MESOCOSM ASSAYS OF OIL SPILL BIOREMEDIATION

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ABSTRACT: Three mesocosms were constructed simulating a sloping intertidal zone in fiberglass tanks (3×1×1 m). Substrate, water, and living organisms from wild unpolluted ecosystems were transferred into the tanks as a natural inoculum for mesocosm development. One-kilogram portions of sediment samples collected from uncontaminated beaches were immersed into Iranian light and placed at two depths (40 and 80 cm below water level) for a total of 12 sediment samples per depth per tank. In each tank, Iranian light was added at a 1:1000 v/v ratio relative to water and installed pumps provided water recirculation (5 m^3/h). The effects of fertilizer type on biodegradation were examined by pouring 330 mL Inipol EAP-22 into the first tank, spreading 330 g of F1 (modified fishmeal) at the water surface of the second one, and no fertilizer to the third one (control).

The effect of various fertilizer additions on hydrocarbon biodegradation was assessed by GC monitoring of n-C_{17}/pristane and n-C_{18}/phytane ratios on days 0, 1, 3, 7, 15 and 30 after mesocosm commissioning, on two replicate samples per mesocosm per depth and at the water surface. Physical and chemical parameters (temperature, salinity, etc.) were also monitored throughout the experiment.

Bioremediation depended on fertilizer added, depth, and sampling date. Inipol produced the most visually dramatic reduction of crude oil: on day 30, the Inipol-assigned mesocosm had the clearest water column of all installed mesocosms. However, based on measurments of the n-C_{17}/pristane and n-C_{18}/phytane ratios, F1 had a better performance. By day 30, F1 increased biodegradation to about 3x the initial value. Similar but less pronounced differences were observed at 40 cm, while no differences were observed at 80 cm for any fertilizer and any sampling day.

INTRODUCTION

Bioremediation, the use of microorganisms to break down hazardous chemicals into nontoxic compounds, is a developing cleanup technology. This technology is most effective in reducing concentration of petroleum products at oil polluted areas (Orzech et al., 1991).

The effectiveness of bioremediation depends on a variety of environmental conditions, including oxygen concentration, indigenous bacteria populations, temperature and nutrient concentration

Many bioremediation techniques have been developed since this technology first appeared in 1967 (Santas et al., 1994). Among them, bioaugmentation, the direct application of microorganisms isolated from the contaminated site or from an off-site vendor, adapted to the specific contaminants and site conditions, cultured, and enhanced. Bioremediation usually involves biostimulation: the addition of oxygen, water and mineral nutrients (combinations of nitrogen, phosphate, and maybe surfactants and trace metals) to accelerate the reproduction of organisms as well as their metabolic activity. Oleophilic additives are preferred in seawater applications because they are dissolved into the oil, facilitating bacterial growth at the oil-water interface (Lacotte et al., 1995).

This paper assesses the bioremediation effectiveness of representative oleophilic fertilizers (Inipol EAP-22, F1) in mesocosms operated outdoors.

MATERIALS AND METHODS

Iranian light was used for all experiments. Table 1 lists the additives used, with their relevant components.

TABLE 1. Additives used and their relevant components in beach simulation experiments

Name	Description	Contents	Source
Inipol EAP-22	Commercially available oleophilic fertilizer	Oleic acid (26.2%), lauryl phosphate (23.7%), 2-butoxy-1-ethanol (10.8%), urea (15.7%), water (23.6%) (Sveum et al., 1994)	Elf Aquitaine, France
F1	Commercially available fertilizer	Modified fish meal	Elf Aquitaine, France (BIOREN project, EUREKA Program)

Mesoscale experiments (Table 2) were performed in continuous-flow 3 m^3 seawater tanks (3×1×1).

The seawater was recirculated at 5 m^3/h, and the average seawater temperature in the tanks was approximately 15°C. Additives were applied immediately after crude oil application. Only bacteria indigenous to the seawater and the sediment were used in these tank experiments.

