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Effects of Wave Action on the Bioremediation of Crude Oil Saturated Hydrocarbons

REGAS SANTAS and PHOTEINOS SANTAS*
OikoTechnics Institute, 50 Kefallenias st., Athens 16 342, Greece

The effects of wave action on the biodegradation of Iranian light crude were investigated in two mesocosm experiments using two different fertilizers (F1, April–May 1997; and Inipol EAP-22, June–July 1997). Three levels of wave action ('high-wave', 'low-wave', 'no-wave') were applied in three mesocosms simulating a typical Mediterranean shore. Biodegradation of saturated hydrocarbons was monitored over a period of one month in surface water and submersed sediment samples ('surface' vs 'shore'). In submersed sediments, maximum biodegradation for both fertilizers (43%-F1; 55%-Inipol) was observed under moderate wave action ('low wave' treatment), followed by the 'high-wave' treatment. Biodegradation was not significant under the 'no-wave' treatment. At the water surface, the highest biodegradation (51%) was observed in the F1-treated mesocosm in the absence of wave action, followed by the 'low-wave' treatment (21%). Inipol application did not enhance biodegradation at the water surface regardless of wave action level. © 2000 Elsevier Science Ltd. All rights reserved.

Keywords: wave action; petroleum hydrocarbons; biodegradation; Mediterranean; Inipol; fish meal; F1; intertidal zone.

Introduction

The mixing of oil slicks by waves has important effects on the physical and chemical nature of the slick. These effects include: (a) rupturing of the slick into bands and streaks and subsequent spreading over a significantly larger area; (b) dramatic evaporation enhancement by breaking waves; (c) dispersion of oil droplets into the water column; and (d) formation of water-in-oil emulsion (mousse). The mousse has a larger volume, increased viscosity and adhesiveness than oil slicks under calm weather conditions. Therefore, under the influence of wave action, oil adheres to surface sediments more readily, but does not penetrate to deeper strata. Moreover, mousse formation greatly decelerates the natural weathering processes. In all cases, the sinking of crude oil is insignificant, since its density rarely exceeds that of seawater, even after extensive weathering (Galt *et al.*, 1991).

Crude oil may contain more than thousands of different hydrocarbons, each with a different weathering rate: the volatile components evaporate quickly; some medium-sized polycyclic aromatic hydrocarbons dissolve in the water to a small extent; some products of photochemical and microbial degradation are highly water soluble.

Two major physical processes occur in the early stages of an oil spill: (a) spreading of the slick due to gravity and surface tension phenomena, and (b) evaporation of the lighter weight hydrocarbons by surface transfer processes. Some of these lighter hydrocarbons, when present in large concentrations, may be toxic for indigenous hydrocarbon decomposer populations, thereby suppressing natural biodegradation. After the evaporation of the lighter hydrocarbons, the biodegradation of the heavier compounds proceeds more rapidly.

The acceleration of natural hydrocarbon biodegradation processes through the addition of nitrogen and phosphorus containing fertilizers has been tested in both marine and terrestrial ecosystems during the last two decades (Atlas, 1981; Lacotte *et al.*, 1995). Water-soluble and oleophilic fertilizers are the two main types of additives used in both laboratory and field experiments. Water-soluble salts containing nitrogen and phosphorus are effective under laboratory conditions (Atlas and Bartha, 1973), but are readily washed away by surface agitation and mixing in the environment (Herrington *et al.*, 1994). The application of N and P sources in oleophilic form is considered to be a more effective nutrient application method, since oleophilic additives remain dissolved in the oil and thus are available at the oil–sediment interface where they enhance bacterial growth and metabolism (Lacotte *et al.*, 1995; Leahy and Colwell, 1990).

*Corresponding author. Tel.: +301-924-1434; fax: +301-924-9308.
E-mail address: santas@hol.gr

Seeding with allochthonous microorganisms previously adapted and culture-enhanced has also been attempted, with controversial results (Texas General Land Office, 1991; Venosa *et al.*, 1991; Madsen and Kristensen, 1997).

