they can be produced by a wide variety of bacteria [7]. Most studies are focused on the production [5,8], characterization [6], optimization [9,10], and application [5,6,11,12] of biosurfactants. The ability of biosurfactants to accelerate the biodegradation of n-alkanes [13–15] and polycyclic aromatic hydrocarbons [8,11,16] has been well documented. However, the effect of biosurfactants on biodegradation of heteroatom compounds has been poorly studied.

Biodegradation is the main pathway for petroleum compounds in surface and background environments for the biogeochemical cycle. In this cycle, diverse bacteria have different effects on the degradation of petroleum compounds, causing different biogeochemical cycle speeds for the four components of petroleum. The relative susceptibility of saturated and aromatic hydrocarbons to microbial alteration is well documented [17,18]. Resins and asphaltenes are considered to be highly resistant to microbial-degradation, most of them are comprised of polycyclic aromatic or naphthenoaroatic nuclei with heteroatoms and alkyl side chains [19]. Nevertheless, some studies have proved that

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resins and asphaltenes can be degraded by microbial alternation. Kirimura et al. [20] demonstrated that *Sphingomonas* sp. CDH-7 can utilize carbazoles (CA) as a sole source of carbon and nitrogen, and the metabolize CA to ammonia via anthranilic acid as an intermediate product. Li et al. [21] reported the biodegradation of C1-carbazoles or C2-carbazoles was influenced by the positions of methyl substitutions.

The distribution characteristics of heteroatom compounds in crude oils and its distillates have been well documented [22–29]. The subjects in all these studies are oils with different degradation degrees. However, studies on the changes occurring during geological processes and biodegradation in the surface environment are limited. Pan et al. [30], obtained different degradation degree oil by aerobic biodegradation simulation experiment and discussed the distribution and biodegradation mechanisms of heteroatom compounds. However, the dominant bacteria may be different according to the properties of the oil and location of the oil reservoir. Moreover, the preference in which different microorganisms utilize heteroatom compound may vary.

Considering above mentions, the objective of this study was to explore the biodegradability of resins. We also studied the effects of different streams, feed (different strains and different combinations of strains and biosurfactant), and the biosurfactant of different strains on the distribution of N- and O-containing compounds using electrospray ionization Fourier transform ion cyclotron resonance mass spectrometry (ESI FT-ICR MS).

2. Materials and methods

2.1. Characteristics of heavy oil

The heavy oil samples were obtained from the Liaohe oil field, China. The viscosity was 15,900 m·Pas at 40 °C. The American Petroleum Institute (API) gravity of the oil was determined to be 19.0. The concentrations of nitrogen, carbon, hydrogen and oxygen were 0.44, 81.37, 10.32, and 3.36 wt%, respectively. As shown in Fig. 1, the relative abundance of the saturated hydrocarbons was high, so we deduced that the oil had not undergone biodegradation.

2.2. Microorganisms and biosurfactants

Strains J1, J2, and J3 were selected from the culture medium in which resins were used as the sole carbon source. Strain BC was screened using the oil-plate method and showed obvious oil spreading diameters. The biosurfactant used in this study was produced by strain BC. The biosurfactant was extracted from the culture medium and purified using the modified acid precipitation and solvent extraction methods [31]. Production and characteristics of the biosurfactant have been described by Cai et al. [12].

2.3. Biodegradation tests

The enrichment medium contained 3.0 g of beef extract, 10 g of peptone, and 5.0 g of NaCl per litre. The four strains were cultivated in 100 mL of enrichment medium for 48 h, respectively. Then 1 mL was inoculated into a flask containing 150 mL of biodegradation media. Biodegradation media contained (g/L): yeast extract, 1.5; $NaNO_3$, 1.5; KCl, 0.5; KH_2PO_4, 1.0; $MgSO_4$, 0.5. The pH of the medium was adjusted to 7.0 by addition of NaOH. The pH of the medium was adjusted to 7.0 by adding NaOH. The heavy oil to be analysed was used as the sole carbon source. All the chemicals used were of analytical grade and purity. Eight groups were set to explore the effect of the microorganism strain and biosurfactant on oil degradation. As shown in Table 1, groups 1–4 contained J1, J2, J3, and BC, respectively. Groups 5–8 had each strain supplemented with the biosurfactant at concentrations of 100 mg/L. Group 0, the uninoculated flask, was served as the control.

