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# Degradation of hexadecane by *Pseudomonas aeruginosa* with the mediation of surfactants: Relation between hexadecane solubilization and bioavailability

Hua Zhong [a, b, d, *], Zhiquan Wang [a, c], Zhifeng Liu [a, c, **], Yang Liu [a, c], Mingda Yu [a, c], Guangming Zeng [a, c]

[a] College of Environmental Science and Engineering, Hunan University, Changsha, 410082, China
[b] School of Water Resources and Hydropower Engineering, Wuhan University, Wuhan, 430070, China
[c] Key Laboratory of Environmental Biology and Pollution Control, Hunan University, Ministry of Education, Changsha, 410082, China
[d] Department of Soil, Water and Environmental Science, University of Arizona, Tucson, AZ, 85721, United States

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ABSTRACT

Degradation of hexadecane by *Pseudomonas aeruginosa* ATCC 9027 mediated with octylphenol ethoxylate (Triton X-100) or sodium dodecylbenzene sulfonate (SDBS) was studied to examine the relation between surfactant pseudo-solubilization and bioavailability for hydrophobic organic compounds (HOCs). For either of the surfactants, degradation of hexadecane in pseudo-solubilized form was delayed compared to hexadecane as a separate phase, showing a reduced availability of pseudo-solubilized hexadecane. When hexadecane with a quantity significantly higher than the pseudo-solubility was used as the carbon source, Triton X-100 enhanced the degradation whereas SDBS still showed inhibitory effect. Degradability test shows that Triton X-100 as the sole carbon source could be partially degraded, whereas SDBS could not. These results indicate that a reduction in HOC bioavailability may occur for surfactant-mediated bioremediation process, likely due to the barrier effect of surfactants at water-HOC interface.



## 1. Introduction

Microbiological removal of hydrophobic organic compounds (HOCs), e.g. petroleum hydrocarbons, polycyclic aromatic hydrocarbons (PAHs) and organic pesticides, has attracted increasing interests in recent years for wastewater treatment (Ania et al., 2007; Zeledon-Toruno et al., 2007; Ding et al., 2008; Lippold et al., 2008; Paria, 2008; Yuan et al., 2010; Janbandhu and Fulekar, 2011; Peng et al., 2015). Limited bioavailability of HOCs is the obstacle for the efficiency of these removal processes (Ghazali et al., 2004; Sarkar et al., 2005). Surfactants are amphiphilic molecules that can increase apparent aqueous solubility of HOCs (Ansari et al., 2013). They have been proved effective in improving bioavailability of HOCs in complex systems for degradation (Zheng et al., 2012; Silva et al., 2014). Such improvement in bioavailability is often assumed to be a result of HOC solubility enhancement in the presence of surfactants.

The effect of surfactants to enhance bioavailability of HOCs by enhancing HOC solubility, however, has yet to be fully resolved. Inhibitory effects were sometimes observed when surfactants were used for enhancing degradation of HOCs (Zhao et al., 2011; Song and Bielefeldt, 2012; Rodrigues et al., 2013). An assumption for such inhibitory effects of surfactants is the barrier effect of surfactants at the surface of solubilized HOC particles, which may block the contact between HOCs and microbial cells and thus uptake of the HOCs by microbial cells (Li and Bai, 2005; Dai et al., 2010).

In these prior studies, however, the dosage of HOCs in cultures was exclusively much higher than their solubility with surfactants, resulting in a variety of carbon source forms, e.g. pseudo-solubilized, emulsified, and separate phase of the compounds (i.e., liquid or solid). The co-existence of these complex forms made it difficult to evaluate the role of surfactant solubilization on the

* Corresponding author. School of Water Resources and Hydropower Engineering, Wuhan University, Wuhan, 430070, China.
** Corresponding author. College of Environmental Science and Engineering, Hunan University, Changsha, 410082, China.
*E-mail addresses:* zhonghua21cn@126.com (H. Zhong), lzf18182002@163.com (Z. Liu).

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HOC bioavailability. In this study, degradation of *n*-hexadecane as a representative of HOCs by *Pseudomonas aeruginosa* ATCC 9027 mediated by an octylphenol ethoxylate (Triton X-100) and sodium dodecylbenzene sulfonate (SDBS) were investigated. Hexadecane is a typical alkane, which belongs to a class of HOCs with simple molecule structures. *P. aeruginosa* ATCC 9027 is a strain that has a strong ability for alkane degradation, which has been demonstrated by the results of many studies (Zhang and Miller, 1992, 1994, 1995; Holden et al., 2002). Triton X-100 and SDBS are typical synthetic surfactants that were widely used for remediation of organic contamination (Yang et al., 2006; Fan et al., 2013; Gitipour et al., 2014). The state of hexadecane in culture was on purpose controlled so that the carbon source was either mass hexadecane without surfactant (a separate phase), or hexadecane in solubilized form with surfactant, or excessive hexadecane with surfactant (multiple forms). The objective is to clarify the relation between surfactant-enhanced solubilization and bioavailability for HOCs.

## 2. Material and methods

### 2.1. Microorganism and chemicals

*Pseudomonas aeruginosa* ATCC 9027 were obtained from the American Type Culture Collection (Rockville, Md.). This strain was received as a lyophilized stock. Before use it was revived on peptone agar slants and activated at 37 °C for 24 h. The surfactants (Triton X-100 and SDBS) and hexadecane (purity≥99%) were purchased from Sigma-Aldrich (St.Louis, Mo., U.S.). Other reagents were of analytical grade and used as received. The water used throughout the experiment is produced by UPT-II-40 (Ulupure, Chengdu, China) with electrical resistivity of 18.2 mΩ cm.

