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Effectiveness of a Bioremediation Product in Degrading the Oil Spilled in the 1991 Arabian Gulf War

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The amount of crude oil spilled into the Gulf during the 1991 Arabian Gulf war was estimated to range between 4 to 6 million barrels. A huge quantity of the spilled oil has polluted the Saudi Arabian gulf coasts and water. Great efforts have been made by the Saudi government and other international organizations to combat the spill.

Bioremediation was one of the suggested methods to degrade the spilled oil. It is a process by which chemical substances are degraded by bacteria and other microorganisms. The use of bacteria and other microorganisms has been successfully applied for the treatment of waste and wastewater in controlled systems. Several research studies have been recently performed to investigate the use of bioremediation for oil-spill cleanup in seawater, freshwater and terrestrial areas (Zobell 1973; Atlas 1981, 1984, 1988; NAS 1985; Bartha 1986). However, there is no clear evidence to demonstrate the efficacy of this technique in oil-spill cleanup over other available chemical and mechanical means. The lack of conclusive information regarding the effectiveness and safety of bioremediation techniques still presents a dilemma to decision makers.

Following the Gulf war, many international companies have shown great interest to introduce their bioremediation products to combat the oil spill. On several occasions, many of these products did not have any convincing scientific evidence concerning their effectiveness in degrading crude oil. The present paper describes the results of a study performed to evaluate the effectiveness of one of the bioremediation agents in degrading the oil spilled in the Gulf under controlled laboratory conditions.

MATERIALS AND METHODS

The effectiveness of a commercially available bacterial product that consists of a mixture of naturally occuring microorganisms to degrade mousse of the Gulf oil spill was performed in the laboratory under controlled experimental conditions. Eighteen fish aquaria (72-L capacity each) were used. Each aquarium was filled with 60 L of Gulf seawater. The aquaria were divided into three groups of six aquaria. To the first group, weathered oil (mousse) collected from an oil spill north

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of Abu Ali Island in the Arabian Gulf, was added to make an oil concentration of 5 g/L of seawater. Oil concentration levels in the second and third groups of aquaria were set at 10 and 20 g/L, respectively. At each concentration level of the oil, the first two aquaria served as duplicate controls containing seawater and oil; the second two aquaria contained seawater, oil and 100 mL of nutrients; and the third two aquaria contained seawater, oil and 100 mL of nutrients, in addition to 2900 mL of the bacterial slurry. A small air pump and air diffuser were used to ensure that the system maintained an aerobic environment. A plastic tube was fixed inside each aquarium at a depth of 40 cm to allow collection of water samples.

Oil and water samples from each aquarium were collected periodically over a period of 120 hr. Water samples (1 liter each) were collected by siphon action using the fixed rubber tubing placed in each aquarium. Oil samples were collected using a stainless steel spatula. In order to keep a constant oil to water ratio, the amount of oil collected from each aquarium was made proportional to every liter of seawater sampled.

One half gram of each oil sample was dissolved in high purity hexane. The volume was made up to 10 mL with the same solvent. The solution was centrifuged to isolate the asphaltenes. A portion of the supernatant liquid was finally transferred into a 1-mL capacity crimp-on vial fitted with a Teflon-lined silicone rubber septum.

Oil hydrocarbons were extracted from 1 liter of seawater samples with two 60 mL portions of methylene chloride. The combined organic phase was concentrated to near dryness using a Kuderna-Danish concentrator, during which time about 5 mL of hexane was added through the Snyder column for a solvent changeover. The concentration step was continued by blowing a gentle stream of nitrogen gas at ambient temperature until the sample volume was less than 0.5 mL. The volume was made up to 1 mL with hexane and the concentrate was finally transferred into a 1-mL capacity crimp-on vial fitted with a Teflon-lined silicone rubber septum. Emulsion was found to form during the extraction of all water samples that contained bacteria. The emulsion was broken down before the concentration step by passing the extract through a bed of anhydrous sodium sulfate supported by glasswool in a glass chromatographic column.

