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PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 9 (8): 1531-1535, 2006
ISSN 1028-8880

Effect of Salinity on Biodegradation of Aliphatic Fractions of Crude Oil in Soil

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Abstract: The effect of different concentrations of NaCl (0-5%) on biodegradation of aliphatic fractions of crude oil in polluted soil has been studied. The relative contribution of volatilization and biodegradation of crude oil and its aliphatic fractions in crude oil-contaminated soil during four months has been investigated. The crude oil reduction was about 12, 41 and 15% in 5% NaCl, 0% NaCl and non-aerated samples, respectively. The reduction ratios of C_{17} to pristine and C_{18} to phytane, as indices of biodegradation, were 75 and 73% in 0% NaCl sample, 28 and 31% in 5% NaCl sample and 46 and 51% in non-aerated sample, respectively. Total aliphatic reduction four months after crude oil contamination was 29 and 7.3% in 1% NaCl and 5% NaCl, respectively. Present results indicated that the presence of NaCl attenuated the oil reduction process presumably through its inhibitory effect on bacterial growth. Increasing NaCl concentration reduced biodegradation rate. We also found biodegradation was the main factor in elimination of crude oil and its aliphatics fraction reduction in comparison to volatilization. We conclude that aeration and removal of NaCl from the contaminated soil are useful approaches in oil removal from contaminated soil.

Key words: Aliphatic fractions, bacteria, biodegradation, crude oil, NaCl, soil

INTRODUCTION

The leakage of crude oil or its products from pipelines, storage tanks and refineries into the soil, is considered to cause damaging effect to the environment and ecosystems. In oil producing countries, crude oil can be a source of major organic pollutants of the soil.

Crude oil components can be divided in to four major groups, including; saturates, aromatics, resins and asphaltenes (Leahy and Colwell, 1990; Colwell and Walker, 1977). In general, saturated alkanes are the most degradable fraction in crude oil (Atlas, 1981; Jobson *et al*., 1972; Walker *et al*., 1976) and those of C_{10} to C_{26} are considered among the first to be degraded by microorganisms (Atlas, 1995).

Biodegradation of oil in contaminated soil is one of the most feasible and economical methods for treatment of the oil contamination in environment (Leahy *et al*., 1990; Atlas, 1981). Major catabolic pathways in biodegradation of crude oil components involve oxygenase enzymes (Cerniglia, 1992; Atlas, 1981), therefore, oxygen plays an important role and is considered to be necessary for biodegradation of crude oil. Biodegradation of petroleum was also studied in extreme environments (Margesin *et al*., 2001a), such as high salinity (Margesin *et al*., 2001b; Ward and Brock, 1978). Many experiments about salinity and the effects of high NaCl concentration on hydrocarbon biodegradation in liquid mediums have been reported (Riis *et al*., 2003; Diaz *et al*., 2002; Mille *et al*., 1991; Ward and Brock, 1978), while only few works about the effects of high salinity in soil have been presented (Jackson and Pardue, 1998; Rhykerd *et al*., 1995).

Biodegradation of oil by microorganisms in the presence of high NaCl and salinity was slow, because high NaCl in medium may disrupt cell membrane, denature some proteins such as enzymes, or change the osmotic force, which any of these factors could be lethal for microorganisms (Kargi and Dincer, 2000; Woolard and Irvine, 1994).

In this study the effect of different concentration of NaCl in biodegradation of crude oil and its aliphatic fractions in soil was studied. Some areas in oil producing countries have salty contaminated soils causing several environmental problems. These results, presented here, would be useful for decontamination of oil pollutants from salty soils.

MATERIALS AND METHODS

Soil preparation: The soil for experiment consisted of 60% clay, 28% slit and 12% sand with 3.4% of organic matters. The soil was dried for 48 h in 50°C and well crushed to extent of homogeneity. Crude oil was added to

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the soil in final concentration of 2% (w/w) and well mixed with crushed soil to make uniform contaminated soil. The soil was divided to equal parts; each part contained 1 kg soil and transferred to a 5 L pail.

Pail 1 designated as control with no moisture, aeration and salt, pail 2 considered as non-aerated, with moisture but no salt. The moisture, aeration and different NaCl (Merck) concentration (0 to 5%) were added to pails 3 to 6 during the experiment. Pail 7 considered as no-oil, which was prepared separately and received moisture, aeration and 5% salt but had no crude oil during experiment. Each sample was prepared as two replicates.

For further study, 50 g contaminated soils kept in -20°C as time zero.

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 the soil, the moisture of soils 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.

