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Diverse effect of surfactants on pyrene biodegradation by a *Pseudomonas* strain utilizing pyrene by cell surface hydrophobicity induction

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ABSTRACT

Pseudomonas aeruginosa is an efficient pyrene degrader that can utilize pyrene through induction of high cell surface hydrophobicity. The effect of three surfactants, Triton X-100, Tween 80 and rhamnolipid JBR 515 on rate and extent of pyrene (100 mg l^{-1}) degradation was evaluated through batch studies. Among the three surfactants Triton X-100 demonstrated highest pyrene solubilization. All surfactants were used at 10 times the critical micelle concentration (CMC) so as to ensure notable enhancement in solubilization. However, growth of *P. aeruginosa* on pyrene and pyrene degradation was inhibited in the presence of Triton X-100 possibly due to accumulation of toxic metabolites formed due to partial utilization of Triton X-100. Tween 80 supported higher rate of pyrene degradation due to higher biomass generation during simultaneous utilization of pyrene and Tween 80 as carbon source. In presence of rhamnolipid JBR 515 preferential degradation of surfactant over pyrene resulted in decrease in specific growth rate and pyrene utilization rate. Maximum biodegradation rates (MBR) for pyrene were 0.458, 1.111 and 0.154 mg l^{-1} h^{-1} in absence of any surfactant, in presence of Tween 80 and rhamnolipid JBR 515, respectively. The results suggest that Tween 80 is the preferred surfactant for remediation of pyrene using *P. aeruginosa* RS1 strain.

1. Introduction

Soil and groundwater contamination with creosote, coal tar or industrial non-aqueous phase liquids (NAPLs) containing hydrophobic organic compounds (HOCs), such as, PAHs is a worldwide problem. Although poor aqueous solubility of high molecular weight polynuclear aromatic hydrocarbons (HMW PAHs) poses a challenge for microbial degradation of these compounds, several microorganisms with good PAH degradation ability have been reported (Margesin et al., 2013; Obayori et al., 2013; Lu et al., 2014). However, the rate and extent of degradation of PAHs are often restricted due to poor aqueous solubility and high hydrophobicity. Sorption to soil and sediments also limits the bioavailability of pyrene (Shuttleworth and Cerniglia, 1995; Seo et al., 2009).

Several studies have been conducted using surfactants to overcome bioavailability limitation in the bioremediation of PAHs. Surfactants are amphiphilic molecules that can enhance the apparent solubility of PAHs by forming micelles when present at concentration above the critical micelle concentration (CMC). Some researchers have reported enhancement in biodegradation of PAHs in presence of surfactants (Prak and Pritchard, 2002; Zheng and Obbard, 2002; Aryal and Liakopoulou-Kyriakides, 2013; Chen et al., 2013). However, sometimes no beneficial effect/detrimental effect have been observed (Laha and Luthy, 1991; Margesin and Schinner, 1999; Mesbaiah et al., 2015). Possible reasons for inhibition in surfactant-mediated degradation include surfactant toxicity to microorganisms due to permeabilization of cell membrane (Willumsen and Karlson, 1998; Avramova et al., 2008), decreased bioavailability of micelle solubilized PAHs (Laha and Luthy, 1991) or preferential utilization of the surfactant over the target PAHs (Kim and Weber, 2003). In a study with four surfactants, Tween 20 (non-ionic), sodium dodecyl sulphate (SDS, anionic), tetradecyltrimethyl ammonium bromide (TTAB, cationic) and Citrikleen (a commercial emulsifier), Bramwell and Laha (2000) observed that mineralization of phenanthrene by *Pseudomonas aeruginosa* decreased as the surfactant concentration increased. This was attributed to enhanced toxicity of solubilized phenanthrene. Yuan et al. (2007) also reported inhibition in biodegradation of phenanthrene and

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other PAHs in presence of common surfactants, such as, Brij 30 and Brij 35. Longer lag phases have been reported in presence of Triton X-100 and Triton N101. Preferential utilization of surfactants as primary substrate is also reported to hinder PAH degradation (Tiehm, 1994; Deschenes et al., 1996). Based on these contradictory results it appears that the impact of surfactants on bioremediation of PAHs is system specific, i.e., it depends on many factors, such as, surfactant type and concentration, substrate type and microorganisms used.

Previous studies mostly highlighted the interaction between HOCs and surfactants and bioavailability of these compounds to the microorganisms (Bramwell and Laha, 2000; Doong and Lei, 2003; Bautista et al., 2009). Later it was identified that the interaction between surfactants and microorganisms also plays an important role in biodegradation of PAHs (Zhang et al., 2013). For biodegradation to take place inside the cells, substrate uptake through biosorption and transmembrane transport is an important step. Cell surface hydrophobicity (CSH) of microorganisms is a crucial factor affecting the uptake mechanism of hydrocarbons and recent studies have conclusively demonstrated the ability of surfactants to alter CSH (Mohanty and Mukherji, 2012; 2013). Some recent studies (Zhao et al., 2011; Zhang et al., 2013) have elucidated the mechanism involved in surfactant induced modification of CSH and have correlated CSH changes with change in rate and extent of PAH degradation. It is typically believed that surfactants modify the cell surfaces of bacteria through adsorption/partitioning of the surfactants (Zhong et al., 2007; Zhang et al., 2013) or release of lipopolysaccaride from gram negative bacteria (Zhang and Miller, 1995; Al-Tahhan et al., 2000; Mohanty and Mukherji, 2008). For better understanding surfactant mediated sorption and changes in CSH, knowledge of the native cell surface properties is important. Generally, surfactants increase the CSH of hydrophilic bacteria, while they reduce the CSH of hydrophobic bacteria (Kaczorek et al., 2008; Zhao et al., 2011). Surfactant properties, such as polyoxyethylene and alkyl chain length are also additional parameter that may affect the extent of CSH induction in any bacterial strain. Zhang et al. (2013) demonstrated that three Tween series surfactants Tween 80, Tween 40 and Tween 20 having the same polyoxyethylene chain length (hydrophilic moiety) and differing only in the alkyl chain (hydrophobic) length exerted different degree of CSH induction in a hydrophilic strain of *Klebsiella oxytoca* PYR-1 during degradation of pyrene. Further work in this area is needed to understand the interaction between surfactant, microbial cells and PAHs to choose appropriate surfactant and bacteria combination for successful clean-up.