The oil and seawater extracts were analyzed by gas chromatography. The aliphatic hydrocarbons in the range of C-10 to C-34 were analyzed using a Varian 6000 Gas Chromatograph (GC) equipped with an FID detector. Results were handled and computed by the DS - 654 Series Data System connected to the GC. The GC oven was programmed from 60 to 290 °C. Ramp rates were set at at 4 °C/min from 60 to 100 °C, 3 °C/min from 100 °C to 120 °C, 2 °C/min from 120 to 250 °C and 3 °C/min from 250 to 290 °C. The column used for the separation of n-alkanes was a SPB-5 fused silica capillary column (30 meters x 0.25 mm id, 0.25 μm film). Nitrogen was used as the carrier gas. Flow rate through the column was set at 1.5 mL/min. Make-up gas flow was maintained at 30 mL/min. The injection technique was splitless, with a split flow of 150 mL/min initiated automatically at

the time of injection. The split valve was opened after 1 min and remained so during the remainder of analysis time.

The GC/MS analyses were performed using a Finnigan OWA-30 GC/MS system. A fused-silica capillary column (30 meters x 0.25 mm id), coated with 0.25-μm layer of SE-30, was used for analysis throughout the study. The mass spectrometer in electron-impact mode was scanned from 45 to 450 amu in 1 second. Helium was used as the carrier gas.

Several criteria have been used by a number of investigators to evaluate the biodegradation of crude oil (Westlake et al. 1974, Ward 1980, Cooney et al. 1985, Pritchard and Costa 1991). These criteria include degradation of the n-alkane fraction of the oil, changes in the C-18/phytane ratio, degradation of selected aromatic hydrocarbons such as phenanthrene and its related homologs and resistive biomarkers, such as hopanes. It is well documented that bacteria and other microorganisms have the ability to biodegrade n-alkane fractions of crude oil, especially those with a carbon number less than 24. It was suggested by a number of investigators that the C-18/ phytane ratio is a useful parameter to monitor microbial degradation. This is because the two compounds have similar vapor pressures and water solubilities. Consequently, losses due to dissolution or evaporation are expected to be similar. However, the branched-alkane phytane was reported to biodegrade at a much slower rate than C-18. Therefore, a decrease in the C-18/phytane ratio suggests microbial degradation. In this study, the following criteria were selected as indicators of microbial degradation: (1) degradation of the n-alkanes of the oil, (2) changes in C-18/phytane ratio of the oil, and (3) changes in the concentration of waterborne oil hydrocarbons. In addition, GC/MS has also been used to confirm the identities of the dispersed oil components in the water column.

RESULTS AND DISCUSSION

Changes in the C-18/phytane ratio of the floating oil were monitored over a period of 120 hr. About 75 oil samples were collected and analyzed by GC.

At an initial oil concentration of 5g/L, the C-18/phytane ratio in the control tanks decreased slightly during the 120-hr study period (Figure 1). However, when nutrients were added, the ratio was found to decrease more rapidly. The sharpest decrease was found in samples seeded with bacteria and nutrients. The decrease in the C-18/phytane ratio, with the addition of nutrients alone could be attributed to the enhancement of oil biodegradation by bacteria indigenous in seawater. However, the addition of bacteria produced a more pronounced effect than that of nutrients only.

The change in the C-18/phytane ratio at 10 g/L oil concentration is shown in Figure 2. Insignificant decrease was observed in the control. This decrease was

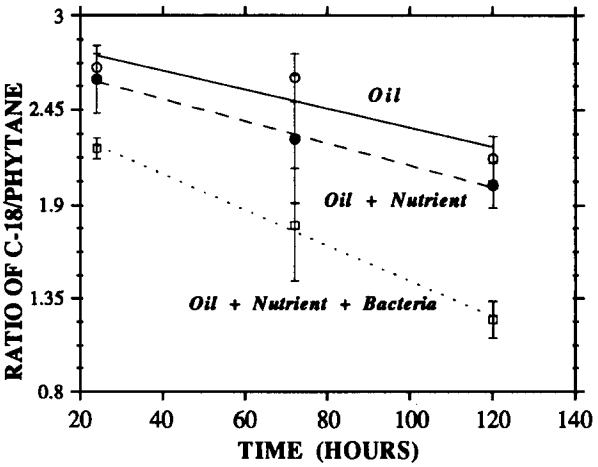

Figure 1. Changes in the ratio of C-18/Phytane with time at an initial oil concentration of 5 g/L.

