



Effect of rhamnolipid biosurfactant on solubilization of polycyclic aromatic hydrocarbons



Shudong Li^{a,b}, Yongrui Pi^{a,b}, Mutai Bao^{a,b,*}, Cong Zhang^{a,b}, Dongwei Zhao^{a,b}, Yiming Li^{a,b}, Peiyan Sun^{c,d}, Jinren Lu^b

^a Key Laboratory of Marine Chemistry Theory and Technology, Ministry of Education, Ocean University of China, Qingdao 266100, China

^b College of Chemistry & Chemical Engineering, Ocean University of China, Qingdao 266100, China

^c Key Laboratory of Marine Spill Oil Identification and Damage Assessment Technology, State Oceanic Administration, Qingdao 266033, China

^d North China Sea Environmental Monitoring Center of State Oceanic Administration, Qingdao 266033, China

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ABSTRACT

Rhamnolipid biosurfactant-producing bacteria, *Bacillus Lz-2*, was isolated from oil polluted water collected from Dongying Shengli oilfield, China. The factors that influence PAH solubilization such as biosurfactant concentration, pH, ionic strength and temperature were discussed. The results showed that the solubilities of naphthalene, phenanthrene and pyrene increased linearly with the rise of rhamnolipid biosurfactant dose above the biosurfactant critical micelle concentration (CMC). Furthermore, the molar solubilization ratio (MSR) values decreased in the following order: naphthalene > phenanthrene > pyrene. However, the solubility percentage increased and followed the opposite order: pyrene > phenanthrene > naphthalene. The solubilities of PAHs in rhamnolipid biosurfactant solution increased with the rise of pH and ionic strength, and reached the maximum values under the conditions of pH 11 and NaCl concentration $8 \text{ g} \cdot \text{L}^{-1}$. The solubility of phenanthrene and pyrene increased with the rise of temperature.

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1. Introduction

Widespread use, improper disposal, incomplete combustion of fossil fuels and biomass, accidental spills and leaks of organic hydrocarbons like petroleum hydrocarbons, organic solvents, and polyaromatic hydrocarbons (PAHs) have resulted in long-term persistent sources of contamination of fossil oils, coal, tar deposits and groundwater, which becomes a major environmental issue because of their adverse effect on human health (Arias et al., 2010; Paria, 2008; Begoña et al., 2015). Polycyclic aromatic hydrocarbons (PAHs) are components of creosote produced during raw petroleum refining, coke production or wood preservation, and the distribution of PAH compounds in the marine sediments of rivers, estuaries, coastlines and sea beds has been commonly investigated (Boonyatumanond et al., 2006; Gong et al., 2014; Page et al., 1999; Pauzi Zakaria et al., 2001; da Silva and Bicego, 2010; Tolosa et al., 2004; Wang et al., 2011). The structure of PAHs is composed of two or more benzene rings covalently bonded together. With the number of rings in the molecular structure increases, water solubility decreases, and hence makes it more difficult to degrade. The toxicity of PAHs including possible mutagenic and carcinogenic

effects may exert potential health risk to urban residents (Colombo et al., 2006; Al-Saleh et al., 2013), as well as it can be bio-accumulated through the food chain and the exposure of humans to PAHs may enhance the risk of cancer and other adverse health effects (Gu et al., 2013; Wan et al., 2007). PAHs also have a strong inhibitory effect on microbial growth (Calder and Lader, 1976) and can destroy the biological membrane and damage DNA, causing genetic information of the cells to be mutated (Kim et al., 2007; Jarvis et al., 2013). As PAHs are so harmful to the environment and human health, it is important to find effective degradation methods.

