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Effect of natural and synthetic surfactants on crude oil biodegradation by indigenous strains

Wei Tian [a], Jun Yao [a,b,*], Ruiping Liu [a], Mijia Zhu [a], Fei Wang [a], Xiaoying Wu [a], Haijun Liu [c,**]

[a] National "International Cooperation Based on Environment and Energy" and School of Civil & Environmental Engineering and, University of Science and Technology Beijing, Beijing 100083, PR China
[b] School of Water Resource and Environmental Engineering, Sino-Hungarian Joint Laboratory of Environmental Science and Health, China University of Geosciences (Beijing), Beijing 100083, PR China
[c] School of Resources and Environment, Anqing Normal University, 1318 Jixian North Road, Anqing 246133, PR China

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ABSTRACT

Hydrocarbon pollution is a worldwide problem. In this study, five surfactants containing SDS, LAS, Brij 30, Tween 80 and biosurfactant were used to evaluate their effect on crude oil biodegradation. Hydrocarbon degrading bacteria were isolated from oil production water. The biosurfactant used was a kind of cyclic lipopeptide produced by *Bacillus subtilis* strain WU-3. Solubilization test showed all the surfactants could apparently increase the water solubility of crude oil. The microbial adhesion to the hydrocarbon (MATH) test showed surfactants could change cell surface hydrophobicity (CSH) of microbiota, depending on their species and concentrations. Microcalorimetric experiments revealed these surfactants exhibited toxicity to microorganisms at high concentrations (above 1 CMC), except for SDS which showed low antibacterial activity. Surfactant supplementation (about 0.1 and 0.2 CMC) could improve degradation rate of crude oil slightly, while high surfactant concentration (above 1 CMC) may decrease the degradation rate from 50.5% to 28.9%. Those findings of this work could provide guidance for the application of surfactants in bioremediation of oil pollution.

1. Introduction

As a result of rapid industrial development, the threat of petroleum contaminations has inevitably increased. The release of hydrocarbon pollutants has caused extensive contamination of surface soils, groundwater, seas and ocean. And this resulted in ecosystem damage through spillages and leakage from underground tanks, steamers, unplugging of oil wells, and abandoned oil refinery sites (Janbandhu and Fulekar, 2011; Prince et al., 2013). Chemical, physical, and biological treatments have been used to remove these hydrocarbon pollutants from the environment. Owing to its environmentally friendly, cost-effective, and efficient advantage compared with physicochemical treatments, bioremediation has gained increasing interest (Bao et al., 2012; Ferradji et al., 2014; Millioli et al., 2009). Some studies have been reported to use bioremediation successfully for spilled oil (Greenwood et al., 2009; Hii et al., 2009).

Numerous aerobic and anaerobic microorganisms were isolated such as algae, bacteria, fungi and yeasts, which were capable of utilizing petroleum compounds as a sole carbon source (Mnif et al., 2014). Crude oil is a complex mixture, and complete oil degradation commonly requires synergistic actions of different microbial species or strains (El–Tarrs et al., 2012). Kumar et al. (2014) reported that pure cultures were able to utilize spent engine oil, but none of the cultures was more efficient than the mixed bacterial consortium, and this result was in agreement with the finding of Díaz et al. (2000) who reported the combination of different cultures producing a synergistic effect and thereby increasing the biodegradation of crude oil.

The bioavailability of hydrocarbon compounds is reduced significantly due to their limited water solubility, which adversely affects their biodegradation in the environment (Megharaj et al., 2011; Millioli et al., 2009). Adding surfactant is a feasible approach to enhance the bioavailability, solubilization, and biodegradation of hydrophobic pollutants. Surfactants can improve hydrocarbon utilization through emulsifications as well as adhesion on microbial cell surfaces (Singh et al., 2007). Biosurfactants have been gaining increasing interest due to their lower toxicity, higher environmental compatibility, biodegradability and stability advantages over chemical surfactants (Benincasa et al., 2010; Thavasi et al., 2011). Thus, both effect of natural and synthetic surfactants should be studied on crude oil biodegradation.

* Corresponding author.
** Corresponding author.
E-mail addresses: yaojun@ustb.edu.cn (J. Yao), liuhj@aqtc.edu.cn (H. Liu).

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Studies showed surfactants could increase hydrocarbon bioavailability by hydrocarbon solubizization or the strengthen of cell surface hydrophobicity (CSH) (Luning Prak et al., 2000; Mulligan, 2005; Obuekwe et al., 2009), which lead to enhance hydrocarbon utilization. However, surfactants are not always beneficial for the degradation system, and it may promote, inhibit or have no effect on hydrocarbon biodegradation (Paria, 2008; Urum and Pekdemir, 2004). There are many reasons responsible for the negative impact, as surfactants may lead to a drastic decrease of the CSH, and usually exhibit toxicity when used at a high concentration, but occasionally utilize as a preferential carbon source (Yu et al., 2007). Furthermore, hydrocarbon biodegradation may be suppressed owing to its unavailability to microbial cells entrapped in surfactant micelles (Chrzanowski et al., 2011; Wang, 2011).

There are many studies about effect of surfactants on hydrocarbon biodegradation, but few of them focus on hydrocarbon biodegradation influenced by surfactants from bacteria in oil production water. This study aims to investigate the overall effect of one biosurfactant and four synthetic surfactants on indigenous microbial community for crude oil biodegradation. The effect of surfactants were evaluated on crude oil solubilization, CSH alteration, and bacterial growth.

2. Materials and methods

2.1. Sampling

Oil production water samples were collected using sterile glass bottles from the Gangxi region in the Dagang oil field, China. The temperature of sampled oil reservoirs ranges from 52 to 65 °C, and the depth is from 948 to 957 m. Samples were stored at 4 °C in the laboratory for further use (Cai et al., 2015).