The high frequency of accidents having devastating ecological effects, as well as the need to clean-up the spills using environmentally acceptable methods call for an increased understanding and further development of bioremediation applications.

The objective of this paper was to investigate the combined effects of a number of factors on saturated hydrocarbon biodegradation at the mesocosm scale. Factors examined include: (a) wave action, (b) type of oleophilic fertilizer, (c) time, and (d) pollution site (water surface vs sediments). The results are the first obtained from the Mediterranean Sea, a region of the world through which large amounts of crude oil are transported every year to different destinations worldwide.

Materials and Methods

Two mesocosm experiments (April–May 1997; June–July 1997) were conducted to assess effects of wave action, fertilizer and time on the biodegradation of Iranian light. The oleophilic fertilizers used were F1 and Inipol EAP-22, with C:N:P ratios of 24:18:3.5 and 62:5:1, respectively.

Each experiment was performed in three 3-m^3 ($3 \times 1 \times 1$ m) fibreglass tanks simulating beach and open-water conditions. The sloping 'beach' end of the mesocosms was constructed by cinderblocks covered with beach pebbles collected from the intertidal zone of a nearby shore of Saronikos Gulf and graded to form a smooth slope (Fig. 1). The tanks were filled with seawater to the topmost layer of the 'beach'. Natural seawater was added periodically to make up for losses due to evaporation and spray. Salinity ranged from 36 ppt at the beginning to 40 ppt at the end of the experiments.

Three levels of wave action ('high-wave': wave height 20 cm, 1 wave per 27 s; 'low-wave': 10 cm, 1 wave per 22 s; 'no-wave', vigorous rippling) were simulated using an adjustable wave generator (Fig. 1) installed 50 cm above the tank water surface. In all three tanks, pumps (rated at 5 m^3/h) installed at the 'open water' end of the tank provided water recirculation. Pump operation and wave action were controlled by a timer (on:off period 2:4 h). The controlled rapid recirculation maintained dissolved oxygen levels in the water near saturation at all times in all mesocosms.

Open-top bags made of polypropylene window-screen (mesh size $\approx$ 1 mm) were filled with 1 kg of beach pebbles each, immersed into 3 L Iranian light and allowed to drain for 30 s. The bags with pebbles were placed at a depth of 10 cm below the water surface of each mesocosm (designated as the 'shore' sampling point), and the remaining crude was spilled on the water surface of each mesocosm.

Fertilizer application followed immediately after the addition of crude oil in each tank. In the first experiment, 120 g F1 were spread on the surface of each of the three tanks and water recirculation was turned on. Four replicate 'surface' samples per tank per sampling day (0, 3, 7, 15 and 30 days after experiment startup) were collected by skimming the water surface. Four replicate 'shore' samples, one from each bag, were collected per tank per sampling day. Each bag was sampled only once but remained in the tank throughout the experiment. Eight samples (4 'shore' plus 4 'surface') were collected per tank per sampling day, for a grand total of 120 samples for the first experiment. Average tank water temperature was 18 ± 2°C. In the second experiment, 300 mL Inipol were sprayed on the surface of each mesocosm and recirculation began by turning pump operation on. The sampling scheme was the same as in

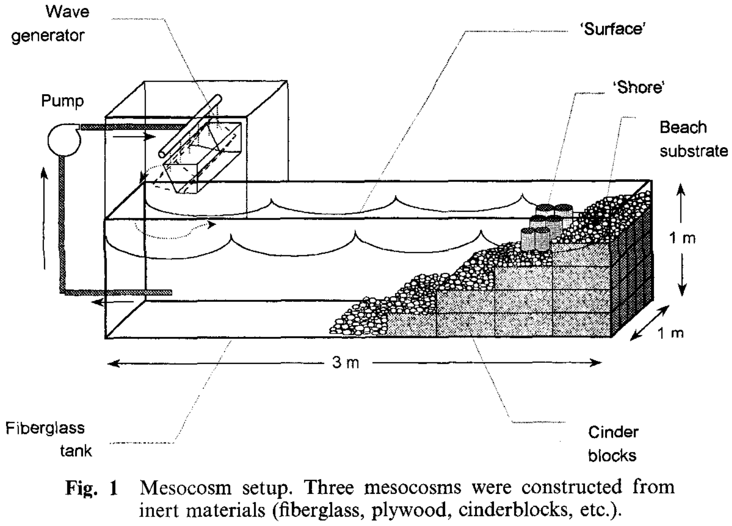

Fig. 1 Mesocosm setup. Three mesocosms were constructed from inert materials (fiberglass, plywood, cinderblocks, etc.).

