Microbial Petroleum Degradation Enhancement By Oil Spill Bioremediation Products

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higher for the nutrient and non-nutrient controls for this batch than for any of the other batches according to the O&G values. An average O&G value for the nutrient control of 66% indicates a reduction of 34% of the initial weight, this value is overcoming the rest of the nutrient controls tested throughout the study by almost 20%. Nutrient levels in the seawater used for this batch show to be higher than for the rest of the batches as shown later in Table 11

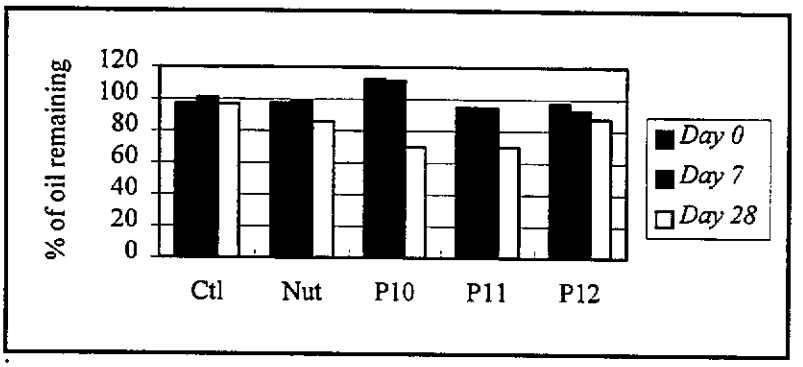
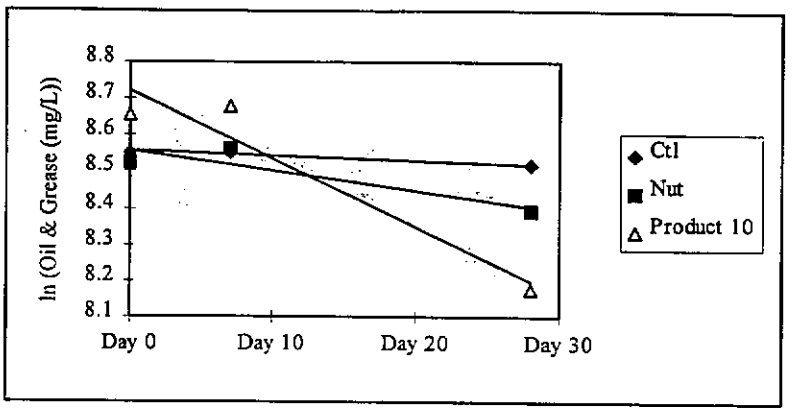


Figure 4– Oil and Grease results (Batch D)

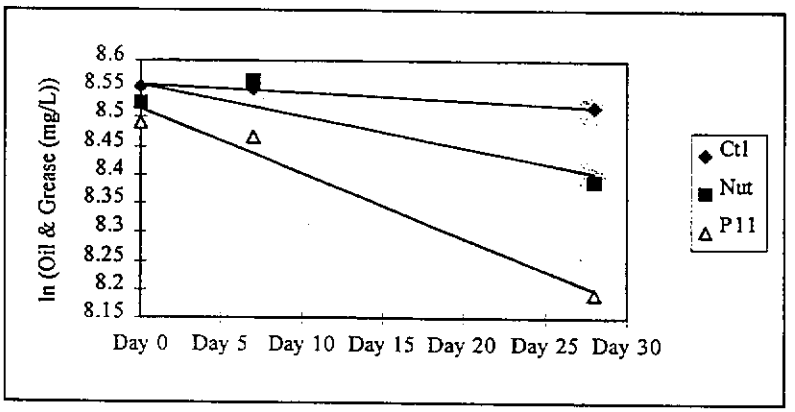
High O&G numbers can be a result of a high production of extractable materials such as biomass or metabolites. As shown in Batch D product 10 is causing an increase in the O&G values at day 0 and 7, with an average value of 11% more of the initial weight. However, microbial counts indicate a high aliphatic degrader population through this period, as will be shown later Figure 16. After 28 days the oil was degraded more extensively by product 10 than by the nutrient control. This suggests that the polar fraction is possibly being increased by the product's contents, on days 0 and 7, but does not imply that the oil is remaining undegraded. Microbial degradation of product 10 could be producing metabolites that are being completely oxidized between day 7 and day 28. Reviews from different articles (Bragg *et. al.* 1993, Venosa *et. al.* 1993, Fedorak, and Westlake, 1981) suggest that absolute degradation rates cannot be drawn exclusively from this analysis.