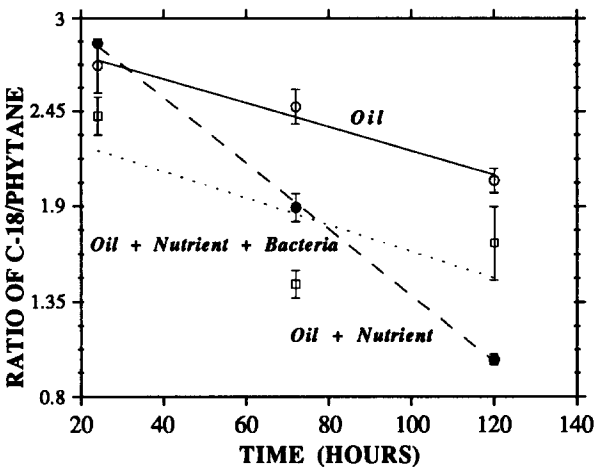

Figure 2. Changes in the ratio of C-18/Phytane with time at an initial oil concentration of 10 g/L.

slightly marked in the presence of bacteria and nutrients. An unexpectedly rapid decrease in the ratio was observed with the addition of nutrients alone. The reason for this phenomenon is not known and needs further investigation.

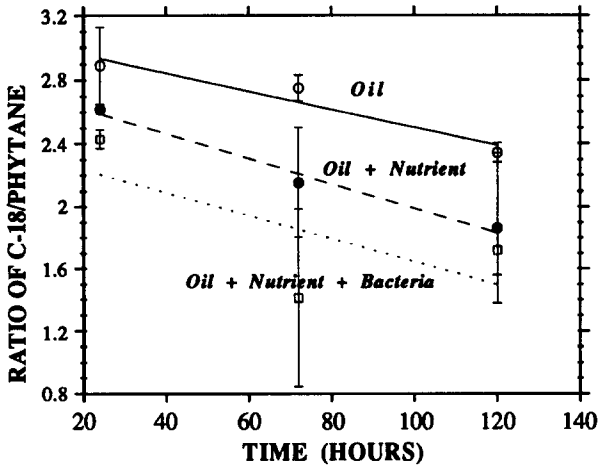

Figure 3. Changes in the ratio of C-18/Phytane with time at an initial oil concentration of 20 g/L.

The change in the C-18/phytane ratio at 20 g/L oil concentration was found to follow exactly the same trend observed at an oil concentration level of 5 g/L, as shown in Figure 3.

The results obtained at initial oil concentrations of 5 and 20 g/L have demonstrated that the addition of nutrients and bacteria to oil has enhanced the biodegradation of the n-alkane fraction of the oil, as indicated by the decrease in the ratio of C-18/phytane. A lesser degree of enhancement was obtained when nutrients alone were added, and microbial degradation of oil was not significant in the absence of nutrients or bacteria. At 10 g/L, addition of nutrients alone was found to enhance microbial degradation more than bacteria addition.

The rates of change over 120 hr in n-alkane concentration of oil samples collected from aquaria which contained oil at an initial concentration of 5 g/L are shown in Figure 4. It can be seen from this figure that the concentrations of these compounds in the oil after 24 hr were lowest in the aquaria treated with bacteria. As time elapsed, a little decrease in the n-alkane concentration was observed after 120 hr. This may indicate that the rate of biodegradation of n-alkanes was most rapid during the first 24 hr. It is interesting to note that the concentration of n-alkanes in oil not treated with nutrients, or bacteria, increased with time. This may be attributed to the loss of considerable amounts of other oil components by emulsification into the water. Therefore, in the absence of microbial degradation attributed to bacteria, n-alkane concentration is expected to increase with time. However, in those cases where nutrients or bacteria were added, the biodegradation of the n-alkane fraction results in concomitant losses of n-alkane fraction in the oil.