the first experiment. Average mesocosm water temperature was 30 ± 1°C.

Oil biodegradation was assessed by measuring the n-C_{17}/pristane and n-C_{18}/phytane indicator ratios (Lee and Levy, 1987; 1989; 1991; Prince *et al.*, 1993; Sveum and Bech, 1994). Biodegradation on day x was the difference in ratio values between day 0 and x divided by the value of day 0 and expressed as a percent. All samples were prepared for gas chromatography as described by Korda *et al.* (1997) and Santas *et al.* (1999).

Total bacteria were enumerated by epifluorescence microscopy on water samples collected from the surface of each of the three mesocosms on days 5 and 10 of the second experiment. Fifteen optical fields per sample were counted and bacterial counts are presented as the average and the range. Efforts to enumerate bacteria at the 'shore' site were erratic due to oil-smears that prevented the counts, and are not reported.

n-C_{17}/pristane and n-C_{18}/phytane ratio data were subject to a three-way ANOVA using wave level ('high-wave', 'low-wave', 'no-wave'), depth ('shore' and 'surface') and time (sampling day) as the sources of variation. The variance associated with any particular source of variation was compared to random variation by means of the F-test. The two indicator ratios yielded comparable results in both experiments. The results from only one ratio (n-C_{18}/phytane) are presented for simplicity.

Results

In both experiments, the artificial oil slick on the surface of the 'high-wave' and 'low-wave' mesocosms was dispersed within 2 h after the spreading of Iranian light and the application of the fertilizers. In the 'no-wave' mesocosms, the slick persisted throughout the 30-day experimental periods. During the intervals of no circulation, an iridescent film formed on the surface of both the 'high-wave' and 'low-wave' mesocosms. This film was washed 'ashore' when pump operation restarted. 'Surface' samples were obtained from this layer and analyzed for the indicator ratios mentioned above.

Wave level had a significant effect on oil degradation in both experiments (Experiment 1: $F = 3.86$, df = 8 and 60, $p < 0.05$; Experiment 2: $F = 2.12$, df = 8 and 90, $p < 0.05$). This effect, however, depended on the combination of sampling site and time (Figs. 2–4).

F1 effects

'Shore'. Hydrocarbon biodegradation was most effective under 'low-wave' conditions. On day 30, a 43% reduction compared to day 0 was observed in this treatment, as measured by the n-C_{18}/phytane ratio (Fig. 2). This value was significantly lower than all values of days 0, 3 and 7, and the values for the 'no-wave' and 'high-wave' treatments on day 15. Hydrocarbon breakdown was lowest in the 'no-wave' treatment (18%). Biodegradation in the 'high-wave' treatment (33%) was intermediate between the other two treatments, although not significantly different than either one (Fig. 2). On day 30, this value was significantly lower than the corresponding values on days 3 and 7 (Fig. 2).