TABLE 2: Description of experiments performed in continuous-flow tanks

Tank	Crude oil	Additive
1	3 L	Inipol EAP-22 (330 mL)
2	3 L	F1 (330 g)
3	3 L	none

Samples were collected from three different points in each tank: water surface, shore sediments(0.40m), and open water sediments (0.80m).

Samples used for gas chromatographic analyses were processed as follows: water samples (~20 mL) were swirled and oil was extracted with 10 mL n-hexane (Merck, *proanalysi*; >99%). Hydrocarbons in sediment samples were extracted with 10 mL n-hexane. Water was removed from the oil/n-hexane solution by sodium sulfate. The supernatant was separated into saturated and aromatic fractions through a silica-gel packed column (2-25 μm particle size). The saturated hydrocarbons fraction was eluted with 1 mL n-hexane and analyzed on a Hewlett Packard 5890

Series II GC, equipped with flame ionization detector (FID) and a splitless injector. Oil biodegradation was evaluated from the n-C_{17}/pristane and n-C_{18}/phytane ratios.

RESULTS

Data from GC analysis for the n-C_{17}/pristane and n-C_{18}/phytane ratios were subjected to a three-way ANOVA. The sources of variation were fertilizer type (F1; Inipol; no fertilizer), depth and sampling day.

n-C_{17}/pristane. The three factor interaction was significant ($F=10.68$; $df=20, 54$; $P<0.05$). Means analysis was performed, 95% Just Significant Confidence Intervals were placed around the means, and the results for each combination of fertilizer type, depth and sampling day are presented in Figures 1-3. On day 15, the surface sample of the Inipol-treated mesocosm was significantly lower than any other mean (Figure 1).

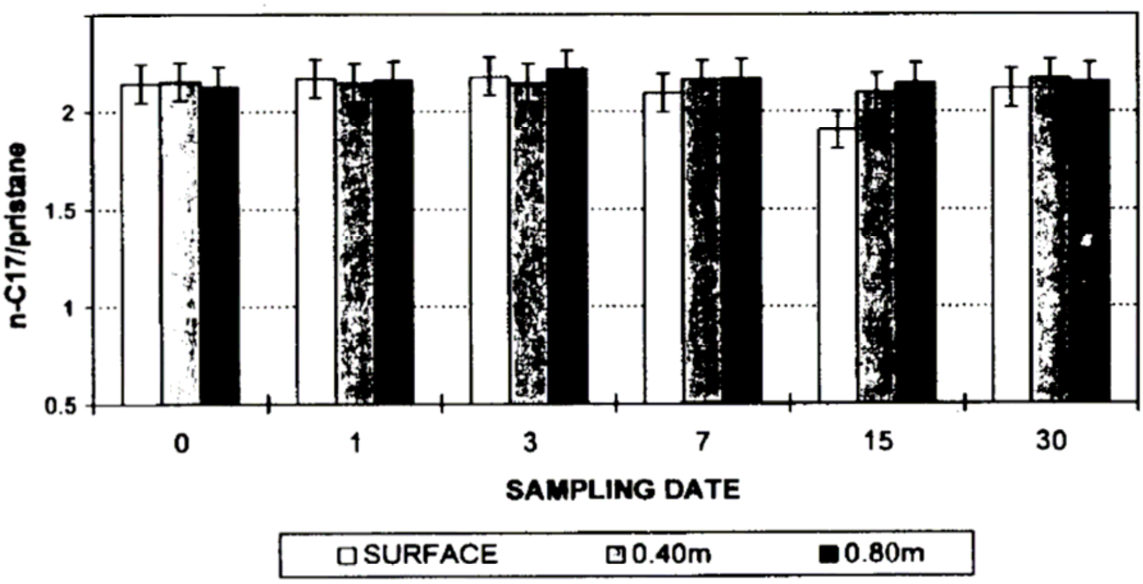

FIGURE 1. Effects of depth and time on biodegradation using Inipol

No significant differences were observed in the F1-treated mesocosm within the first week after commissioning (Figure 2). The n-C_{17}/pristane ratio at the water surface on day 15 was significantly lower than day 7, and the n-C_{17}/pristane ratio on day 30 was significantly lower than any other value. At mid-depth, the n-C_{17}/pristane ratio on day 15 was not significantly different from day 7, while on day 30 this ratio was significantly lower than on day 15. At the deep layer, no significant changes were observed throughout the project.