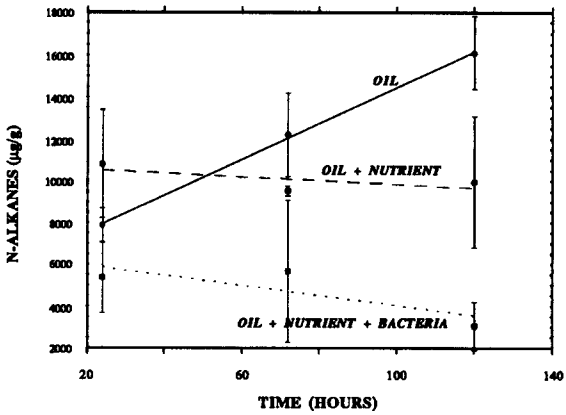

Figure 4. Changes of n-alkane concentration in oil as a function of time at an initial oil concentration of 5 g/L.

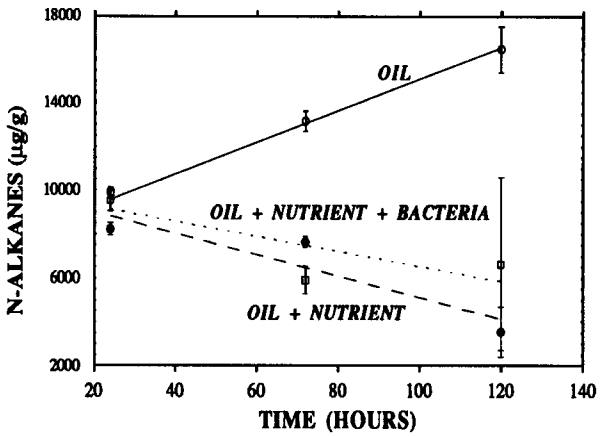

Figure 5. Changes of n-alkane concentration in oil as a function of time at an initial oil concentration of 10 g/L.

The effect of biodegradation on n-alkanes at an initial oil concentration of 10 g/L was more pronounced. As can be seen from Figure 5, the n-alkane concentrations of the oil after 24 hr were close to each other. However, as time elapsed, a significant decrease was observed. This decrease was slightly enhanced in presence of nutrients alone, as compared with that found in the presence of nutrients and bacteria. These results are in good agreement with the observation on the C-18/phytane ratio discussed above.

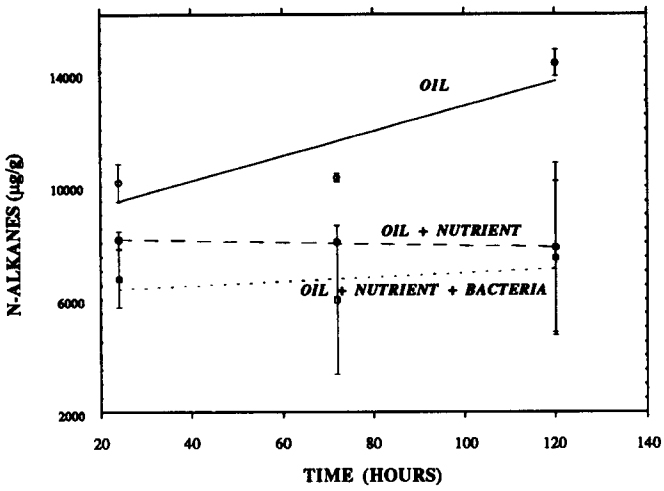

Figure 6. Changes of n-alkane concentration in oil as a function of time at an initial oil concentration of 20 g/L.

