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The Effect of Triton X-100 on Biodegradation of Aliphatic and Aromatic Fractions of Crude Oil in Soil

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Abstract: Crude oil is one the most common organic pollutant of the soil. The spillage of crude oil in the soil can be harmful to living organisms. Certain microorganisms are able to biodegrade crude oil and use it as sole carbon source. Some detergents were used to help the biodegradation of crude oil by microorganisms. In this study Triton X-100 was used to determine its effect in biodegradation of aliphatic and aromatic fractions of heavy crude oil in soil during 4 months. Different concentration of Triton X-100 (0 to 0.25%) was added to crude oil-contaminated soil with 2% (w/w) crude oil as final concentration. Present results demonstrated that in 0.025% of Triton X-100 the reduction of total crude oil, total aliphatic and total aromatic fractions were high, while in 0.05 to 0.25% the reduction reached to its minimum value. The higher reduction of phenanthrene, anthracene, fluoranthene and pyrene was observed in 0.025% Triton X-100 while it was lower in 0.25% followed by 0.1% Triton X-100. The low reduction of C_{17}/pristane and C_{18}/phytane in 0.25% Triton X-100 suggested low bioavaibility of aliphatic compounds in this concentration.

Key words: Biodegradation, crude oil, soil, Triton X-100

INTRODUCTION

The leakage of crude oil in soil can damage environment and ecosystems. Crude oil contains aliphatic and aromatic fractions which some of them are toxic for living organisms (Armstrong *et al*., 2004; Gibbs, 1997; Hammond *et al*., 1976)). Crude oil can be biodegraded by some bacteria and alga (Atlas, 1981; Leahy and Colwell, 1990). The aliphatic fractions are the first compounds to be degraded by bacteria.

Some aromatics are also biodegraded by microorganisms (Cerniglia, 1992). Various factors can enhance the biodegradation of crude oil and its components; such as fertilizer, pH, salinity and some surfactants. Some reports indicated that the presence of surfactants inhibits biodegradation (Laha and Luthy, 1991; Tiehm, 1994). Surfactants may reduce the adhesion of bacteria to hydrophobic surfaces (Stelmack *et al*., 1999). This mechanism may be important for the biodegradation of virtually insoluble contaminants and therefore the use of surfactants may not be beneficial. Other report suggested that the preferential utilization of surfactants by PAH degraders was responsible for the inhibition observed in the biodegradation of the hydrocarbons (Deschenes *et al*., 1996). Some other reports indicated that the presence of surfactants enhances biodegradation (Bury and Miller, 1993; Doong *et al*., 1996). The most important effect of surfactants on the interactions among soil and pollutants is stimulation of mass transport of the pollutant from the soil to the aqueous phase (Volkering *et al*., 1997).

Surfactants can help solubilize non-polar material in liquid phase and increase its biodegradation. Triton X-100 is a non-ionic detergent widely use in membrane proteins extraction. This compound may be toxic to microorganisms in high concentration. The effect of this detergent on microbial oil biodegradation was studied, especially in aromatics fractions (Wong *et al*., 2004; Kim *et al*., 2001).

In this report the effect of different concentrations of Triton X-100 on biodegradation of aromatic and aliphatic fractions of crude oil in soil was studied during four months.

MATERIALS AND METHODS

Soil preparation: The soil consisted of 60% clay, 38% slit and 12% sand with 3.4% of organic matters. The soil was

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Table 1: Total colony count (cfu g^{-1} soil) in month 2 and 4 after the start time

Months	Control	0%	0.01%	0.03%	0.05%	0.10%	0.25%	No-oil
0	2.2×10^4	2.2×10^4	2.2×10^4	2.2×10^4	2.2×10^4	2.2×10^4	2.2×10^4	$2.2\times10_4$
2	1.9×10^4	6.7×10^7	6.6×10^7	5.4×10^7	9.8×10^7	5.7×10^7	5.9×10^7	2.1×10^7
4	3.0×10^4	9.5×10^7	8.5×10^7	8.1×10^7	10.0×10^7	6.0×10^7	8.5×10^7	1.8×10^7

dried for 48 h in 50°C and well crushed to extent of homogeneity. Heavy crude oil (API = 18) was added to soil in final concentration of 2% (w/w) and well mixed with crushed soil to make uniform contaminated soil. The soil was divided into equal portions; each portion contained 500 g soil and transferred to a 2 L pail. Solubilized Triton X-100 with final concentrations of 0.01 to 0.25% was added to the soil (v/w).

Pail 1 designated as control with 2% crude oil but no moisture, aeration and Triton X-100. The moisture, aeration, crude oil (2%) and different Triton X-100 (Merck) concentrations (0 to 0.25%) were added to pails 2 to 6 during the experiment. Pail 7 considered as no-oil, which was prepared separately and received moisture, aeration and 0.25% Triton X-100 but had no crude oil during experiment. Each sample was prepared as two replicates.