Induced aeration was done by mixing the wet soil every other day in all pails except the pails number 1 and 2. All the pails were incubated in room temperature (25°C) during experiment.

Crude oil extraction from soil: Extraction of crude oil from soil was conducted according to Hutchinson *et al.* (2001) with some modifications. Two samples from each duplicate were taken for crude oil extraction and further preparations.

GC analysis: After extraction of oil by the 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 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.

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

Effect of crude oil and salt in microbial population: The colony count was done each two months and compared with the time zero (Table 1). The microbial population reduced in crude oil contaminated soil and also in NaCl

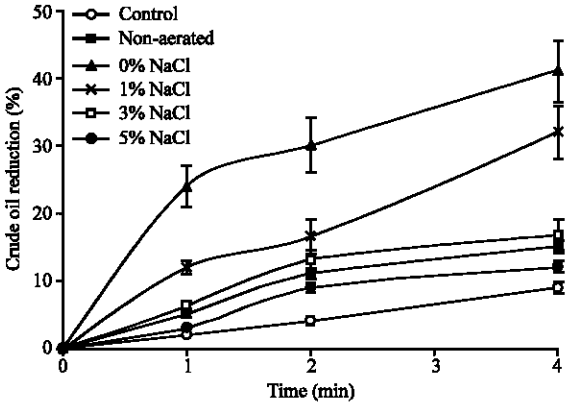

Fig. 1: Reduction of total crude oil during 4 months in different conditions. In 0% NaCl sample the reduction was higher and in 5% NaCl sample the reduction was lower. (Data are the means±SD, n = 4, $p<0.05$)

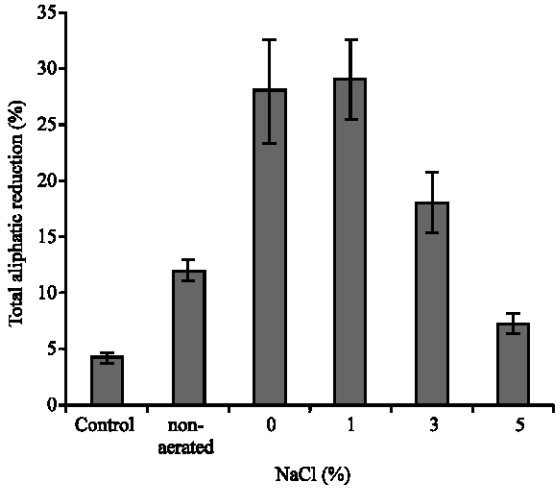

Fig. 2: Reduction of total aliphatic fraction from crude oil after 4 months. In 1% NaCl and 0% NaCl samples, the reduction was higher than other sample. (Data are the means±SD, n = 4, $p<0.05$)

Table 1: The total colony count (cfu g^{-1} soil) was determined at time 0, 2 and 4 months. The reduction of microorganisms was higher in the presence of 5% NaCl. Average values given±Standard deviation (SD), n = 2

Time (month)	Control	Non-aerated	0%	1%	3%	5%	No-oil
0	$39.0\times10^{8}\pm8$	$39\times10^{8}\pm8$	$39.0\times10^{8}\pm8$	$39.0\times10^{8}\pm8$	$39\times10^{8}\pm8$	$39.0\times10^{8}\pm8$	$39.0\times10^{8}\pm8$
2	$7.6\times10^{7}\pm4$	$23\times10^{6}\pm7$	$19.0\times10^{8}\pm3$	$3.4\times10^{8}\pm2$	$11\times10^{5}\pm5$	$8.4\times10^{3}\pm3$	$4.5\times10^{3}\pm2$
4	$4.5\times10^{6}\pm2$	$3\times10^{6}\pm1$	$3.3\times10^{7}\pm2$	$18.0\times10^{7}\pm4$	$14\times10^{5}\pm5$	$5.3\times10^{3}\pm2$	$6.8\times10^{3}\pm1$