Surfactants are amphiphilic compounds that can reduce the free energy of the system by replacing the bulk molecules of higher energy at the interface. The term surface-active agent or “surfactant” represents a heterogeneous and long-chain molecule containing both hydrophilic (head) and hydrophobic (tail) moieties (Paria, 2008). They contain a hydrophobic portion with little affinity for the bulk medium and a hydrophilic group attracted to the bulk medium. Consequently, surfactants are used to lower surface tensions and increase solubility. They are used for their detergency power, wetting ability and foaming capacity in petroleum industry (Al-Sabagh et al., 2003), mineral flotation (Asplin et al., 1998; Koopal et al., 1999) and pharmaceutical industries (Madunić-Čačić et al., 2008; Christiansen et al., 2011; Nogueira et al., 2011). But the introduction of surfactants into the environment can lead to contamination concerns. Consequently, the toxicity of the

* Corresponding author at: Key Laboratory of Marine Chemistry Theory and Technology, Ministry of Education, Ocean University of China, Qingdao 266100, China.

E-mail address: mtbao@ouc.edu.cn (M. Bao).

surfactant and its potential degradation products needs to be carefully considered prior to the selection of surfactant (Edwards et al., 2003).

Biosurfactants are mainly produced by bacteria or yeast, and also available from plants, animals, including human (Paria, 2008). Most microbial surfactants are complex molecules, comprising different structures that include lipopeptides, glycolipids, polysaccharide protein complexes, fatty acids and phospholipids (Thavasi et al., 2008). In the past few decades, biosurfactants have gained more attention and more potentially effective over synthetic surfactants because of their advantages such as high specificity biodegradability and biocompatibility (Torres et al., 2011), low toxicity, ecological acceptability and ability to be produced from renewable and cheaper substrates (Desai and Banat, 1997; Nitschke and Pastore, 2004; Paria, 2008). Therefore, there have more environmental applications in recent years about biosurfactants. Most of the studies focused on their application in the remediation of wastewaters; soils contaminated by heavy metals and hydrophobic organic compounds, such as petroleum hydrocarbons and pesticides (Sponza and Gök, 2010; Zeftawy et al., 2011; Thavasi et al., 2011; Shin et al., 2006; Mulligan and Wang, 2006; Wang and Mulligan, 2009; Cao et al., 2013). A recent study done by Zhou (Zhou et al., 2011) about saponin, a plant-derived non-ionic biosurfactant, described its application in enhancing solubilization of PAHs; it showed that the solubility of PAHs was highly dependent of pH and ionic strength of the solution; the molar solubilization ratio (MSR) of saponin for phenanthrene was about 3–6 times of those synthetic non-ionic surfactants, and decreased about 70% with the increase of solution pH from 4.0 to 8.0, but increased linearly when NaCl concentration increased from 0.01 to 1.0 M; saponin is more effective in enhancing PAH solubilization than synthetic non-ionic surfactants and has potential application in removing organic pollutants from contaminated soils.

In this research, we analyzed the structure of a biosurfactant that was produced from oil degradation bacteria *Bacillus Lz-2*. The corresponding influences of a number of environmental factors (biosurfactant concentrations, pH, ionic strength and temperature) were investigated. The results improve the understanding and prediction of the enhanced solubilization of rhamnolipid biosurfactant for PAHs and provide valuable information for the application of biosurfactants in the remediation of organic contaminated water. The main components of the oil are alkanes, cyclanes and aromatic hydrocarbons. Aromatic hydrocarbons are difficult to be degraded. Rhamnolipid biosurfactant can enhance the solubilization of PAHs in experiment (Zhao et al., 2015), so it can work on other simple components in the oil. Rhamnolipid biosurfactant enhances certain solubilization on oil and achieves certain auxiliary degradation effect. So it can be applied to the marine oil spill.

2. Methods and materials

2.1. Chemicals

Naphthalene, phenanthrene and pyrene (purity >98%) were obtained from Aldrich Chemical Company. They were chosen as the three PAH probes because they are petroleum-, coal- and chemical industry-related organic contaminants that are frequently found in surface and subsurface environments. Dichloromethane and sodium hydroxide were purchased from the Chinese Medicine Group Chemical Reagent Co., LTD. Sulfuric acid was purchased from Tianjin Yao Hua Chemical Reagent Co., LTD. Sodium chloride was used to investigate the effect of ionic strength on PAH solubilization. HCl and NaOH were employed in studying the effect of solution pH on PAH solubilization.