For further study, 10 g contaminated soils kept in -20 as zero time.

Soil factors: For each 1000 mg of crude oil about 150 mg of nitrate (NH_4NO_3) and 30 mg of phosphate (KH_2PO_4) were added to all pails (Rosenberg and Ron, 1996).

The pH of soil was determined 7.4 for soil-distilled water slurry (1:5, w/vol).

After determination of water holding capacity (field capacity) of soil, the moisture of pails was adjusted about 30% by adding distilled water to samples (except pail 1). The soil water content was measured with gravimetric method during the experiment.

Mixing the wet soil every other day in all pails (except number 1) induced aeration. All the pails were incubated in room temperature (25°C) during experiment.

Crude oil extraction from soil: Two samples from each pail were used for crude oil extraction. Extraction of crude oil was conducted according to Hutchinson (2001) with some modifications.

GC and HPLC analysis: After extraction of crude oil by mentioned method, the residue was dissolved in 5 mL n-hexane (Merck) and filtered. The sample was loaded to 1×25 cm column filled with 20 cm Silica Gel and 5 cm Na_2SO_4 (Merck). The column was pre-washed by n-hexane. Thirty milliliter of n-hexane was used as mobile phase to release aliphatic fractions. The fraction collected and the solvent was evaporated. The residue was weighted to determine the amount of total aliphatic of each sample. The residue of each sample was dissolved in 150 μL of n-hexane and 1 μL was injected to gas chromatography column equipped with FID detector and fused silica capillary column. The carrier gas was H_2 and the injection temperature was 300°C and that of detector was 330°C. The total peak areas of the hydrocarbons were taken as a quantitative amount of their concentration and compared with the time zero. To release aromatic fraction from the column, 30 mL of n-hexane/dichlromethane (1:1, v/v) was used and the aromatics fraction was collected and the solvent was evaporated. The residue was weighted to determine the amount of total aromatic of each sample. The residue was dissolved in 5 mL acetonitrile and 20 μL was injected to HPLC column, with water/acetonitrile (1:2, v/v) as mobile phase and flow rate of 1 mL min^{-1} which equipped by UV detector at 254 nm. Some PAHs such as phenanthrene, anthracene, fluoranthene and pyrene were prepared as standard and injected to HPLC column. The regions of their exit from the column were used to localize them in main graphs.

The total peak area of each compound in the graphs was used to determine the reduction of PAHs and compared with the time zero.

Total colony count: Determination of total colony count in soil was done by *pure-plate* method every two months and compared with the time zero. From each pail, 1 g of soil was dissolved in 9 mL of autoclaved NaCl solution (9 g L^{-1}) and serial dilution was prepared for each sample. Diluted samples were transferred to Nutrient agar (Merck) plates. The plates incubated at 30°C for 48 h and the colonies were counted by screening the plates.

RESULTS

Total colony count: Total colony count showed that in the presence of both Triton X-100 and crude oil, the population of bacteria increased. In contrast in no-oil sample the population of bacteria was slightly lower than other samples. There was no significant difference on colony count in the samples containing Triton X-100. No increasing observed in control sample during 4 months (Table 1).

Crude oil reduction: The effect of Triton X-100 in crude oil reduction was shown in Fig. 1. The higher reduction (33%) was observed in 0.025% of Triton X-100 followed by 0.01 and 0% of Triton X-100. The lower reduction

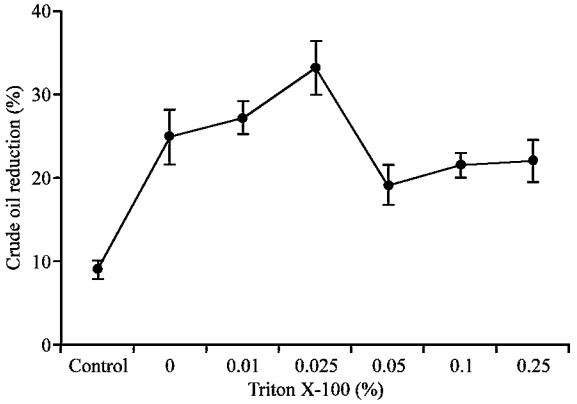

Fig. 1: Reduction of crude oil in the presence of different concentration of Triton X-100. (Data are the means±SD, n = 4, p<0.05)

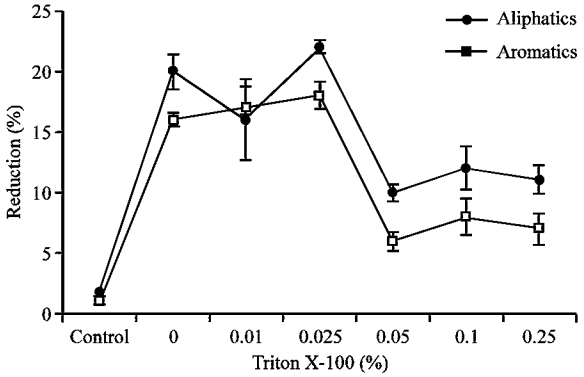

Fig. 2: The reduction of aliphatic and aromatic fractions in different concentration of Triton X-100. (±SD, n = 4, p<0.05)