Treatment	Slope	R square
Control	-0.0013	0.9505
Nutrient	-0.00563	0.8041
Product 10	-0.01859	0.9228

Figure 10– Ln concentration change with time for product 10 (P10) as compared with the nutrient and non-nutrient control

Figure 10 suggests a lag phase for product 10 between day 0 and 7, after this period the microbial population shows a high degradation rate, achieving a final degradation extent higher than that of the nutrient and non-nutrient control.



Treatment	Slope	R square
Control	-0.0013	0.9505
Nutrient	-0.00563	0.8041
Product 11	-0.01126	0.9715

Figure 11– Ln concentration change with time for product 11 (P11) as compared with the nutrient and non-nutrient control

The rate of oil removal is an important factor to consider when comparing the performance of each product. Table 7 presents a summary with the different rates of oil removal as well as the average.

Product	Rate	Non-nutrient control	Nutrient control
Product 1	0.007	0.00013	0.004
Product 3	0.012	0.00013	0.004
Product 6	0.014	0.002	0.005
Product 8	0.017	0.0003	0.014
Product 10	0.018	0.00013	0.005
Product 11	0.011	0.00013	0.005
Average	0.013	0.0005	0.006

Table 7-- Rates of oil removal for the products passing the O&G criteria (mgof oil/L-Day)

According to these results the average half-life of the petroleum mixture for this specific experiment is approximately 40 days. Prior studies suggest a half-life for petroleum mixtures of approximately 2 months (Stewart *et. al.*, 1993).

Toxicity Assay Results

The toxicity of a particular oil depends on its water-soluble components or on its direct contact with marine organisms (Atlas and Bartha, 1972). Petroleum hydrocarbons may have a variety of sublethal effects on microorganisms. The presence of these inhibitory substances in oil can, however, delay or prevent the biodegradation of otherwise suitable hydrocarbon substrates. Compounds such as tar or creosote used widely as preservatives against fungal attack can be present in oil. Surgical quality petroleum ether is used in some countries as an alternative to ethanol for skin disinfection prior to minor surgery. Petroleum degradation is sometimes inhibited by these compounds when they are solubilized in the aqueous phase (Bartha and Atlas, 1977).

As shown in Table 9 product 11 passed all the analyses; GC-MS data is presented for this product in APPENDIX F - GC-MS Results. Products marked with one star * are the ones showing a statistically lower O&G value as compared with the nutrient control. Products marked with ** meet the O&G criteria and are among the 30% with the highest oil removal extent in terms of O&G. Products passing these criteria were further evaluated using the toxicity results. The number of products proceeding to GC-MS analysis was limited to one product. Table 10 presents a summary of these results. This table assumes that all products passed tier 1, however is only considering the six products passing the O&G criteria

Tier 1	Tier 2 (O&G)	Tier 2 (Toxicity)	Tier 2 (Highest 30 %)	GC-MS analysis
Product 11	Product 11	Product 11	Product 11	Product 11
Product 10	Product 10	Failed	Product 10	Not analyzed
Product 8	Product 8	Failed	Product 8	Not analyzed
Product 6	Product 6	Failed	Product 6	Not analyzed
Product 3	Product 3	Product 3	Failed	Not analyzed
Product 1	Product 1	Product 1	Failed	Not analyzed

Table 10-- Summary of the elimination process for the products passing the O&G criteria

Nutrient Analysis

Seawater used for each batch was analyzed for total nutrient levels of nitrogen and phosphorous. Several studies have concluded that concentrations of N and P are limiting with respect of hydrocarbon degradation (Atlas, 1981). Possible differences in the seawater nutrient levels could be possible sources of variability. Table 11 summarizes the results for Total Kjeldahl Nitrogen and Phosphorous, TKN and TKP, respectively.

Polar fraction composition remained almost constant for products 7, 8 and 9 as presented in Figure 31. For the control treatment this composition increased almost 50 % of its initial value may be due to the production of biomass and biodegradation intermediates.

Figures 32-34 show the composition of aliphatics, aromatics, and polars for batch D. As presented earlier for batches A and B, the aliphatic fraction is being degraded more severely than the aromatic fraction. The same results are found in the next two figures. Microbial counts for aliphatic degraders (Figure 16) show a higher number for product 10, with a value of 4.06 E7 at day 28, as compared with the rest of the treatments in this batch, with values in the order of 10^6 at the most. This is reflected as a decrease in the aliphatic fraction composition from a 100% to 46% after 28 days.