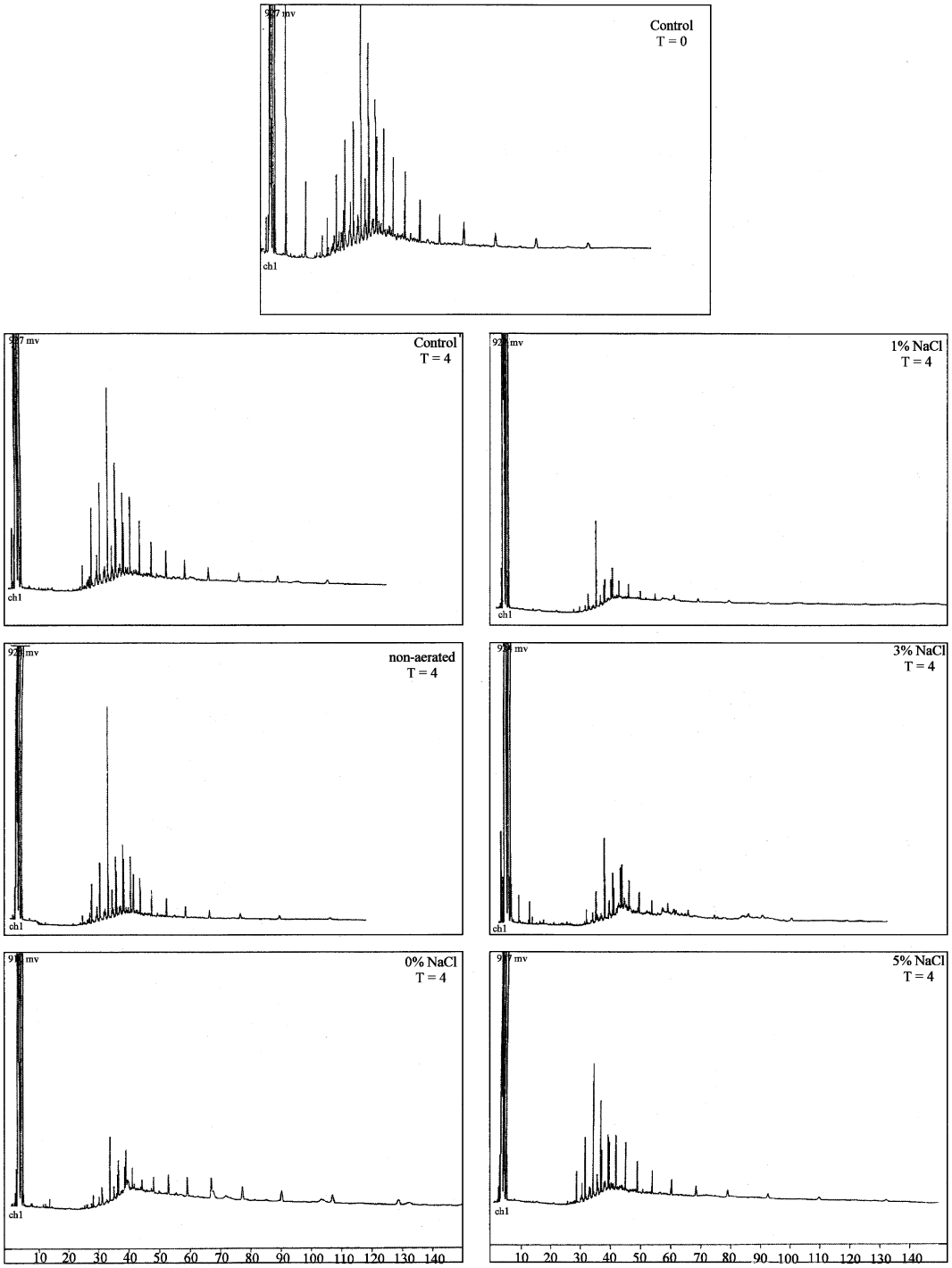

Fig. 3: GC pattern of samples at start point (T = 0) and after 4 months (T = 4). The reduction of aliphatic fractions in 0% NaCl and 1% NaCl samples was higher, while it was lower in non-aerated and 5% NaCl samples

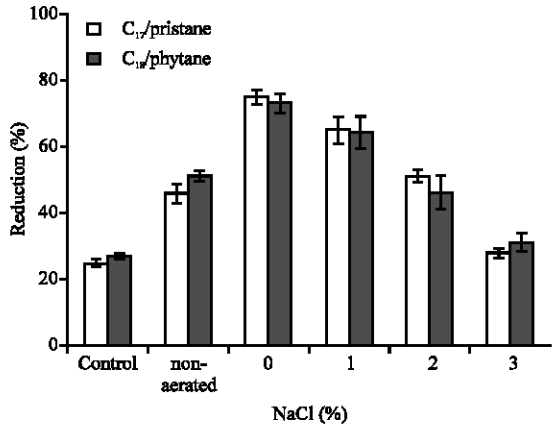

Fig. 4: Reduction of C_{17}/pristane and C_{18}/phytane in different concentration of NaCl (n = 2, $p<0.05$)

containing samples. The reduction was high in 5% NaCl and no-oil samples. In 0 and 1% NaCl the population was higher than other samples.

