BIOREMEDIATION OF OILED BEACH SEDIMENTS: ASSESSMENT OF INORGANIC AND ORGANIC FERTILIZERS

Kenneth Lee and Gilles H. Tremblay
Department of Fisheries and Oceans
Maurice Lamontagne Institute
P.O. Box 1000
Mont-Joli, Quebec G5H 3Z4
Canada

Susan E. Cobanli
BDR Research Limited
P.O. Box 652, Station M
Halifax, Nova Scotia B3J 2T3
Canada

The effects of inorganic (ammonium nitrate and triple superphosphate) and organic (fish bone-meal) fertilizers on the biodegradation rates of Venture condensate within a sand-beach environment were assessed over 333 days. Field results showed that the organic fertilizer stimulated microbial growth and metabolic activity to the greatest extent. However, based on chemical analysis of residual oil concentrations and composition, the application of inorganic fertilizers was the superior bioremediation strategy. This paradox between microbiological and chemical results was attributed to the selective growth of different bacterial populations, the preferential use of components within the organic fertilizer over oil by the indigenous microflora, and the production of toxic metabolic by-products from the degradation of the organic fertilizer.

Microbial degradation is a major mechanism for elimination of petroleum pollutants from the aerobic marine environment.[18] Large-scale field tests to evaluate the potential of bioremediation techniques to accelerate natural oil-degradation rates by the addition of nutrients were conducted in Alaska following the *Exxon Valdez* oil spill incident.[2] The addition of both water-soluble and oleophilic fertilizers was found to accelerate the rate of oil biodegradation up to a factor of five or more, depending on the nitrogen concentration in the pore water of the intertidal sediments. Based largely on the results of these studies,[4,19,20] the scientific community has recently declared that bioremediation has come of age.[26] Research is now concentrated on the development and refinement of bioremediation products and operational guidelines.

Previous field studies by the Department of Fisheries and Oceans, Canada,[11,13 - 15] have

shown that the simple additions of agricultural fertilizers (ammonium nitrate, super triplephosphate, sulphur-coated urea) could enhance the natural biodegradation rates of hydrocarbons stranded in coastal sediments. Compared to other nutrient products (for example, oleophilic formulations), and/or methods (such as seeding by the addition of specific microbial inocula) for the enhancement of natural oil biodegradation rates, agricultural fertilizers offer several advantages. These products are commercially available, they are inexpensive, and they can be easily applied with conventional equipment.[2,13]

This paper summarizes the results of a field experiment comparing the effectiveness of inorganic and organic agricultural fertilizer formulations, as bioremediation agents, to treat Venture condensate stranded in a sand-beach environment.

Materials and experimental methods

Test oil and bioremediation agents.

Venture condensate, recovered from an exploratory well on the Scotian Shelf, off the coast of Nova Scotia, is a liquid of low viscosity and high volatility containing a high proportion of low-molecular weight hydrocarbons.[24] The granular forms of commercial inorganic fertilizers used in this study were ammonium nitrate (N:P:K ratio - 33-0-0) and triple superphosphate (N:P:K ratio - 0-46-0). The organic fertilizer used (Seagreen®, N:P:K ratio - 6-10-1; National Sea Products Ltd., Canada), consisted of fish bonemeal containing a small amount of fish oil as a binding agent (Table 1). Fish bonemeal (approximate composition: 45% protein, 10% fat, 45% ash) contains many trace elements (including Fe, Mn, Cu, Zn, and B) and organic growth factors not found in the chemical fertilizers.[23]

Field site and sampling procedures.

Field studies were conducted in the intertidal zone of a low-energy beach located on the eastern shore of Nova Scotia, Canada.[11] On day 0 (August 6, 1992), replicate in-situ sediment enclosures, constructed of 240 Mm mesh fabric, were filled with 7 liters of sieved wet sand collected from the study site. Venture condensate was added to selected enclosures at a concentration of 3 percent (w/v). Following treatment and initial subsampling of the sediments, the enclosures were anchored (2 m apart, 3 cm below the beach surface), midway between the high-water and low-water marks.

Application of nutrient treatment to the experimental enclosures was not initiated until day 12 (Table 2). Thus, prior to nutrient treatment, the concentration of toxic components (for example, low molecular weight hydrocarbons) within the sediments was reduced by dissolution and evaporation processes, and the indigenous bacterial population had become adapted oil metabolism.[11] At subsequent sampling periods, fertilizers were reapplied to specific enclosures designated to receive periodic nutrient treatments after the collection of samples for microbiological and chemical analyses. Routine sampling of the enclosures and nutrient additions ceased on day 123 (December 7, 1992) due to inclement weather and ice conditions. Final subsampling and removal of the enclosures occurred on July 5, 1993 (day 333).