The objective of this study was to investigate the impact of the two non-ionic surfactants, Triton X-100 and Tween 80 and a biosurfactant rhamnolipid JBR 515, on biodegradation of pyrene by the *P. aeruginosa* RS1 strain that could utilize pyrene effectively in the absence of surfactants (Ghosh et al., 2014). Although various strains of *Pseudomonas* are reported to degrade LMW PAHs, only a few strains can utilize four ring PAHs, such as, pyrene, as sole source of carbon and energy. Hence, it is important to study the response of such strains to surfactants that are often added to facilitate degradation in contaminated sites. Additionally the CSH of the bacteria in absence and presence of surfactants was determined to gain insight regarding the mechanism of microbial interaction with surfactants during degradation of pyrene. In these studies, CSH was determined based on both bacterial adhesion to hydrocarbon (BATH) assay and contact angle measurement. In most of the surfactant-mediated PAH degradation studies BATH assay i.e., adherence of bacteria to n-hexadecane has been performed. BATH assay provides a relative measure of percent adherence of bacteria to non-aqueous phase liquids which may be affected by several solution phase parameters, such as, pH, re-suspension buffer, ionic strength (Chakraborty et al., 2010) and also by specific affinity to the chosen non-aqueous phase liquid (NAPL) (Mohanty and Mukherji, 2008). In contrast, the cell surface contact angle values are generally unaffected by such factors and provide a more reliable measure of CSH.

2. Materials and methods

2.1. Chemicals

Pyrene (98%) was purchased from Sigma Aldrich Chemicals Pvt. Ltd. Chemical surfactants Triton X-100 (98%) and Tween 80 (98%) were obtained from Merck India Pvt. Ltd., Mumbai, India. Biosurfactant rhamnolipid JBR 515 was gifted by Procter and Gamble, U.S.A. It was a 15% solution in water and consisted of a mixture of monoramnolipid and dirhamnolipid. Chemical structures of the surfactants are provided in electronic supplementary information (Fig. S1, ESI). Mineral media components, nutrient broth and bacteriological agar were supplied by Hi-media Laboratories, Mumbai, India.

2.2. Bacterial strain and growth condition

P. aeruginosa RS1 used in this study was isolated from a consortium enriched from tank bottom sludge collected from an oil refinery in Mumbai, India (Jasmine and Mukherji, 2014) using the oil extracted from sludge as sole substrate in mineral media. The gene bank accession number for this strain is KF751345. *P. aeruginosa*, RS1 exhibited good growth on oil extracted from sludge. Later, RS1 strain demonstrated ability to utilize pyrene as sole source of carbon and energy over the pyrene concentration ranges 25–500 mg l^{-1} (Ghosh et al., 2014). The composition of mineral media used for all the studies are as described by Mukherji et al. (2004).

2.3. Pyrene solubilizing capacity of various surfactants

The CMC values of Triton X-100, Tween 80 and rhamnolipid JBR 515 are 0.149, 0.016 and 0.125 g l^{-1} (Mohanty, 2010), respectively. Stock solutions of 1000 CMC was prepared for all the three surfactants separately and were sterilized by filtering through 0.2 μm pore size nylon filter (Pall Corporation, U.S.A). Pyrene solubilizing capacity of the three surfactants, i.e., Triton X-100, Tween 80 and rhamnolipid JBR 515 was determined. Aqueous solubility of pyrene is 0.13 mg l^{-1}. For this study, pyrene (400 μl) was added from a pyrene stock (2.5%, w/v) solution in dichloromethane (DCM) to achieve a final concentration of 100 mg l^{-1} in a 500 ml Erlenmeyer flask. After complete evaporation of the solvent, 100 ml mineral media and the required quantity of each surfactant was added from 1000 CMC stock so as to obtain a concentration of 2, 4, 10, 20 and 30 CMC. Duplicate flasks were kept for each surfactant–concentration combination. The flasks were incubated in a rotary shaker at 120 rpm and 28 °C for 24 h. These studies were performed under aseptic conditions so as to avoid any biological growth. After 24 h, 5 ml of sample was pipetted out from duplicate flasks with glass pipette and filtered through 0.2 μm filter to remove all the suspended crystals of pyrene (Mahanty et al., 2009). The filtrate contained the aqueous pyrene solubilized by the surfactant. The filtrate was further diluted by HPLC grade methanol where necessary and the solubilized pyrene concentration was determined using HPLC equipped with fluorescence detector (Ghosh et al., 2014).