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ScienceDirect
Journal of Colloid and Interface Science 305 (2007) 361–365
JOURNAL OF Colloid and Interface Science
www.elsevier.com/locate/jcis

Note

Enhanced aqueous solubilization of tetrachloroethylene by a rhamnolipid biosurfactant

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Received 31 July 2006; accepted 15 October 2006

Available online 19 October 2006

Abstract

A rhamnolipid biosurfactant produced by *Pseudomonas aeruginosa* ATCC 9027 was isolated, purified and characterized in terms of its ability to mobilize and solubilize tetrachloroethylene (PCE) for potential use in surfactant-enhanced aquifer remediation (SEAR) applications. Using a drop volume method, the PCE–biosurfactant steady-state interfacial tension was determined and found to be ca. 10 mN/m which is not low enough to cause significant PCE nonaqueous phase liquid (NAPL) mobilization. It was observed that the biosurfactant partitioned significantly into PCE at aqueous concentrations higher than the critical micelle concentration (CMC). After accounting for rhamnolipid partitioning into the PCE phase, a weight solubilization ratio (WSR) of 1.2 $g_{PCE}/g_{rhamnolipid}$ was determined and through this mechanism the biosurfactant significantly improved the apparent aqueous solubility of PCE.

Keywords: Biosurfactant; Distribution coefficient; Interfacial tension; *Pseudomonas aeruginosa*; Rhamnolipid; Tetrachloroethylene

1. Introduction

Surfactant-enhanced aquifer remediation (SEAR) of tetrachloroethylene (PCE), a common groundwater pollutant, has been repeatedly demonstrated at field scale [1–4]. SEAR has gained much attention as a viable alternative to the traditional pump-and-treat methods that are used for cleaning groundwater contaminated with nonaqueous phase liquids (NAPL) [5,6]. In this approach, the addition of surfactant serves two roles: (1) increase the apparent solubility of the dissolved NAPL and (2) reduce the interfacial tension between the aqueous and NAPL phase to mobilize entrapped NAPL.

The interest in biosurfactants is fueled by the advantage that they are readily biodegradable [7] and as a result more attractive from an environmental perspective for SEAR processes. There is also the potential opportunity to produce the biosurfactant in situ, which may result in an economically feasible long-term approach to aquifer remediation. With this in mind, we evaluated the physical properties of a rhamnolipid biosurfactant and its suitability for the enhanced solubilization of PCE.

Rhamnolipids are anionic biosurfactants produced by *Pseudomonas* sp. bacteria [8]. The chemical composition of the rhamnolipid produced depends on its source. They are mainly composed of one or two rhamnose sugars as the hydrophilic moieties and long chain fatty acids as the lipophilic moieties. Much of the current research has focused on environmental applications of rhamnolipids [16]. The enhanced dispersion and biodegradation of octadecane [9], the biodegradation of phenanthrene [9] and its removal from soil [10], and the removal of various hydrophobic compounds [11], metals [12], and crude oil [13] from soil have been studied. Further, the influence of rhamnolipid on the solubilization and mobilization of hexadecane [14] and the partitioning of hexadecane in multiphase systems [15] have been examined. The solubilization of chlorinated solvents by rhamnolipids has not been previously reported.

This paper describes the characterization of a rhamnolipid biosurfactant produced by *Pseudomonas aeruginosa* ATCC 9027 in view of its potential use in SEAR processes. This work builds on earlier work [17] aimed at understanding the contribution of surfactants to the dissolution of PCE in porous media.

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0021-9797/$ – see front matter
doi:10.1016/j.jcis.2006.10.026

2. Experimental

2.1. Biosurfactant production and characterization

Pseudomonas aeruginosa ATCC 9027 was obtained from the American Type Culture Collection (Rockville, MD) and maintained as slants on *Pseudomonas* Medium A [18]. The rhamnolipid biosurfactant was produced as described by Zhang and Miller [9]. The crude biosurfactant was purified by silica gel column chromatography using a chloroform–methanol gradient. The purity of the biosurfactant was assessed using thin-layer chromatography (TLC) on Kieselgel 60 F254 aluminum backed plates and a chloroform–methanol–water (65:15:2 v/v/v) solvent system. Detection on the TLC plates was based *p*-anisaldehyde [19] and anthrone [20] spray reagents. The composition of the sugar moiety was determined by TLC as described by Rapp et al. [21]. The composition of the fatty acid moiety was characterized by fatty acid methyl ester (FAME) analysis. Methanolysis of the fatty acids was conducted as per Minnikin et al. [22] and the extracts were analyzed by gas chromatography using a HP-1 column (Agilent Technologies), helium as the carrier gas (1.0 ml/min) and flame-ionization detection. The oven temperature was held at 150 °C for 4 min then ramped to 250 °C at 4 °C/min. Biosurfactant concentration was determined by quantifying the rhamnose moiety using the method of Gibbons [23].

