

Selection of surfactants for enhancing diesel hydrocarbons-contaminated media bioremediation

A. Franzetti*, P. Di Gennaro, G. Bestetti, M. Lasagni,
D. Pitea, E. Collina

*Dep. Environmental Sciences, University of Milano-Bicocca,
piazza della Scienza 1, 20126 Milano, Italy*

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Abstract

The use of surfactants represents a valuable method to enhance the access of the microorganisms to low-soluble and recalcitrant compounds in bioremediation techniques. The choice of surfactants is the first step of feasibility studies for this application. So far, no defined procedures are present in literature to select the most suitable surfactant for the treatment of a specific contaminated site. Furthermore, the characterisation of physico-chemical parameters is important to understand the reason of successes and failures. In this paper a step procedure to select and characterise a commercial surfactant to be used in bioremediation enhancement of hydrocarbon-contaminated media was developed. Among the commercial surfactants, the procedure was applied to alkyl polyethoxylates (Brij family) and sorbitan derivatives (Tween family). The selection resulted in the application of Brij 56 and Tween 80 as biodegradation-enhancer in different lab scale systems for remediation of diesel contamination. In liquid systems, Tween 80 greatly increased biodegradation of highly branched and high-molecular weight hydrocarbons, while Brij 56 enhanced degradation of highly branched hydrocarbons. Based on these results, the potential applications and the limitations of these surfactants at full scale level were estimated.

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1. Introduction

Bioremediation techniques could represent an economical and environmental sound tool for managing sites polluted by organic wastes. Application of bioremediation technologies for hydrocarbon-contaminated soil is often limited by presence, within the mixture of contaminants, of high recalcitrant and low bioavailable compounds. Compounds characterised by low solubility and high hydrophobicity tend to be strongly sorbed to organic matrix and to be low available to microorganisms for degradation. It has been demonstrated that slow release of these compounds from soil and free phase to water phase could represent a rate-limiting factor for bioremedia-

tion processes, leading to impossibility in reaching the target of remediation [1,2]. Extent of limitation depends on pollutant characteristics like K_{oc} value and on soil parameters like clay content, Cation Exchange Capacity (CEC) and, in particular, organic matter. Furthermore, biodegradation of complex mixtures of hydrocarbons is often characterised by preferential degradation of linear and low-molecular weight hydrocarbons leading to accumulation of recalcitrant compounds [3].

For these reasons, suitable methods to overcome metabolic and bioavailability drawbacks are needed to successfully remediate contaminated wastes. To enhance bioavailability of contaminants, surfactants may be useful due to their capability to increase water solubility and mass transfer. Many applications of surfactants are reported in literature for remediation of hydrocarbon-contaminated soil both in solid and slurry systems [4–7]. Before application, an environmental fate assessment of surfactant is strictly recommended to avoid unacceptable accumulation of surfactant in soil system [8].

* Corresponding author. Tel.: +39 02 6448 2927; fax: +39 02 6448 2996.

E-mail addresses: andrea.franzetti@unimib.it (A. Franzetti),
patrizia.digennaro@unimib.it (P. Di Gennaro), giuseppina.bestetti@unimib.it
(G. Bestetti), marina.lasagni@unimib.it (M. Lasagni),
demetrio.pitea@unimib.it (D. Pitea), elena.collina@unimib.it (E. Collina).

A key step in evaluation of applicability of a commercial surfactant in soil remediation is the choice of surfactant. So far, no defined procedures are present in literature for the selection of the most suitable surfactant for the remediation of a specific contaminated site.

In this work, the selection of a surfactant to enhance diesel hydrocarbon biodegradation was carried out by a step procedure considering: (i) the physico-chemical properties of surfactants; (ii) the biodegradability of surfactants; and (iii) the influence of the surfactants on the biodegradation rate and yield of contaminants in different systems. Among the commercial surfactants, non-ionic surfactants are considered less toxic and more biodegradable than anionic and cationic ones [9]; furthermore, among non-ionic surfactants, the use of alkyl phenol polyethoxylates is discouraged because their biodegradation leads to formation of alkyl phenol, more toxic and persistent than parent compounds [10]. For this reason, the selection of surfactant was carried out among alkyl polyethoxylates (Brij family) and sorbitan derivatives (Tween family).