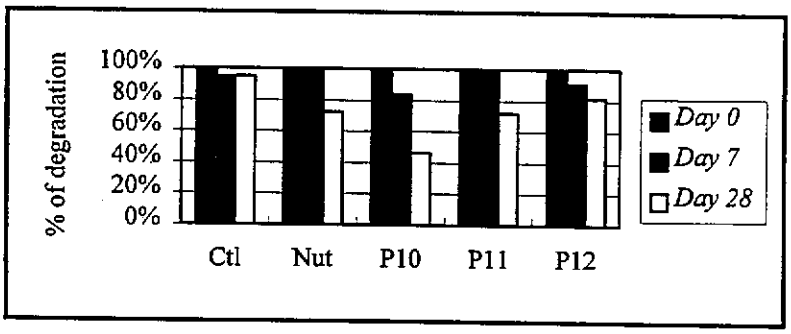


Figure 32-- Aliphatic fraction composition through time (% of degradation (Batch D))

Products 10, 11, and 12 are decreasing in aliphatic and aromatic composition up to 50% for the aliphatic fraction and 25% for the aromatic. It is clear from these results that the oil is being degraded, and therefore, changing in composition. However, the aliphatic fraction is being degraded at a greater extent than the aromatic fraction, as mentioned before. Product 10 is showing a significant extent of hydrocarbons removal as presented in Figure 33 and Figure 33 for product 10, however, is presenting a level of toxicity greater than that exhibited by the control treatment.

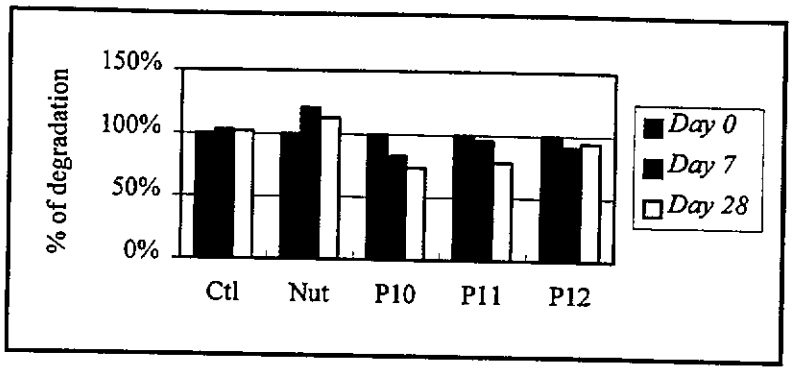


Figure 33-- **Aromatic fraction composition through time (% of degradation (Batch D))**

As presented in Figures 23 through 33 show the average of aliphatic fraction biodegraded was 34%, while only 21% of the aromatic fraction showed to be biodegraded.

Hydrocarbon degradation is being enhanced by several treatments, however, toxicity levels are also being increased as compared with the control treatment. Toxicity could be increased by the solubilization of petroleum hydrocarbons in the aqueous phase. However, solubilization and dispersion of petroleum hydrocarbons will result in an increase of the surface area available for microbial colonization (Atlas, 1981). From these conclusions, a reevaluation of the protocols used is suggested for further evaluations.

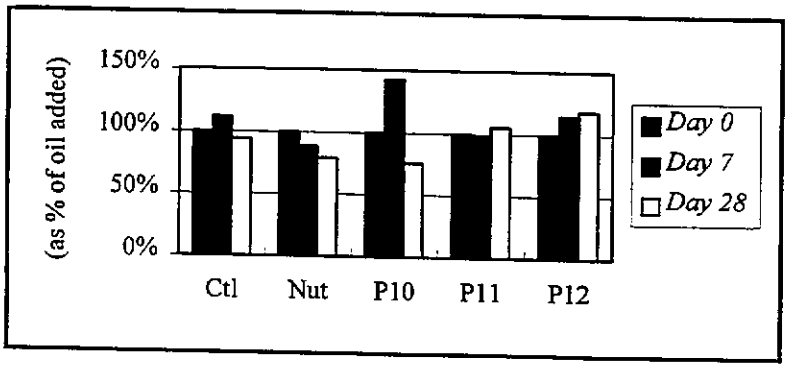


Figure 34-- **Polar fraction composition through time as a percentage of the amount initially present (Batch D)**