BIODEGRADATION OF ANIONIC SURFACTANTS IN THE BIOREMEDIATION OF OIL-POLLUTED SOIL

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ABSTRACT

Surfactants are applied as emulsifiers or solubilizers by treatment of polluted soil. The problem of secondary pollution has arisen as result of the surfactant-enhanced remediation of a polluted soil contaminated with hydrophobic organic compounds. Several studies have shown that the surfactants are biodegradable in aerobic conditions and the biodegradability depended on the chemical properties and concentration of surfactant. A study of the leaching of surfactants from the soil is important, as it is difficult to identify the reason for the reduction of concentration of pollutants in the soil: is it degradation or leaching? The experiments were carried out with a fine sandy soil in column tests and $CaCO_3$ was added to increase soil pH. The soil was treated twice with the bioremediation agent SR-100. The soil pH, the concentrations of anionic surfactant and petroleum hydrocarbons at the different depths of soil were determined. The microbial activity of soil fractions was evaluated by respirometer. The concentration of surfactants was determined colorimetrically as Methylene Blue active substances (MBAS). The concentration of anionic surfactants decreased in the upper layer of the columns, but it increased in the lower layers. It indicated the leaching of the anionic surfactants from soil during experiments of 60 days. The amounts of residual surfactants were lower in the samples of polluted soil in comparison with unpolluted soil. The samples of lower soil fractions had higher microbial activity in comparison with upper fractions. Soil pH was measured as pH_{H2O}, pH_{KCl} and pH_{CaCl2} instead of the pH of soil solution, because soil was too dry. The pH of fine sandy soil was 5.8 and during the experiment the value of pH increased in the lower layer of soil till $pH_{H2O} = 7.5$.

KEYWORDS

Anionic surfactants; Column tests; Leaching; Petroleum hydrocarbons; Respirometry; Bioremediation

1 INTRODUCTION

Surfactants are amphiphilic molecules and they are used as emulsifiers or solubilizer to enhance of biodegradation of hydrophobic compounds [1, 2]. The surfactants contaminating the soil can discharge from the wastewater treatment plant (WWTP) and from the seewage sludge used as a fertilizer in the agriculture, because the anionic surfactants can accumulate in

sewage sludge [3]. Anionic surfactants cause the pollution of ground and surface water and its accumulation can be toxic to biota [2, 4]. The problem of secondary pollution has arisen as result of the use of surfactants for the surfactants-enhanced remediation of a polluted soil [1, 2, 5-8]. The indigenous microorganisms produce a variety biosurfactants which help to increase the surface area of the hydrophobic compounds for the growth of microorganisms [9-11]. The elimination of surfactants becomes important for the successful use of the surfactants-based technologies. Several studies show that surfactants are easily biodegradable in aerobic conditions, but are less degradable under anaerobic conditions [12-15].

Besides the biodegradation of surfactants, it is important to study the leaching of pollutants and surfactants from the soil. In situ bioremediation usually requires a long time, and the leaching occurs by rainwater. Due to the leaching it is difficult finally to identify the reason for the reduction of concentration of pollutants in the soil: is it degradation or leaching? Behavior of linear alkylbenzene sulfonates (LAS) in sandy soils were studied by Küchler and Schnaak in a field trial and lysimeter studies [16].

The main goal of this work was to study the degradation and leaching of anionic surfactants in the columns of sandy soil. The determination of the concentration of anionic surfactants, pH and microbial activity of soil at the different depths of soil was the aim of our study. The concentration of petroleum hydrocarbons was determined to evaluate the process of bioremediation. The sandy soil was chosen due to the fact that porous soils containing gravel and sand are the most favourable for the application of bioremediation due to better oxygen supply.