The first phase of selection estimated the environmental fate and toxicity of a wide group of surfactants belonging to the cited families to reduce the number of surfactants for laboratory characterisation. For this purpose, K_{oc} and *Daphnia magna* EC₅₀ were estimated by a molecular descriptor and a QSAR model. In the second step, laboratory experiments were conducted to evaluate soil sorption and biodegradability of surfactants and Brij 56 and Tween 80 were selected. In the last part of the research, the effect of these surfactants on biodegradation of diesel fuel was studied in liquid cultures and in slurry and solid phase systems. Liquid phase experiments were carried out using a diesel hydrocarbon-degrading microorganism named Ap. The latter experiments were performed with diesel-contaminated soil in bench scale slurry phase bioreactor and solid phase columns. To assess the effect of selected surfactants on the final composition of residual hydrocarbon mixture, an elaboration of the chromatographic data obtained in the chemical analyses was developed.

2. Materials and methods

2.1. Soil characterisation

In this study soil from a diesel fuel-contaminated site was used. Two kind of soil samples were collected with the same characteristics apart from the presence of contaminants: one in the contaminated zone and one in an uncontaminated part of the site. In the former, hydrocarbons in the mixture were in the range of C₁₂–C₃₀ and the concentration was 1700 mg/kg_{dw}. The characterisation of the soil is already reported in a previous work [8] and here summarised. Content of organic carbon of 11.4 g/kg_{dw}; granulometric composition as follows (less than 250 μm: 3%; 250–500 μm: 13%; 500–850 μm: 31%; 850–2000 μm: 53%). Value of Cation Exchange Capacity (CEC) was determined as 7.34 ± 0.01 cmol(+)/kg. Heterotrophic bacteria were determined as 1.5 × 10⁶ CFU/g_{dw}.

2.2. Screening of surfactants by estimation of K_{oc} and toxicity

K_{oc} values of surfactants among alkyl polyethoxylates (Brij family) and sorbitan derivatives (Tween family) were evaluated by a modified first-order molecular connectivity index (MCI) [11]. Toxicity of alkyl polyethoxylate surfactants was estimated by a specific QSAR model, that estimated the toxicity of each specific molecule on the basis of the number of ethoxylate groups and the length of alkyl chain [12].

2.3. Measurement of surfactants

Brij surfactants were analysed by CTAS method [13]. Briefly, non-ionic surfactants react with aqueous cobalt thiocyanate to give a cobalt-containing product. The concentration of this product, extracted in an organic liquid, is measured spectrophotometrically.

2.4. Surfactant sorption experiments

Surfactant sorption experiments were conducted preparing 100 ml Erlenmeyer flasks filled with 1 or 2 g of soil sample (previously sterilised by addition of sodium azide) and 10 ml of saline surfactant–water solution (NaCl 1 g/l). Five flasks at different concentrations were prepared for each surfactant. Initial concentrations of surfactants were 0.5, 1, 2, 3, 4 mg/l. Flasks were left at 20 °C and 100 rpm for 24 h to reach sorption equilibrium (equilibrium time was determined by preliminary tests) and then centrifuged at 8100 rpm for 10 min. Supernatant solution was analysed for surfactant concentration as described above (Section 2.3) and sorbed concentration was calculated by difference.

2.5. Biodegradability of surfactants

Biodegradability of the surfactants was assessed by respirometric tests both in liquid culture and solid phase. Experiments were performed in Biochemical Oxygen Demand (BOD) apparatus (VELP Scientifica, Italy) and ratio between actual BOD and theoretical BOD (BOD_{Th}) was calculated over time. For experiments in liquid cultures, bottles were filled with 125 ml surfactant solutions in mineral bacteriological medium M9 [14], and 2 ml of soil bacteria inoculum at optical density at 540 nm (OD₅₄₀) equal to 1, as an indicator of bacterial biomass concentration. Bacterial inoculum was obtained as previously described [8]. Different concentrations of surfactants (500, 250, 100, 50 mg/l) were tested to evaluate the biodegradability in different conditions. To evaluate the biodegradation of surfactants in presence of soil bacteria and of surfactant sorption on soil, solid phase respirometric test were carried out. Bottles were filled with 5 g of soil and 2 ml of saline (NaCl 1 g/l) surfactant water solution to reach desired surfactant concentration in soil. Initial concentrations of surfactant in soil were 0.5 and 2 g/kg. Other bottles without surfactant were assembled to measure basal activity of inoculated bacteria. Bottles were incubated at 20 °C. BOD was calculated by

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