Total crude oil reduction: The measurement of total crude oil in samples after 4 months (Fig. 1) showed that the highest reduction of total crude oil occurred in 0% NaCl (41%), followed by 1% NaCl (32%), while the lowest reduction observed in 5% NaCl (12%). Increasing NaCl concentration decreased the total crude oil reduction. In non-aerated sample the reduction (15%) was lower than 3% NaCl (16.5%), but higher than 5% NaCl. Because no oil was extracted from no-oil sample, in further results this sample was not mentioned.

Reduction of total aliphatic fractions: The reduction of total aliphatic fractions in samples is shown in Fig. 2. The maximum reduction was in 1% NaCl (29%), slightly lower was 0% NaCl (28%). The GC pattern of the samples (Fig. 3) also showed significant reduction of aliphatic fractions in 0 and 1% NaCl. In contrast, minimum aliphatic reduction was observed in 5% NaCl (7.3%). The GC pattern of 5% NaCl also showed low reduction of aliphatic in comparison to 0 and 1% NaCl. The reduction of total aliphatic decreased by increasing NaCl in samples. In non-aerated sample the total aliphatic reduction (12%) was higher than 5% NaCl (7.3%) but lower than 3% NaCl (18%). This could be also compared by GC pattern of these samples (Fig. 3).

Reduction of pristane and phytane: The ratios of C_{17}/pristane and C_{18}/phytane were used as indices to follow the rate of biodegradation (Fig. 4). The reductions of these ratios were high in 0% NaCl, followed by 1% NaCl. These ratios decreased with an increase of the NaCl concentration and were lower in 5% NaCl sample. In non-aerated sample the reductions were more than 5% NaCl and nearly close to the 3% NaCl.

DISCUSSION

Present results mainly shows the effect of NaCl on biodegradation of crude oil and its total aliphatic fractions in soil and also compares the effect of volatilization and biodegradation of these reductions. The reduction of the number of colonies in the samples, suggested that the presence of crude oil and NaCl in soil is responsible for the reduction of the population of microorganisms. The comparison of microbial population in 0% NaCl and no-oil samples (Table 1) suggested that the main factor which decreased the number of colonies was the presence of NaCl. The high NaCl concentrations are believed to be responsible for inducing the osmotic shock to some bacteria, causing plasmolysis and inhibiting various physiological processes and macromolecule biosynthesis (Csonka, 1989). As result, the microbial population in 3% and especially in 5% NaCl decreased in comparison to other samples.

The reduction of microorganisms in the samples reduced the biodegradation effect of the bacteria. This phenomenon was observed in 3 and 5% NaCl (Fig. 1 and 2). In these samples, the reduction of crude oil and its aliphatic fractions were decreased in comparison to 0 and 1% NaCl in which the microbial population was higher than other samples. The effect of salinity in reducing the crude oil biodegradation has been mentioned in some reports within liquid mediums (Diaz *et al*., 2002; Mille *et al*., 1991; Ward and Brock, 1978), but there is no report about the effect of salinity on biodegradation of aliphatic fractions of oil in soil. Biodegradation and volatilization are two main factors for elimination of crude oil and its components from the soil. In our experiment, soil induced aeration was important for volatilization and the growth of bacteria. To understand which factor (Biodegradation or volatilization) had more effect on reduction of crude oil and its aliphatic fractions, the non-aerated sample was prepared. The ratios of C_{17}/pristane and C_{18}/phytane were usually used to follow the biodegradation rate (Seklemova *et al*., 2001). In present samples the low reduction of ratios of C_{17}/pristane and C_{18}/phytane in 5% NaCl showed that in this sample the biodegradation reached to its minimum value (Fig. 4), while in 0 and 1% NaCl the high reduction of these ratios showed that biodegradation was active in these samples. This suggested that in 5% NaCl sample, the aliphatic fractions were mainly reduced by volatilization while in 0 and 1% and in non-aerated samples biodegradation was the main factor for aliphatic fractions.

These results obtained under laboratory condition (without direct sunlight and wind) show the effect of biodegradation in the reduction of crude oil and its aliphatic fractions to be higher than volatilization. Sunlight and wind are important for photo oxidization and weathering of crude oil (Nicodem *et al*., 1997). In conclusion, our results suggest that in salty, oil-contaminated soil with more than 3% NaCl, aeration could enhance volatilization but not biodegradation. Reduction of NaCl from contaminated soil to concentrations of 1% is a useful approach in removal of oil and its aliphatics fractions from contaminated soil by biodegradation.

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