'Surface'. Saturated hydrocarbon biodegradation was most effective under 'no-wave' conditions. The highest biodegradation (51%) was observed on day 30 for this treatment (Fig. 3). On day 30, biodegradation under the

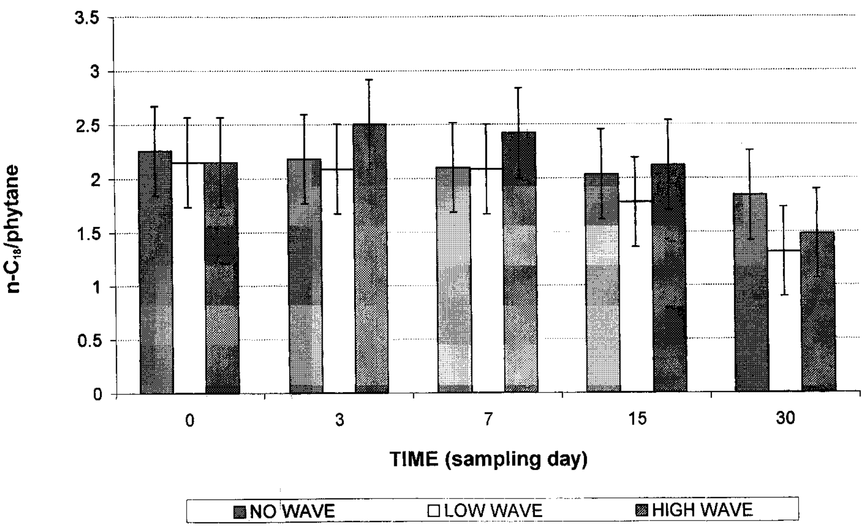

Fig. 2 Effects of time and wave action on n-C_{18}/phytane ratio on shore sediments receiving F1. Each mean is the average of $n = 4$ values. Means analysis of mesocosm assays (means ±95% confidence intervals). Overlapping of confidence intervals indicates no significant differences at the $p = 0.05$ level.

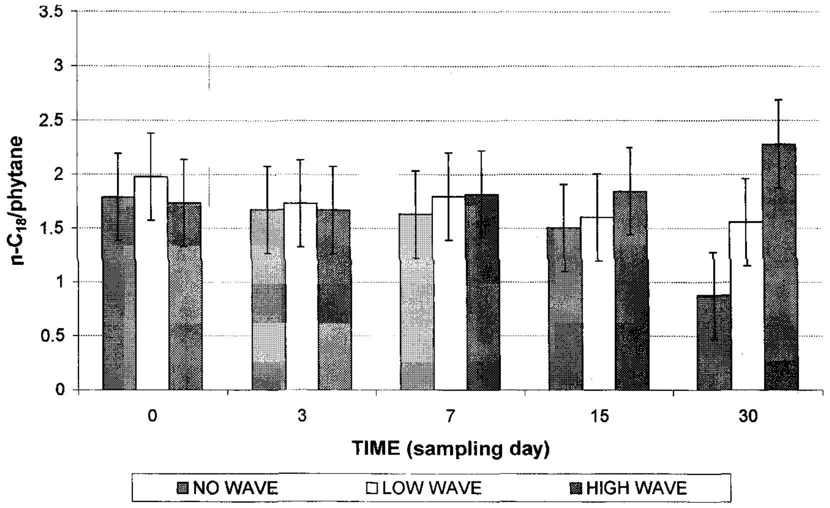

Fig. 3 Effects of time and wave action on n-C_{18}/phytane ratio on water surface receiving F1. Each mean is the average of $n = 2$ values. The most dramatic differences are observed on day 30. Means analysis of mesocosm assays (means ±95% confidence intervals). Overlapping of confidence intervals indicates no significant differences at the $p = 0.05$ level.