No significant differences were observed in the control mesocosm (Figure 3).

n-C_{18}/phytane. The three factor interaction including fertilizer type, depth and sampling day was significant ($F=24.84$; $df=20, 54$; $P<0.05$), meaning that the effects of fertilizer type on the n-C_{18}/phytane ratio depend on the combination of depth and sampling day.

No significantly different n-C_{18}/phytane values were observed on days 0-7 across depths for the F1 treated mesocosms (Figure 5). On day 15, the surface and

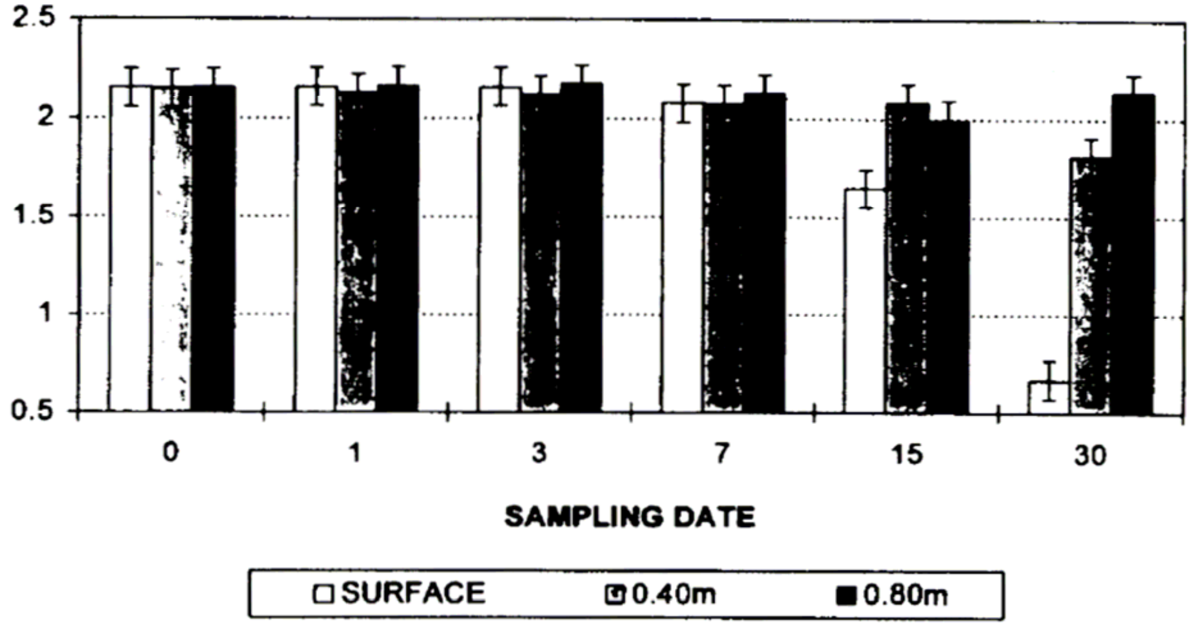

FIGURE 2. Effects of depth and time on biodegradation in F1-treated mesocosms.