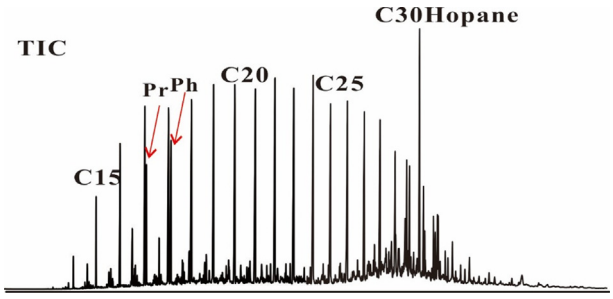

Fig. 1. Total ion chromatograms (TIC) of the saturated hydrocarbons in the studied oil.

After cultivation, the residual oil was extracted twice using dichloromethane as the solvent, then evaporated using a rotary evaporator. The DCM extraction recovery ranged from 90% to 110% (average 94%) The oil sample was deasphalted using n-hexane and then fractionated using column chromatography (silica gel: alumina of 3:2 v/v) into saturates, aromatics, and resins using n-hexane, dichloromethane/n-hexane (2:1 v/v), and dichloromethane/methanol (98:2 v/v) as respective eluents. The SARA separation recovery ranged from 93% to 108% (average 98%). The biodegradation rates were determined gravimetrically as Eq. (1).

$$\text{Biodegradation}\ (\%) = \frac{W_0 - W_1}{W_0} \times 100 \quad (1)$$

where W0 is the weight of oil or fraction in the blank control group (KB) and W1 is the weight of oil or fraction in the treated group.

2.4. GC–MS and FT-ICR MS analysis

Saturate and aromatic hydrocarbons were carried out with gas chromatography-mass spectrometry (GC–MS) (Agilent 6890/5975, the USA). Helium was used as a carrier gas. The gas chromatograph (GC) operating conditions for saturates were 50 °C–120 °C at 20 °C/min and then to 310 °C at 3 °C/min. The oven temperature for aromatics was at 80 °C, held for 1 min, then increased to 300 °C at 3 °C/min, and held for 18 min. The injector temperature was programmed at 300 °C. The injection volume was 1.0 μL. And the syringe size was 10.0 μL. The mass spectrometer (MS) was operated in electron impact ionization (EI) mode with 70 eV electron energy and a scanning range of 50–600 Da.

The resins were analysed using ESI FT-ICR MS (Bruker Apex ultra, Germany) with a 9.4 T superconducting magnet. The operating procedure for the experimental analysis has been described by Shi et al. [25,26]. Each sample was dissolved in toluene: methanol (1:1, v/v) to form a solution with a concentration of 10 mg/mL. The solution was injected at 3 μL/min into the ESI source using a syringe pump. Prior to injection, $5*10^{-9}$ mol/L of octadecane-D_{35} acid was added to serve as an internal standard. The ESI source was operated in negative-ion mode. The voltages at the emitter, capillary column front end, and capillary column end were −4.0 kV, −4.5 kV and −320 V, respectively. Ions were accumulated in the ion source for 0.1 s in a hexapole, after which they passed through a single quadrupole before accumulating for 1 s in a collision cell filled with argon bath gas. The delay in the transfer of ions from the collision cell to an ICR cell by electrostatic focusing was set to 1.2 ms. The mass range was set to m/z 200–1000 and the data size was set to 2 M words. The time domain datasets of 256 acquisitions were co-added.

2.5. Mass calibration and data analysis

Data analysis was performed using custom software, which has been described by Hughey et al. [23]. Compounds with the same heteroatom class and its isotopes with different double bond equivalents (DBE) and carbon numbers were searched within a set ± 0.001 Kendrick mass defect (KMD) tolerance [32].

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