2 MATERIALS AND METHODS

2.1 Chemicals and soil

During the bioremediation the soil was treated twice with the bioremediation agent SR-100 (E-Tech, USA) which is offered on the market for bioremediation of oil-polluted soil [17]. It contains 9.18% anionic surfactants as Methylene Blue active material (MBAS) and nutrients: 0.24% phosphorus and 0.49% nitrogen. Limestone powder ($CaCO_3$) was obtained from Rakke Lime factory (Estonia). The Methylene Blue (MB), Sodium Dodecylsulphate (SDS), n-Hexane and Chloroform were the analytical grade.

The experiments were carried out with fine (diameter 0.2-4 mm) natural sandy soil from Kloogaranna beach (northwestern Estonia). It was artificially contaminated with used diesel oil and the concentration of hydrocarbons was about 500-600 mg HEM/kg DS. The limestone powder was added 10 vol% into soil and it was used for the increasing pH of the fine soil. The columns with the unpolluted sand and the mixture of the contaminated sand and limestone powder were used to compare the leaching of hydrocarbons and surfactants from the different soils. The values of the soil pH determined in distilled water are presented in *Table 1*.

Table 1. The values of the soil pH used in the experiments.

Soil	pH_{H2O}
Unpolluted sandy soil	5.80
Polluted sandy soil	6.17
Polluted sandy soil + CaCO3	7.91

2.2 Chemical analysis

The colorimetric method was used for determination of surfactants [18, 19]. The soil samples (1g) were mixed with 200 ml distilled water and after the agitation a water phase sample of 10 ml was taken. It was mixed with 5 ml of a 1mM MB solution and 5 ml of chloroform in a separatory funnel. The chloroform phase with dissolved colored complex was separated and the absorbance of the chloroform solution was measured at 654 nm by the spectrophotometer KFK-3 (USSR). The concentration of anionic surfactants was calculated by calibration curve as the MBAS. The concentration of the surfactants in the leachate was determined in the same way. Each analysis was repeated at least three times.

The hydrocarbons were extracted from soil samples with n-Hexane and the concentration of the hydrocarbons was determined gravimetrically as HEM by the USEPA methods 1664 [20].

The most appropriate medium for measuring soil pH would be one that the best simulates the characteristics of the soil solution [21]. Studied soil samples were dry, and because soil pH was measured as pH_{H2O}, pH_{KCl} and pH_{CaCl2} instead of the pH of soil solution. Soil pH was measured in each of deionised water, $0.01M$ $CaCl_2$ and $1M$ KCl at a 1:5 soil:solvent ratio [21, 22] using Jenway pH Meter.

2.3 Bioremediation experiments

The experiments in bioremediation were carried out in the Plexiglas columns and each column contained 2.2 kg soil. In the experiments the columns with soil were treated twice with the 80 cm^3 diluted solution of SR-100 (4% dry solids) and every week 30 ml aerated distilled water was added to the column to moisten the soil and to supply the soil with oxygen to model the natural conditions (rain).

The leachate was collected and the concentration of the leached surfactant and hydrocarbons were determined at the end of the experiment. After the end of the experiments the contents of the columns were divided into four equal fractions by volume and the concentrations of the surfactant and hydrocarbons were determined for each fraction of soil. The pH of the each soil fraction soil was also measured.

The microbial activity of soil was evaluated by the respirometry of different soil samples. The respirometric OxiTop® system (WTW, Germany) was used to measure the oxygen consumption of the soil samples [23].