2.2. Microorganisms and culture conditions

The microbial consortium used throughout this study was obtained from oil production water. Bacterial 16S rRNA clone library analysis revealed that microbes in production water were mainly α-proteobacteria, and culturable microorganisms included genus *Sphingomonas* and genus *Pseudomonas*. The clone analysis also indicated that archaea in production water were mainly *Methanosarcina* (unpublished results). A number of studies demonstrated that genus *Sphingomonas* was one of the dominant species in the microbial communities of PAH-contaminated soil and water samples (Brito et al., 2006; Saul et al., 2005; Shokrollahzadeh et al., 2008). And genus *Pseudomonas* was known to have an important potential to degrade PAHs and alkanes (Molina et al., 2009). An enrichment procedure was performed in 150 ml Erlenmeyer flasks containing 50 ml mineral salts medium (MSM) and 1% w/v crude oil as the only carbon source. The MSM medium contained NaCl 10 g, NH_4Cl 0.5 g, KH_2PO_4 0.5 g, K_2HPO_4 1.0 g, $MgSO_4$ 0.5 g, KCl 0.1 g, $CaCl_2$ 0.02 g and $FeCl \cdot 4H_2O$ 0.02 g in 1000 ml deionized water, and the pH was adjusted to 7.0 with 1.0 M NaOH/HCl before sterilization under 121 °C for 30 min. The flasks were incubated at 30 °C and 160 rpm on a rotary shaker for 7 days. The culture was enriched by 4 consecutive inoculations of 5 ml inoculum to 50 ml fresh sterilized MSM medium. Cells were harvested by centrifugation and washed three times with saline solution. Then, cells obtained from the plates were stored in LB medium that contained 10 g of NaCl, 10 g of tryptone and 5.0 g of yeast extract in 1000 ml deionized water at pH 7.0 with 15% of glycerol at −80 °C for later inoculation (Liu et al., 2014).

To prepare the seed culture, 1 ml bacterial stock culture aforementioned was incubated in LB medium for 24 h. The obtained cells were washed with the potassium phosphate buffer (pH 7.2) and centrifuged again at 5000 g for 15 min at 4 °C. The process repeated 3 times and then bacteria resuspended in potassium phosphate buffer to measure the optical density at 600 nm by spectrophotometer (Shimadzu UV-1800,Tokyo, Japan). The final bacteria solution was ready for the crude oil biodegradation.

2.3. Surfactants

In this study, two anionicsynthetic surfactants i.e. sodium dodecyl sulfate (SDS, chemically pure, formula $C_{12}H_{25}NaO_4S$, molecular weight 288.38) and sodium dodecyl benzene sulfonate (SDBS/LAS, analytical reagent, formula $C_{18}H_{29}NaO_3S$, molecular weight 348.48), two non-ionic synthetic surfactants i.e. Tween 80 (chemically pure, density (at 20 °C) 1.05–1.10 g/ml) and Brij 30 (high grade purity, formula $C_{32}H_{66}O_{11}$, molecular weight 626.85), and a biosurfactant were used. The critical micelle concentration (CMC) of surfactants was determined with a JYW-200B tension meter (Chengde, China) using the ring method (Abouseoud et al., 2008) at about 25 °C.

2.3.1. Extraction and characterization of biosurfactant

The biosurfactant was produced by *Bacillus subtilis* strain WU-3 (accession number KF894965 in the Genbank), which was previously isolated in our laboratory. The strain WU-3 was inoculated in the production medium containing peptone 10 g, glucose 15 g, $MgSO_4$ 0.4 g, NH_4Cl 1.07 g, KCl 1.49 g, Tris–HCl 18.9 g per liter and incubated at 30 °C, and 160 rpm for 72 h. The extraction and purification procedure of the biosurfactant was carried out as described by Chandankere et al. (2013). Fourier transform infrared spectroscopy (FT-IR) analysis was conducted to identify the structural groups of the biosurfactant. 1.0 mg of isolated biosurfactant was powdered with 1.0 g of KBr, and then pressed under 7500 kg for 30 s to result a translucent KBr pellet. FT-IR spectrum was recorded on a Nexus 670 system (ThermoNicolet, USA) within the range of 4000–400 cm^{-1} wave number. All datas were corrected with background spectrum.

2.4. Crude oil solubilization assay

Procedures for solubility determination were described as follows: 100 ml sterilized surfactant solution with various concentrations (0.1 times, 0.2 times, 0.5 times, 1 times and 2 times of CMC) was poured into 250 ml Erlenmeyer flasks, and 0.5% w/v crude oil was also added into each flask. Flasks were sealed with a cork to prevent any loss from solution and incubated in a vertical position for 48 h at 30 °C with a shaking of 200 rpm in dark. Preliminary experiment showed that 48 hours was sufficient to reach the solubilization equilibrium. For the solubilization analysis, the solution was transferred to a separating funnel to keep balance for 1 h. Then 50 ml bottom aqueous phase was transferred to a clean separating funnel, and a few drops of sulfuric acid (1:1) was added in to demulsify the solution. The crude oil in solution was extracted by 10 ml hexane for 2 times. Hexane phases were combined, and then passed through anhydrous sodium sulfate to remove moisture. The solution was placed in a 25 ml volumetric flask, and hexane was added in until the lower liquid level reached the scale line. Concentration of crude oil in the hexane extract was measured spectrophotometrically at 227 nm. Experiments were performed in triplicates, while control experiments were also conducted in parallel without biosurfactant in the assay sample.

2.5. Cell surface hydrophobicity

In order to investigate the cell surface hydrophobicity (CSH) of the microbial community, microbial adhesion to the hydrocarbon