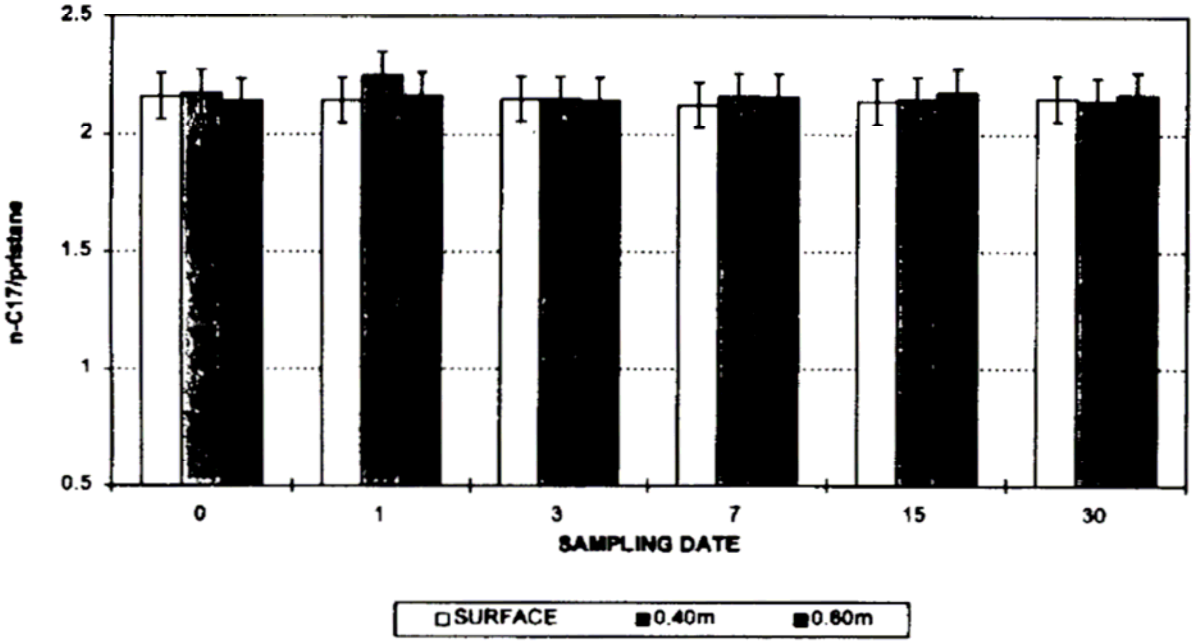

FIGURE 3. Effects of depth and time on biodegradation in the control mesocosm.

deepest waters had significantly lower n-C_{18}/phytane values than the respective ones of day 7, while there was no significant difference in the n-C_{18}/phytane values at mid-depths. On day 30, the surface waters had the lowest values recorded (0.65), while the mid-depth value was significantly lower than the respective one on day 15. In contrast, the n-C_{18}/phytane value at the deepest water was significantly higher than the respective one on day 15, and not significantly different from the values of days 0-7.

In the control tank the n-C_{18}/phytane value for the sediment-water interface was significantly higher on day 1 than the same value for the water surface on day 15 (Figure 6).