The effect of biodegradation on n-alkanes at an initial oil concentration of 20 g/L is depicted in Figure 6. This figure shows that the biodegradation of the n-alkane fraction followed the same degradation pattern observed at an oil concentration of 5 g/L.

It is worth to mention that after 24 hr at an initial oil concentration of 5 g/L, the decrease in the concentration of n-alkanes with the addition of nutrients alone is significantly less than that of nutrients and bacteria combined. This difference was much less at 10 and 20 g/L initial oil concentrations. This may indicate that bioremediation works more effectively at low oil concentrations. At higher oil concentrations, the differences were too small to preferentially recommend the use of bacteria seeding over nutrient addition only. Moreover, at 10 g/L it was found that the addition of nutrients alone exhibited more enhanced bioremediation.

Visual observation of the progress of the bioremediation experiment has shown that waters in the control aquaria were clear over the entire period of the experiment. The waters in the aquaria containing nutrients became turbid and brownish in color. This color was deeper in the aquaria containing nutrients and bacteria. The oil floating on the surface of the water was also found to be affected. The least affected was the oil in control aquaria. The oil in the aquaria containing the nutrients became brownish and small bubbles were formed. The brownish color of the oil was deeper in the aquaria containing bacteria and large air bubbles were formed at the surface of the water.

Gas chromatographic analysis of about 70 water samples collected from various aquaria has clearly shown that very limited amounts of oil were dispersed and/or dissolved in water in the control aquaria over 120 hr. However, oil was found to be dispersed into the water in those cases where nutrients or bacteria were added. The concentration of the dispersed compounds was found to increase considerably with time as indicated by the pattern of change in the unresolved complex envelope of various chromatograms shown in Figure 7. The results of this experiment demonstrate that the addition of nutrients or bacteria results in the dispersion of considerable amount of oil components into the water. The identities of the dispersed materials in the water column were determined by analyzing extracts of the water by GC/MS technique.The results of the analysis have demonstrated the presence of many organic compounds that form a considerable proportion of crude oils. A list of oil hydrocarbons identified in the water samples by GC/MS is shown in Table 1.

Table 1. Oil hydrocarbons identified using GC/MS in water samples where bacteria and nutrients were added.

Compound	Quantitation Ion	Confirmation Ion	% Relative Abundance of Confirmation Ion
Alkanes	85		
C-3 Naphthalenes	170	155	
C-2 Fluorenes	194	179	25
C-3 Fluorenes	208	193	
Dibenzothiophene	184	152,139	15
C-1 Phenanthrene/Anthracene	192	191	60
C-2 Phenanthrene/Anthracene	206	191	
C-3 Phenanthrene/Anthracene	220	205	
Naphthobenzothiophene	234		
C-1 Naphthobenzothiophene	248		
C-2 Naphthobenzothiophene	262		
C-3 Naphthobenzothiophene	276		
Fluoranthene/Pyrene	202		
C-1 Fluoranthene/Pyrene	216	215	60
C-2 Fluoranthene/Pyrene	230		
Chrysene/Triphenylene	228	226	30
C-1 Chrysenes	242	241	
C-2 Chrysenes	256	241	
C-3 Chrysenes	270	255	
Hopanes (191 family)	191		
Sterenes (217 family)	217		
Indeno(g,h,i)pyrene	276	277,138	25,30
Benzo(1,2,3)perylene	276	277,138	25,20