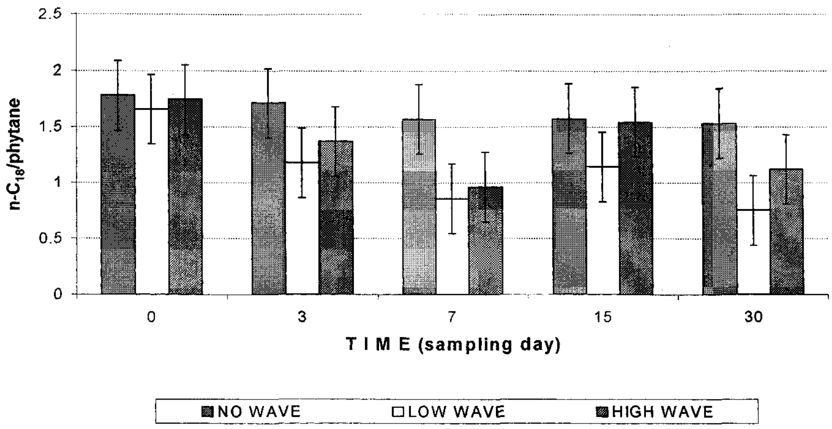

Fig. 4 Effects of time and wave action on n-C_{18}/phytane ratio on shore sediments receiving Inipol. Each mean is the average of $n = 4$ values. Means analysis of mesocosm assays (means ±95% confidence intervals). Overlapping of confidence intervals indicates no significant differences at the $p = 0.05$ level.

calm water conditions (no-wave) was significantly lower than any other treatment on any other day except the now-wave and the low-wave on day 15 and the high-wave value on day 30 (Fig. 3).

Inipol effects

'*Shore*'. Similarly to F1, hydrocarbon biodegradation with Inipol as the nutrient supplement was most effective under 'low-wave' conditions. The lowest value of the n-C_{18}/phytane ratio (0.75) was observed on day 30 (55% biodegradation; Fig. 4).

On day 7, biodegradation under the 'low-wave' conditions was significantly higher compared to day 0, or to the 'no-wave' conditions on days 3 and 7 (Fig. 4). On day 15, biodegradation under the 'low-wave' treatment was significantly higher than any treatment on day 0, as well as the 'no-wave' treatment on day 3 (Fig. 4). On day 30, biodegradation under the 'low-wave' treatment was significantly higher than on day 0, the 'no-wave' treatment for all days, and the 'high-wave' treatment on day 15 (Fig. 4).

Biodegradation in the 'high-wave' treatment was intermediate between the other two treatments. On day 7, biodegradation for the 'high-wave' treatment was 45%. This value was significantly lower than all values on day 0, and the 'no-wave' values on days 3 and 7 (Fig. 4). On day 30, the n-C_{18}/phytane ratio was significantly lower than the value for the 'no-wave' and 'high-wave' treatments on day 0 (Fig. 4).

TABLE 1
Bacteria counts in the second experiment (for all counts, $n = 15$).

Wave treatment	Day 5 Mean number of colonies/ml – (range)	Day 10 Mean number of colonies/ml – (range)
'No-wave'	8.85×10^6 [$7.89 \times 10^6 - 9.81 \times 10^6$]	1.28×10^7 [$1.16 \times 10^7 - 1.41 \times 10^7$]
'Low-wave'	6.58×10^6 [$5.99 \times 10^6 - 7.17 \times 10^6$]	1.07×10^7 [$9.12 \times 10^6 - 1.24 \times 10^7$]
'High-wave'	6.36×10^6 [$5.46 \times 10^6 - 7.26 \times 10^6$]	7.62×10^6 [$6.37 \times 10^6 - 8.87 \times 10^6$]

'*Surface*'. No statistically significant differences in biodegradation were observed at this sampling point between days or treatments.

The above results show that biodegradation proceeds in the following order:

Site	Fertilizer	Biodegradation order
Shore	F1, Inipol	'low-wave' > 'high-wave' > 'no-wave'
Surface	F1	'no-wave' > 'low-wave' > 'high-wave'
	Inipol	No differences

Bacteria counts obtained from the 'surface' of the three mesocosms in the second experiment (data unavailable for the first experiment) are summarized in Table 1. Substantial bacterial counts were observed in all three mesocosms on both days. Higher bacterial counts on day 10 compared to day 5 indicate favourable growth conditions within the tanks. The highest and lowest numbers of bacteria were observed in the 'no-wave' and 'high-wave' treatments, respectively.