2.2. Interfacial tension measurements

Steady-state interfacial tension between the biosurfactant and PCE was determined using a drop volume method [24]. Rhamnolipid solutions (0–175 mg/l) were prepared in 10 mM phosphate buffer (pH 7). Twenty ml of each rhamnolipid solution was allowed to equilibrate with 20 µl of PCE prior to analysis. Approximately 3 ml of the equilibrated rhamnolipid solution was placed in a glass cuvette. The tip of a capillary ($r_{cap} = 0.0135$ cm) connected to a motor-driven syringe was immersed into the biosurfactant solution and PCE was dispensed at a constant rate of 4.4 ml/h. The total number of drops dispensed for a predetermined volume was counted to determine the average drop volume. The correction factor of Wilkinson [25] was used since the drop detached at its neck and not the tip of the capillary.

2.3. Rhamnolipid distribution coefficient

Aqueous rhamnolipid solutions (0–225 mg/l) were prepared in 10 mM phosphate buffer (pH 7). The rhamnolipid solutions were combined with PCE in a 1:1 ratio (v/v). The biphasic mixtures were agitated at 100 rpm on an orbital shaker for a minimum of 48 h. The samples were centrifuged at 1000*g* for 30 min to break the emulsion and separate the phases. The concentration of rhamnolipid in the aqueous phase was measured before and after equilibration with PCE. The equilibrium rhamnolipid concentration in the organic phase was determined by mass balance.

2.4. PCE solubility

Aqueous stock solutions of rhamnolipid (0–175 mg/l) were prepared in 10 mM phosphate buffer (pH 7). Twenty ml of each rhamnolipid solution was combined with 20 µl of PCE resulting in a biphasic system. A minimal amount of PCE was used to limit the potential of rhamnolipid partitioning into the organic phase [17,26]. The samples were agitated at 100 rpm on an orbital shaker for 48 h. The samples were allowed to settle for 24 h then 5 ml of the upper aqueous layer was carefully removed and extracted with 1 ml of methyl *tert*-butyl ether in the presence of 2 g of sodium sulphate and 50 µl of a surrogate standard (decafluorobiphenyl, 10 mg/ml in acetone) [27]. The upper organic layer was analyzed by gas chromatography using a DB-5 column (J&W Scientific), helium as the carrier gas (1.0 ml/min) and flame-ionization detection. The oven temperature was held at 60 °C for 5 min then ramped to 200 °C at 20 °C/min.

3. Results and discussion

3.1. Biosurfactant characterization

The chemical character of the biosurfactant was evaluated using a combination of TLC and FAME analysis by gas chromatography. The sugar moiety of the biosurfactant was determined by TLC using both glucose and rhamnose as sugar standards. Aqueous solutions of each standard along with a mixture of the two sugars were initially analyzed by TLC to verify if sufficient resolution could be obtained. The standards were well separated with a resolution of 1.7; glucose and rhamnose had retention factors (R_f) of 0.44 and 0.55, respectively. The rhamnolipid sample was chromatographed along with the authentic standards and had an anthrone positive spot with a R_f of 0.56 which was consistent with rhamnose. Analysis of the fatty acid methyl ester profiles of the biosurfactant's lipid moiety indicated that the major fatty acid components were β-hydroxydecanoic acid (ca. 75%) and methyl-3-hydroxydodecanoic acid (ca. 8%). This information suggested that biosurfactant was a rhamnosyl-β-hydroxydecanoyl-β-hydroxydecanoic acid which was consistent with the monorhamnolipid biosurfactant produced by Zhang and Miller [9]. Based on this analysis, a rhamnolipid molar mass of 504 g/mol was assigned for subsequent calculations. The preparation was 80–90% biosurfactant based on the rhamnose content and assuming that only monorhamnolipid was present.

3.2. Interfacial tension measurements

Interfacial tension measurements between PCE and aqueous rhamnolipid solutions were performed to characterize the surface active properties of the biosurfactant. The data was then used to estimate the critical micelle concentration (CMC). The rhamnolipid solutions were prepared in phosphate buffer to control pH which is known to have a significant influence on the morphology of the surfactant aggregates [28,29]. Micelles