3 RESULTS AND DISCUSSION

3.1 Concentration of surfactants in the upper layer of soil

The columns with soil were treated with the 80 cm^3 diluted solution of SR-100. During the experiments the concentrations of the anionic surfactants were measured regularly in the upper layer of the column. The determined concentrations of anionic surfactants as MBAS in the upper layer of soil are presented in *Figure 1*.
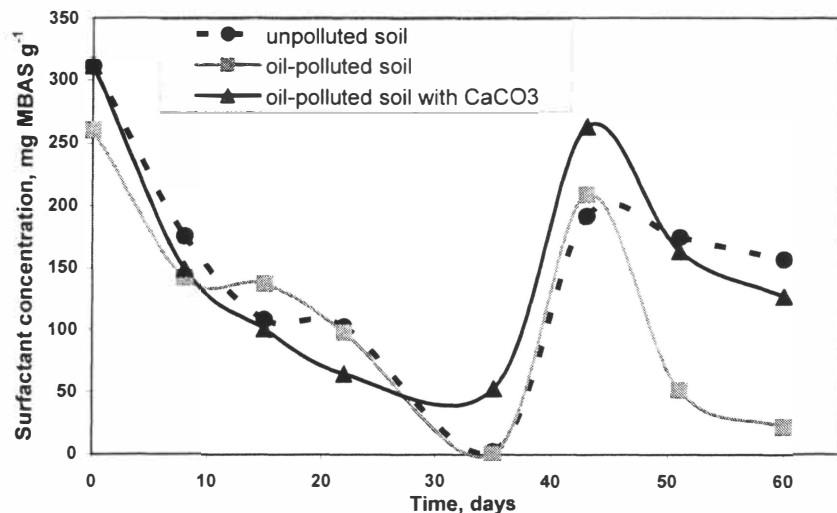
Figure 1. Concentration of the anionic surfactants in the upper layer of soil columns.

First time the concentration of the surfactants was detected, when the added solution of surfactants was immersed completely into soil. The surfactants were washed out from the upper layer during the first 35 days and the columns were treated again with the same amount of solution of SR-100 to study the effect of the recommended amount of SR-100 [17] during 60 days. The initial concentration of surfactants and its reduction in the upper layer were similar for all soil samples. After the second treatment the rate of decrease in the concentration of surfactants was the highest in the case of oil-polluted soil.

3.2 Concentrations of surfactants in the different fractions of soil columns

At the end of experiments all four fractions of soil samples were analyzed and the values of concentrations of the anionic surfactants are presented in *Table 2*. The decision about contamination of soil is made by the concentration of pollutants mainly, and because the residual concentrations of the surfactants were determined in the experiments.

Table 2. Concentration of the residual surfactants (mg MBAS/kg soil) in the fractions of the soil columns.

Soil Fraction	Unpolluted soil	Polluted soil	Polluted soil + $CaCO_3$
1 (upper)	27.6 (±3.2)	83.7 (±4.8)	134.5 (±9.4)
2	46.1 (±4.2)	160.9 (±8.3)	105.8 (±6.1)
3	262.5 (±19.6)	284.6 (±15.2)	343.9 (±18.5)
4 (lower)	1597.1 (±51.7)	2107.6 (±36.5)	2119.5 (±48.4)

The concentrations of residual surfactants in the different fractions of the soil columns (*Table 2*) have a clear trend: lower fraction contained more surfactants. This trend indicated the leaching of the surfactants from upper fractions into lower ones. The highest concentrations of residual surfactants were determined in the most fractions of the mixture of soil and $CaCO_3$ but the difference was not significant. It can be explained by the adsorption of surfactants on the fine particles of soil and $CaCO_3$ powder. The highest amount of anionic surfactants was determined in the leachate of column of polluted soil (5.5% of added surfactants). The addition of $CaCO_3$ decreased the leaching of anionic surfactants (2.8% of added surfactants).

The calculation of mass balance of the anionic surfactants showed that the biggest amount (36%) of anionic surfactants was degraded in the column of the polluted soil. In the columns of unpolluted soil and the mixture of polluted soil and $CaCO_3$ the degraded amount of the surfactants were 11% and 18%, respectively. By the low porosity of fine soil not enough amount of oxygen was diffused into the soil to achieve the aerobic conditions, which are needed for the surfactants degradation.

At the end of experiments the concentrations of total petroleum hydrocarbons (as HEM) in the soil fractions were also determined. The microorganisms used hydrocarbons as substrate biodegradation of hydrocarbons is well studied. During 60 days of current experiments 40-50% of initially added petroleum hydrocarbons were degraded by calculation of mass balance. The dry residual of the leachate from the columns contained about 5% of hydrocarbons.