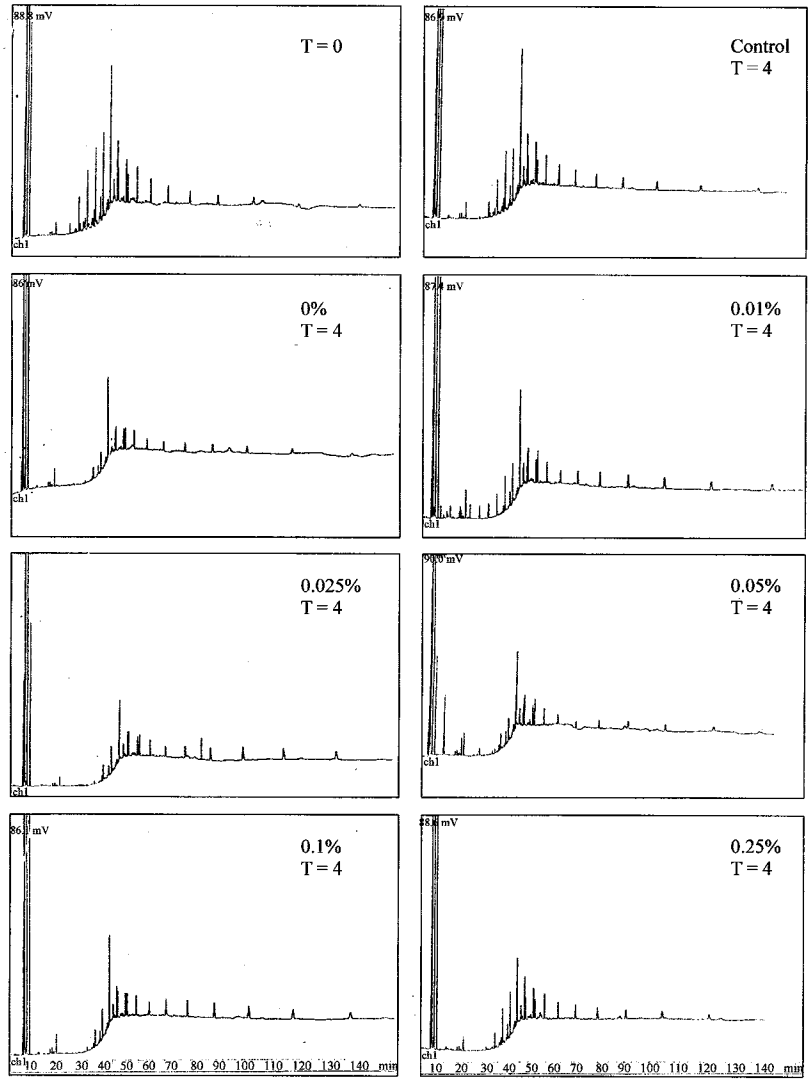

Fig. 3: GC pattern of the samples, in start time (T = 0) and after four months (T = 4)

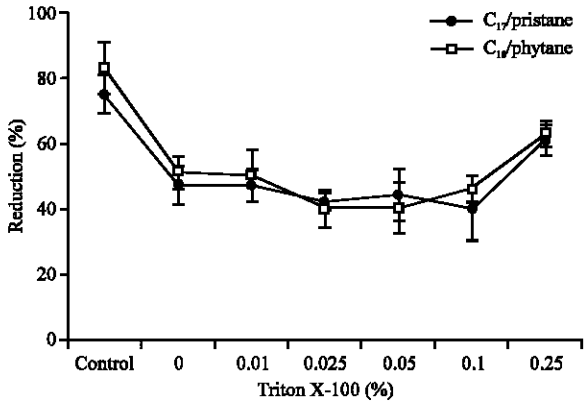

Fig. 4: The reduction of C_{17}/pristane and C_{18}/phytane after 4 months. (±SD, n = 2, $p<0.05$)

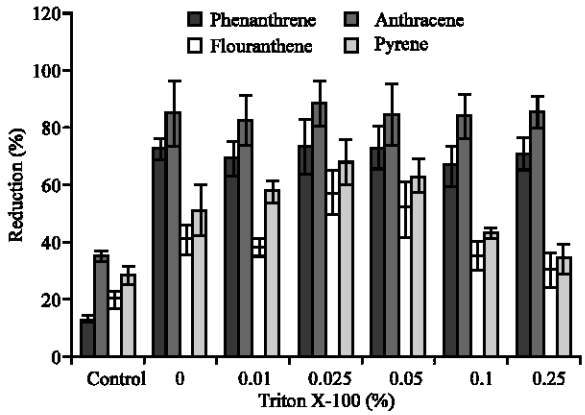

Fig. 5: Reduction of some PAHs after 4 months in the presence of different concentration of Triton X-100. (±SD, n = 2, $p<0.05$)

(19%) was seen in 0.05% of Triton X-100 followed by 0.1% and 0.25% of Triton X-100. There was also 9% reduction in control sample.