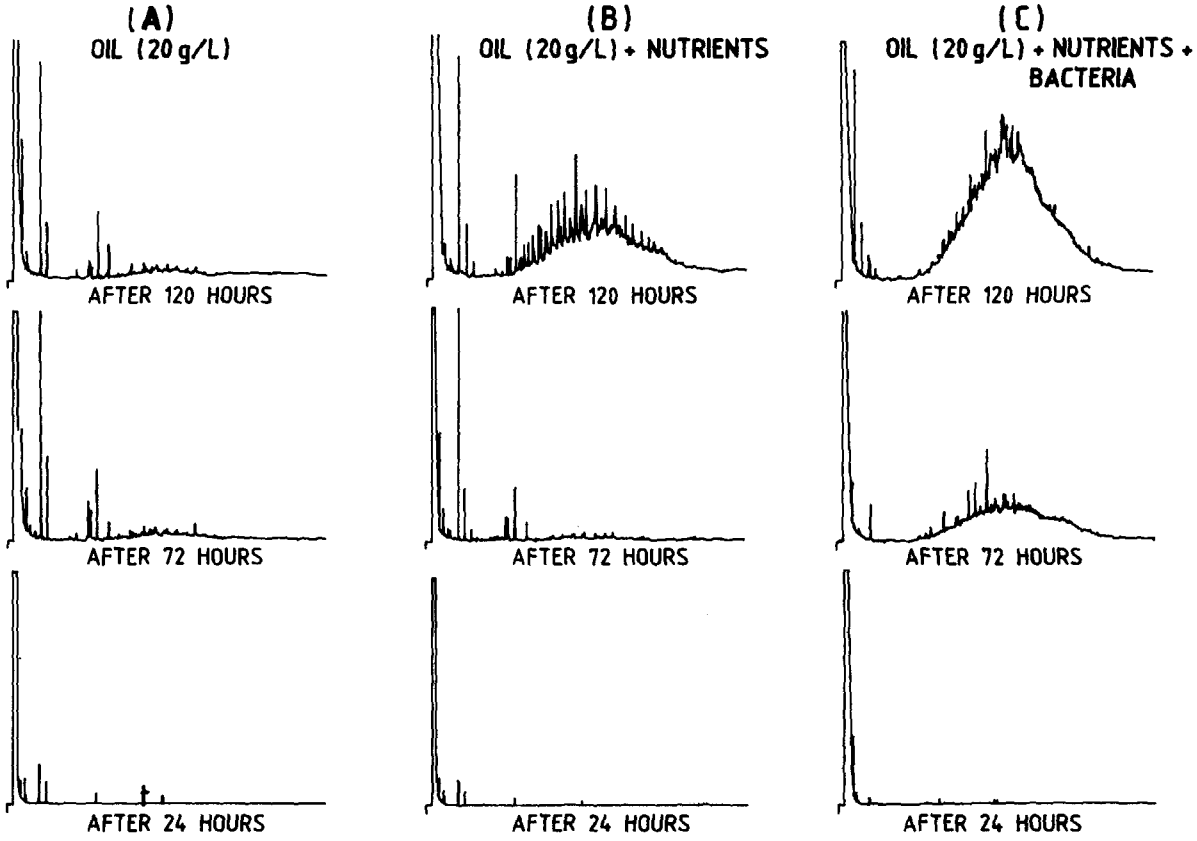

Figure 7. Gas chromatograms obtained for oil hydrocarbons extracted from 1-liter water samples, containing (A) oil, (B) oil and nutrients, and (C) oil, nutrients and bacteria, collected at different sampling times.

The results of this study have shown that, under laboratory conditions, bacteria can degrade some fractions of the oil as indicated by changes in the C-18/phytane ratio and n-alkane concentration. Biodegradation depends on the amount of nutrients and/or bacteria relative to that of oil. At an oil concentration of 10 g/L, the addition of nutrients enhances oil degradation slightly more than the case where bacteria were added. At high oil concentration (20 g/L), the degree of biodegradation produced by the addition of nutrients alone was similar to that obtained in the presence of nutrients and bacteria. The addition of nutrients alone can enhance the biodegradation of spilled oil. The addition of nutrients or bacteria, resulted not only in the degradation of the n-alkane fraction of the oil but also in the dispersion of considerable quantities of its non-alkane components into the water.

It follows that if this bioremediation technique is applied in open waters, marine organisms will be exposed to more toxic fractions of the dispersed oil in the water column. Consequently, the adverse effects of oil pollution on the marine environment may be aggravated. However, this may not be the case if the technique is employed to combat oil pollution on contaminated coastal areas.

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