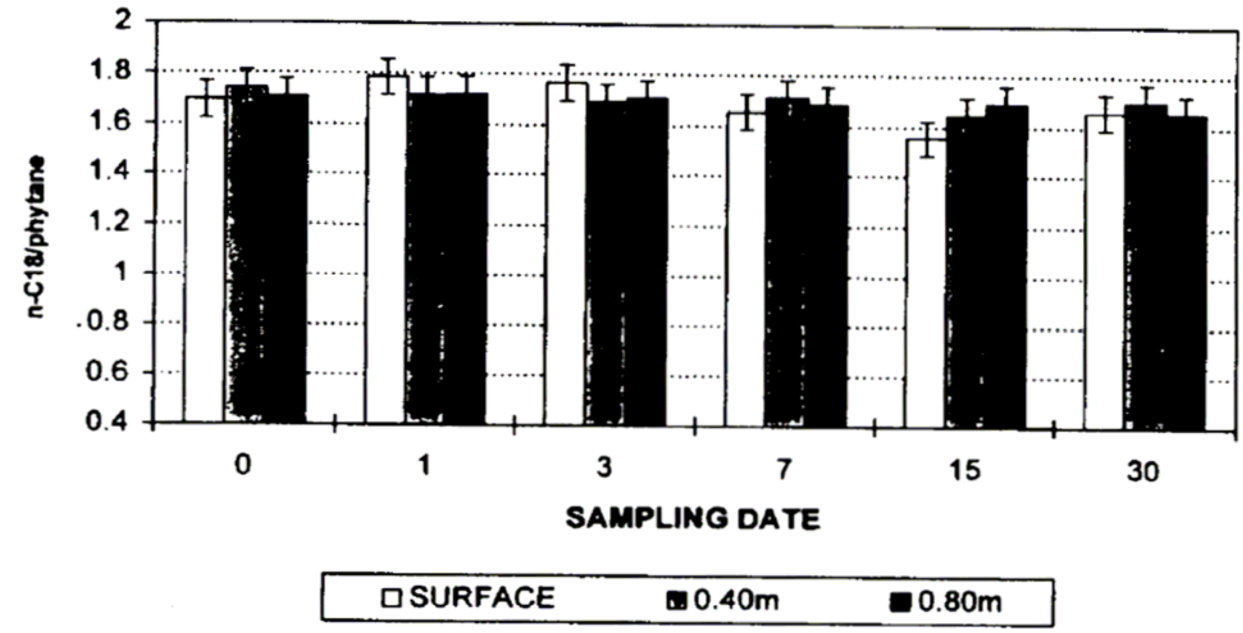

FIGURE 4. Effects of depth and time on biodegradation using Inipol.

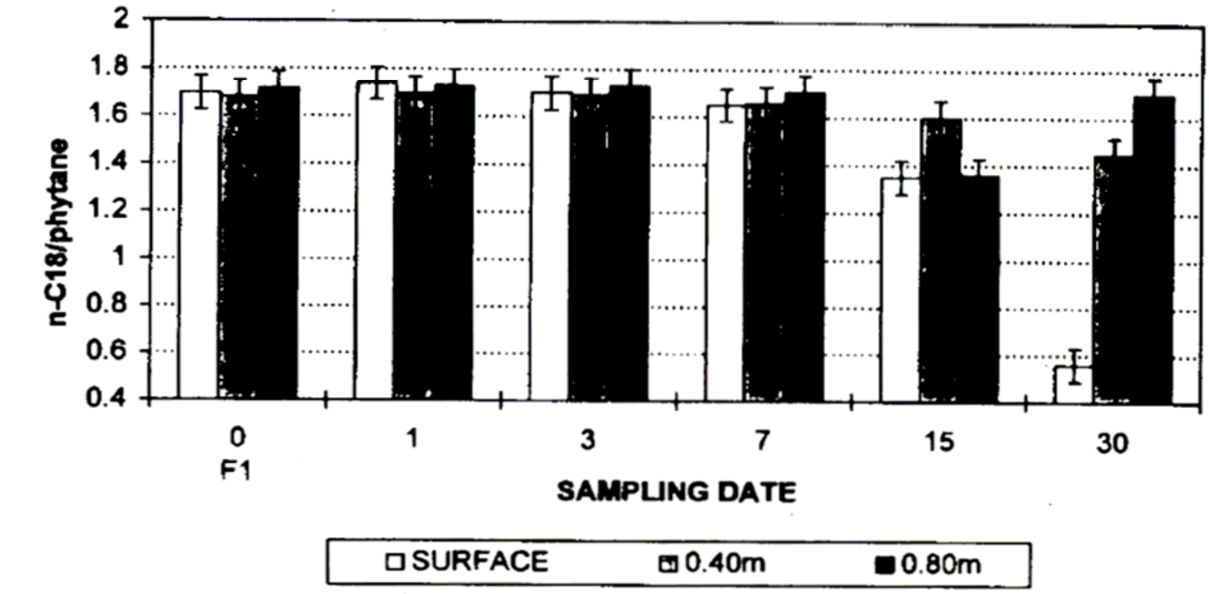

FIGURE 5. Effects of depth and time on biodegradation using F1.