3.3 pH and microbial activity of soil fractions

At the end of experiments the pH of the each soil fraction soil was also measured. Studied soil samples were too dry to get the soil solution, and because soil pH was measured as pH_{H2O}, pH_{KCl} and pH_{CaCl2} instead of the pH of soil solution. The measured pH values of soil fractions are presented in *Table 3*.

Table 3. pH values of different soil fractions at the end of experiments

Soil fraction	Unpolluted soil			Polluted soil			Polluted soil + $CaCO_3$		
	pH_{H2O}	pH_{KCl}	pH_{CaCl2}	pH_{H2O}	pH_{KCl}	pH_{CaCl2}	pH_{H2O}	pH_{KCl}	pH_{CaCl2}
1	6.20	6.54	6.48	6.17	6.42	6.28	7.37	8.71	6.55
2	6.60	6.43	6.21	6.29	6.36	6.23	7.69	8.52	6.60
3	6.42	6.14	6.00	6.43	6.43	6.35	8.27	9.04	6.71
4	7.53	7.01	6.77	7.05	6.85	6.61	8.03	8.97	6.88

The measured pH values had no clear trend for the different fractions, but the pH values of the lower fractions were the highest. The indicated the leaching of surfactants, hydrocarbons, their intermediates, and soil components from upper fractions into lower ones. This trend indicates that it is also important to take soil samples from the lower layer of soil to evaluate

the effect of different addtives on the bioremediation of the polluted soil. The complete explanation of pH values can be the aim of the next studies.

The microbial activity of soil was evaluated by the respirometry and the respirometric OxiTop® system was used to measure the oxygen consumption of the soil fractions. The rate of oxygen consumption was calculated by the respirometric data and the first order rate constants are presented in *Table 4*.

Table 4. The first order rate constants (kl, min-1) of oxygen consumption

Soil fraction	Unpolluted soil	Polluted soil	Polluted soila+ $CaCO_3$
1	0.058	0.088	0.084
2	0.082	0.096	0.086
3	0.079	0.092	0.081
4	0.284	0.167	0.155

The oxygen consumption of the soil fractions affirm that microorganisms were also washed out from upper fractions into lower ones by added water. The upper soil fraction had aerobic conditions, but the rate of oxygen consumption in that fraction was much lower than in the lower fractions. The columns had the opening in the bottom and oxygen diffused into lower fraction of soil. The second fraction had little higher rate of oxygen consumption than the third one because oxygen diffused into this fraction through upper fraction. Third fraction had anaerobic conditions and microbial activity was lower. Similar situation was appeared by the measured values of surfactants concentrations (*Table 2*). The mixture of polluted soil and $CaCO_3$ had lower microbial activity because the hydrocarbons could be adsorb onto particle of $CaCO_3$ and it decreased bioavailability of hydrocarbons.

4 CONCLUSIONS

The aim of this work was to study the biodegradation of anionic surfactants during bioremediation in the oil-polluted sandy soil. The concentration of the anionic surfactants, pH and microbial activity of soil were determined at the different depth of soil columns. The limestone powder was added to increase the soil pH.

The initial concentration of the surfactants and its reduction were similar for all soil samples, but the reduction was slightly slower for the mixture of soil and $CaCO_3$. The concentrations of residual surfactants in the different fractions of the fine soil column have a clear trend: the lower fraction contained more surfactants indicating the leaching of surfactants. The calculation of mass balance of the anionic surfactants showed that the biggest amount (36%) of anionic surfactants was degraded in the column of the polluted soil. The columns of sandy soil were less porous and not enough oxygen was diffused into the soil to achieve the aerobic conditions which are needed for the surfactants degradation.

The time of experiments was not enough for the complete biodegradation of petroleum hydrocarbons and the reduction of the hydrocarbon concentration was about 50%. The

measured pH values had no clear trend for the different fractions, but the pH values of the lower fractions were the highest. It indicated the leaching of surfactants, hydrocarbons, their intermediates, and soil components from upper fractions into lower ones

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