Aliphatic and aromatic fractions: Figure 2 shows the reduction of aliphatic and aromatic fractions in different Triton X-100 concentrations. The higher reduction was observed in 0.025% of Triton X-100 and its lower reduction was seen in the concentration of over 0.05% followed by 1 and 0.25% of Triton X-100. Figure 3 shows the GC pattern of aliphatic fractions of samples. The reduction of aliphatics had not significant difference in the samples and the pattern seems to be nearly equal in all samples. The GC pattern in control sample showed minor reduction of aliphatic fractions in comparison to other samples.

Reduction of ratio of pristane and phytane: The reduction of C_{17} to pristane and C_{18} to phytane usually use as indices of monitoring biodegradation. The lowest ratios were observed in 0.025% and the highest ratios were seen at control followed by 0.25% Triton X-100 (Fig. 4). However in 0 to 0.1% of Triton X-100, there was no significant difference in these ratios and it seems to be nearly equal.

PAHs reduction: The study of HPLC patterns of the samples and the measurement of some PAHs was shown in Fig. 5. The reduction of phenanthrene, antheracene, fluoranthene and pyrene were measured after four months and compared with time zero. The reduction of phenethrene and antracene was nearly equal in all the samples except in control. The reduction of fluoranthene and pyrene was higher in 0.025% followed by 0.05 and 0.01% of Triton X-100. Their reduction was lower in 0.25% followed by 0.1% of Triton X-100.

DISCUSSION

High microbial population in soil is important for biodegradation process (Atlas, 1995; Leahy and Colwell, 1990). In present experiments, the samples with both crude oil and Triton X-100, the population of microorganisms was increased. In no-oil sample and 0% Triton X-100 it could be seen that the microbial population was also increased. This suggests that both Triton X-100 and crude oil could be used as a carbon source by microorganisms. The high concentration of Triton X-100 in no-oil and 0.25% samples could not decrease the number of colonies suggesting that Triton X-100 was not harmful to microorganisms of the soil in this concentration. The higher reduction of crude oil and its aliphatic and aromatic components occurred in 0.01 and 0.025% of Triton X-100, which were close to CMC of Triton X-100 (0.015%). Some reports have indicated that both nonionic and anionic surfactants increase the solubility of hydrocarbons by forming micelles, the surfactants begin to assemble into micelles at the Critical Micelle Concentration (CMC) and the interiors of the micelles provide a hydrophobic environment to solubilize nonpolar compounds, such as hydrocarbons (Edwards *et al*., 1991; Guerin and Jones, 1988; Jafvert, 1991; Kohler *et al*., 1994; Roy *et al*., 1994).

In concentrations with much greater than CMC of Triton X-100 (0.05-0.25%), the reduction of total crude oil as well as total aromatics and total aliphatics had been decreased. It has been reported that addition of Triton X-100 at a concentration greater than its CMC inhibited adhesion of bacteria to solid surface, which in turn prevented degradation of both hexadecane and naphthalene (Efroymson and Alexander, 1991; Ortega-Calvo and Alexander, 1994).

The ratio of C_{17}/pristane and C_{18}/phytane was used as indices of biodegradation (Seklemova *et al*., 2001). The higher reduction of these ratios in 0 to 0.1% Triton X-100 samples suggested that the biodegradation was responsible for aliphatic reduction. The lower reduction of these ratios in 0.25% sample determined that biodegradation have had little effect in crude oil reduction. This suggested that the bioavaibility of aliphatic and aromatic compounds has been reduced in 0.25% Triton X-100 in comparison to other samples.

Two factors reduce the amount of crude oil and its fractions in soil, biodegradation and volatilization (Atlas, 1981; Leahy and Colwell, 1990; Nicodem *et al*., 1997).

The reduction of phenanthrene and anthracene was nearly equal in all treated samples; this suggests that these compounds were reduced by volatilization as well as by biodegradation. On the other hand, the higher reduction of fluorathene and pyrene in 0.025% Triton X-100, in comparison to 0.25% Triton X-100 (in which the biodegradation decreased), showed that biodegradation was the main factor for their reduction.

In conclusion, whilst the high concentration of Triton X-100 had no effect on microorganism population of the soil, it could reduce the bioavaibility of aromatic and aliphatic fractions.

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