2.2. Purification and identification of the biosurfactant

The biosurfactant used in this study was a rhamnolipid surfactant obtained from a bacterial strain, which had been isolated from oil polluted water collected from Dongying, China. The isolated bacteria

was 99% similar to *Bacillus* based on its 16S rRNA gene sequence (1342 bp) and was named *Bacillus Lz-2* (GenBank accession number KC256826). The critical micellar concentration (CMC) of this biosurfactant is 240 mg·L⁻¹.

The mineral salt medium (MSM) with supplement of sodium citrate 30 g·L⁻¹, peptone 5 g·L⁻¹, NaCl 5 g·L⁻¹; pH = 7, T = 37 °C was used for bacterial growth. For inoculation, the strain was incubated in autoclaved MSM for a week on DHZ-D constant temperature shaker (Taicang Lab-Line Instruments, China) at 150 rpm. After centrifuged at 12,000 r·min⁻¹ at 4 °C for 20 min, the supernatant was adjusted to pH 2.0 with 1.0 M sulfuric acid, prior to biosurfactant extraction using equal volume of ethyl acetate. The organic phase was separated and the solvent was evaporated to concentrate the biosurfactant. The biosurfactant was then dried using a rotary evaporator. The dried product was washed with absolute ethanol three times completely to remove residual pigments.

The IR spectra were recorded on Bruker IFS66 V FT-IR spectrometer (KBr and polyethylene pellets). The FT-Raman and SERS spectra were obtained on a Bruker IFS66V NIR-FT instrument equipped with a FRA 106 Raman module. A Nd:YAG laser at 1064 nm with an output of 300 mW was used for excitation. The detector was a Ge-diode cooled to liquid nitrogen temperature. One thousand scans were accumulated with a total registration time of about 30 min. The spectral resolution was 6 cm⁻¹.

HPLC–MS characterization of the biosurfactant was achieved using the quadrupole ion-trap mass spectrometer (1290–6430, Agilent, USA). The scanning type was positive/negative ion scanning with scan range *m/z* 200–1500. The fragmentor and capillary voltage were set to 110 V and 4.5 kV, and the drying gas temperature was 350 °C with the flow 9 L·min⁻¹. HPLC–MS separation of the rhamnolipid mixtures utilized a reverse phase ZOBARX SB C18 column (5 μm C18, 100 × 2.1 mm, Agilent) and a binary gradient mobile phase comprising HPLC grade H₂O as mobile phase A and methanol as mobile phase B. Initially, the proportions were 30% A and 70% B. The flow rate of 0.2 ml min⁻¹ and an injection volume of 1 μL were used throughout.

2.3. Preparation of standard solution

0.2 g of each PAH compound was dissolved in 100 mL of dichloromethane to prepare a stock solution of 2 g·L⁻¹. The solution was then stored at –10 °C.

The PAHs were quantified using 7 levels of external standards obtained by serial dilutions of stock solutions at a concentration range 2–14 mg·L⁻¹ of naphthalene, 0.4–2.8 mg·L⁻¹ of phenanthrene, 0.2–2.6 mg·L⁻¹ of pyrene.

2.4. PAH detection and quantification

Detection and quantification of different PAHs (naphthalene, phenanthrene and pyrene) were carried out using Ultraviolet Spectrophotometry (UV2450, Shimadzu).

2.5. Solubilization experiments

All experiments were carried out in 100 mL glass conical flasks with Teflon-lined screw caps. The effects of biosurfactants on the solubilization behaviors of naphthalene, phenanthrene and pyrene were examined. For the solubilization experiment, excess PAHs were added to the conical flask and 25 mL background solution. The background solution was comprised of appropriate biosurfactant with 0.01 M NaNO₃, which was a biocide system. The pH was adjusted to 7 at a constant ion concentration. The conical flasks were placed on a reciprocal shaker at 30 °C, 100 rpm for 48 h to reach solubilization equilibrium. Preliminary experiments showed that 48 h were enough for the solubilization of phenanthrene to reach equilibrium, and phenanthrene degradation or adsorption by the tubes was negligible. The suspensions

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