DISCUSSION

F1 was the most effective and fastest-acting treatment (Figures 2 and 5). The n-C_{17}/pristane and the n-C_{18}/phytane ratios at the water surface on day 15 were dramatically lower than the previous days. Within 30 days, the original concentrations of n-C_{17} and n-C_{18} had been reduced by 75% and 65%, respectively, compared to the initial values.

On day 30, visual inspection of the Inipol-assigned mesocosm revealed the clearest water column of all installed mesocosms. After mesocosm decommissioning, it was observed that some of the oil had moved from the water surface towards the beach and had been permanently attached on the cinder blocks used as support for the beach gravel. With regard to the n-C_{17}/pristane and n-C_{18}/phytane ratios.

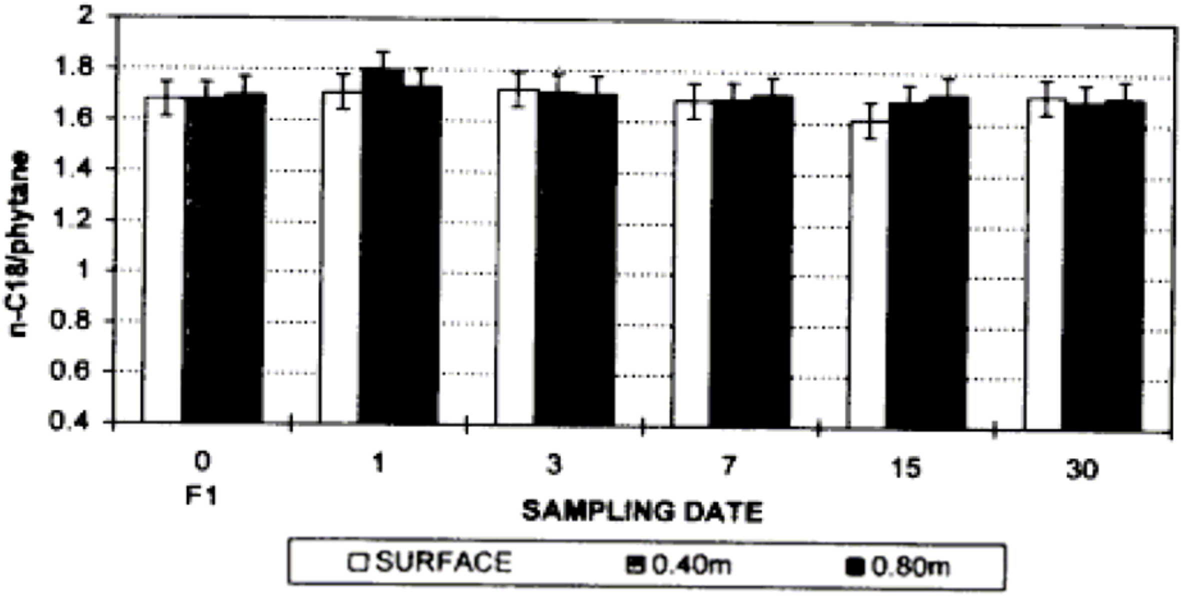

FIGURE 6. Effects of depth and time on biodegradation in the control mesocosm.

Inipol application had less dramatic effects. A possible explanation for this may be that Inipol was sprinkled on the water surface, as opposed to the usual application on the beach.

Unlike the Inipol mesocosm, the degraded oil on the water surface of the F1-treated mesocosm had a non-uniform distribution: there were patches of a thin, clear crust of undegraded material, while some other areas were covered by dark, thick oil residues. A thick crust of petroleum fractions resistant to biodegradation floated on the water surface.

ACKNOWLEDGMENTS

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