### 2.2. Determination of critical micelle concentrations (CMCs) of Triton X-100 and SDBS

Triton X-100 or SDBS were dissolved in mineral salt medium (MSM, constituents are (g/L): $NH_4Cl$, 5; $KH_2PO_4$, 2.5; $Na_2HPO_4$, 5; $MgSO_4 \cdot 7H_2O$, 0.5). The initial pH was adjusted to 6.8 with NaOH) and diluted to a series of concentrations. Surface tensions of the solutions were measured by the surface tension meter (JZ-200A, Chengde, China). CMCs of the surfactants was obtained by analyzing the relation between surface tension and surfactant concentration using the method described by Yuan et al. (2007).

### 2.3. Solubilization of hexadecane by Triton X-100 and SDBS

Two (2) mL hexadecane was pipetted to a 500-mL Erlenmeyer flask and spread on the bottom by slowly rotating the flask. Then 250 mL of MSM solution with surfactant at concentration of 5 × CMC was added into the flask, and the flask was shaken at 30 °C for 72 h on a reciprocal shaker running at 100 rpm. The solution was transferred into a separatory funnel and stood still overnight to allow complete floating of mass hexadecane to the top of the aqueous phase. Then the bottom solution was collected at a very low and steady flow rate (approximate one drop per 10 s). The above separation process was repeated, and finally the pseudo-solubilized hexadecane solution with 5 × CMC surfactant was obtained. The concentration of hexadecane, $C_{5\times CMC}$, was measured using gas chromatography method described by Zhong et al. (2014).

### 2.4. Degradation experiment

Firstly the strain (*P. aeruginosa* ATCC 9027) was enriched for 8 h in seed medium on a gyratory shaker running at 200 rpm and 37 °C. Constituents of the seed medium were (g/L): peptone, 5; beef extract, 10; NaCl, 5; and pH 6.8. The seed was then inoculated at a ratio of 3% (v/v) into MSM with 1% (v/v) hexadecane as carbon source, and cultured at 200 rpm and 37 °C. This is for production of hexadecane-acclimated inoculum for degradation experiment.

Totally there were 5 sets of culture medium, with different forms and concentrations of hexadecane and/or surfactant as the carbon source. These sets of culture medium are: (1) Pseudo-solubilized hexadecane in the presence of 5 × CMC surfactant. The concentration of hexadecane was $C_{5\times CMC}$. (2) Mass hexadecane added as the sole carbon source and no surfactant. The equivalent concentration of hexadecane was $C_{5\times CMC}$; (3) Only surfactant at the concentration of 5 × CMC and no hexadecane. (4) Surfactant at concentration of 5 × CMC and hexadecane in great excess to the pseudo-solubility. The equivalent concentration of hexadecane is 10 × $C_{5\times CMC}$. (5) Large quantity of mass hexadecane as sole carbon source. The equivalent concentration of hexadecane is 10 × $C_{5\times CMC}$.

10-mL plastic centrifuge tubes were used as the bioreactors. 2 mL of culture medium were placed in the tubes. Mass hexadecane was added using μL-level pipette when needed. Then 60 μL of hexadecane-acclimated culture was inoculated into every tube. The tubes were then plugged with sterile cotton and incubated with gyratory shaking at 30 °C and 200 rpm to start the degradation process. Every two day the tubes were unplugged on a clean bench and the cultures were exposed to the air for 30 min to enhance replenishment of oxygen. At predetermined time points, three tubes were sacrificed for cell protein and hexadecane concentration analysis. The tubes were centrifuged at 5600 × *g* for 10 min, and the supernatant was decanted into another 10-mL centrifuge tube. The inner wall of the centrifuged tube was washed using 3 mL ethanol so that the attached hexadecane was also washed into the new tube. The culture supernatant in the new tube was used for hexadecane analysis. The cell pellet in the cultivation tube was examined for protein content. Triton X-100 or SDBS concentration were also analyzed when they were used as the sole carbon source.

Hexadecane concentration and cell protein concentration were measured using gas chromatography and UV spectrometry methods, respectively, as described by Zhong et al. (2014). The cobalt thiocyanate method (Mata-Sandoval et al., 2001) and methylthionine chloride method (Porcel et al., 2001) were used for Triton X-100 and SDBS analysis, respectively.

## 3. Results and discussion

Dependence of MSM surface tension on surfactant

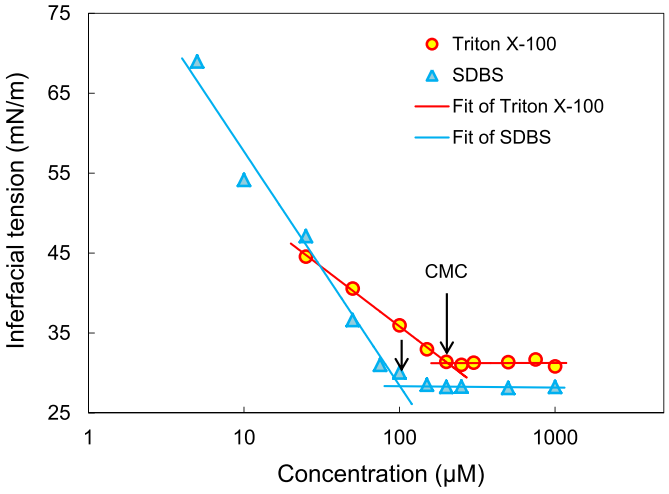


**Fig. 1.** Surface tension of MSM solution in the presence of Triton X-100 and SDBS.