Discussion

Some of the questions generated during oil-spill cleanup attempts include: (1) the distinction between correlation and causation, (2) practical considerations in difficult-to-control, large-scale experiments, (3) discrepancies between laboratory and field results, etc. The controlled conditions of laboratory experiments often result in impressive but unrealistic assessment of the factor(s) in question. Field attempts, on the other hand, are often inconclusive due to the complexity of the open systems used and the intricacy of the biological processes involved. To address such issues, this study strived for a realistic mid-scale simulation of typical local average conditions. Mesocosms are attempts to upscale laboratory experiments and combine the advantages of reasonable control under a more complex set of interacting environmental parameters, such as season, temperature, solar radiation, etc. In addition to their economic and technical manageability, mesocosm experiments are free of the many legal and social issues stemming from full-scale trials. The mesocosm approach, with all inherent strengths and weaknesses, was chosen herein to investigate the interaction of wave action, fertilizer type and pollution site on hydrocarbon biodegradation.

The most usual waves reaching the Greek shorelines are 'spillers' (maximum height rarely exceeding 2–3 m; typical frequency 1 wave/15–20 s) with foaming crests (Wetzel, 1975). Larger waves occur 9–10 days throughout the year on the average. The two wave levels employed in this study simulate the most frequent type of waves on the Greek intertidal zone.

Biodegradation efficiency in the mesocosm 'wave-splash' zone was highest under moderate wave action. The accelerated biodegradation in the 'shore' samples of the 'low-wave' treatment may be explained by: (a) improved oxygenation, and (b) increased physical contact between bacteria, substrate and oil. The scouring action of larger waves results in increased attrition of microbial decomposer populations growing on oiled sediments and hence to lower biodegradation enhancement. Evidence for the stressful conditions generated by the 'high-wave' treatment comes from the observed erosion of the artificial shore in the mesocosms: much of the sediment around the enclosures (Fig. 1) was washed away to the bottom of the 'high-wave' tanks. In contrast, very few and no pebbles were displaced in the 'low-wave' and 'no-wave' mesocosms, respectively. In the natural environment, heavy wave action may cause severe substrate erosion thereby reducing the numbers and/or effectiveness of surface-attached, oil-degrading populations.

At the 'surface' of the F1-treated mesocosms, 'no-wave' provided the most effective treatment. Biodegradation followed a steady decline and reached 51% within 30 days (Fig. 3). This suggests a greater abundance of or enhanced activity by oil-degrading bacteria at the oil–water interface in calm water conditions. This was confirmed by bacterial counts (Table 1).

Wave action may also affect biodegradation indirectly through the accelerated volatilization of small organic compounds that are highly toxic to oil-degrading bacteria (Galt *et al.*, 1991). The elimination of such toxic compounds may accelerate biodegradation of larger organics.

Experiment 2 took place in the summer, under considerably higher temperatures compared to experiment 1 (30°C and 18°C, respectively). The difference in temperature, rather than fertilizer, probably accounts for the slightly higher biodegradation observed during Experiment 2 (summer, Inipol). F1 has been a more effective treatment than Inipol in other mesocosm experiments performed under the same temperature (Korda *et al.* 1997; Santas *et al.* 1997).

The assessment of biodegradation, by monitoring the n-C_{17}/pristane and n-C_{18}/phytane indicator ratios, was based on the assumption that the biodegradation of pristane and phytane was negligible during the 30-day experimental period. Over a 90-day period, and under controlled conditions of aeration and nutrient additions, a 30% pristane biodegradation has been reported (Samson *et al.*, 1994). Therefore, the results presented herein are probably a conservative estimate of the biodegradation efficiency of the two fertilizers, since pristane and phytane are at the denominator of the two indicator ratios. Analytical procedures using more stable biomarkers, such as hopanoids (Venosa *et al.*, 1997), would have likely shown a higher biodegradation in all treatment combinations, but probably would not affect the comparison between treatments during the 30-day duration of this study.

In conclusion, evidence presented in this paper shows that: (a) moderate wave action accelerates hydrocarbon biodegradation in sediments of the intertidal zone, (b) large amounts of mixing energy have a less pronounced beneficial effect in sediments, but (c) inhibit biodegradation at the water surface.

Further research should be directed towards quantifying the relation between the mechanical energy of waves, the capacity of microbes for attachment to different sediment types and their oil degrading efficiency.

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