Microbiological analyses.

Relative heterotrophic activity, bacterial productivity, and potential hydrocarbon degradation

rates in the beach sediments were monitored by determining the microbial uptake and/or respiration rates of the radio-labeled organic substrates L-[^{14}C(U)]-glutamic acid, methyl-[^{3}H]thymidine and n-[1-^{14}C]hexadecane.[11,14]

Chemical analyses.

Sediment samples for oil analysis were collected from each experimental enclosure on a routine basis during the experiment. The samples were immediately frozen (-15 ° C), in hexane-rinsed glass jars for storage until laboratory analysis. Changes in the concentration and composition of remaining Venture condensate in the sediments were determined by capillary gas chromatography.[15]

Surface waters over the experimental enclosures were routinely analyzed during the experiment for the concentrations of nitrate, nitrite, phosphate, and ammonia.[7] In addition, samples were collected prior to each nutrient reapplication event for the determination of potential nutrient release rates. To determine a nutrient release value, 7.0 mL of standard reference seawater was added to 5.0 g of sediment in a 50 mL beaker, 6 hours before the recovery of the supernatant for nutrient analysis. Data were corrected to account for the contribution of nutrients from the reference seawater.

Results

Field observations.

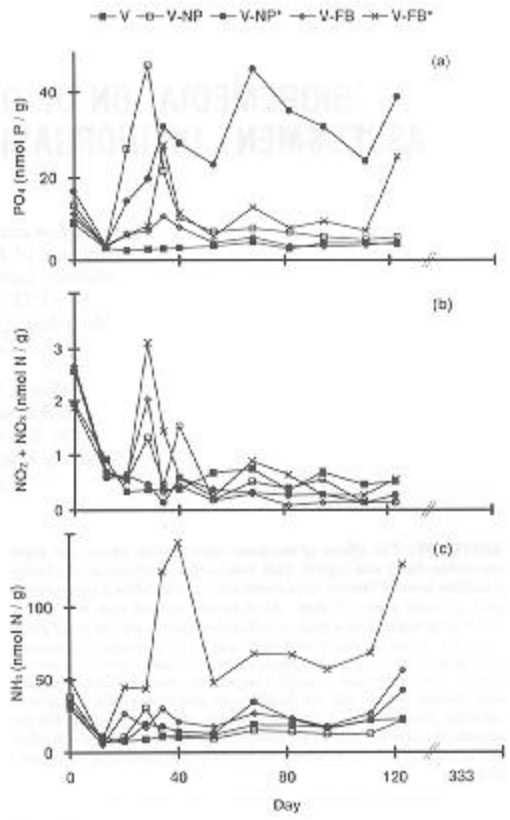
During the experimental period, variations in surface seawater temperature (2 - 20.5 ° C), salinity (28.48 - 30.69%), and nutrient concentrations (0.09 - 1.29 Mmol/L nitrate; 0.36 - 0.67 Mmol/L phosphate, and 0.30 - 4.97 Mmol/L ammonia) were recorded.

Nutrient data.

Data on potential nutrient release rates from the sediments showed that the application of the inorganic fertilizer (triple superphosphate) rapidly elevated interstitial water phosphorus concentrations (Figure 1a). Periodic applications of the fertilizer sustained elevated phosphorus concentrations throughout the study period. Results from the enclosure treated with a single application of the fish bonemeal illustrated the slow-release characteristic of the organic fertilizer (peak on day 34). Although the N:P ratio and concentrations were similar between the organic and inorganic fertilizers, lower phosphorus release rates were observed in the sediments treated with the organic fertilizer approximately two weeks after nutrient reapplication. Nevertheless, continuous phosphorus release was observed during the study period in all enclosures treated with periodic additions of fertilizer.

Considering that the potential for nitrogen release after each ammonium nitrate addition is on the order of 2,600 nmol N/g, extremely low values for nitrate and/or nitrite release (<2 nmol N/g) were observed in sediments recovered immediately prior to each nutrient reapplication event. This evidence suggested rapid physical loss, or utilization rates for nitrate and nitrite (Figure 1b) from the fertilized sediments within the experimental enclosures. While nitrate is readily assimilated by microorganisms, it may have also been removed from the enclosures by dispersion and dilution due to its solubility. The highest concentrations of nitrate and nitrite release were observed in the enclosures treated with the organic fertilizer between day 20 and day 40 (the period of highest bacterial productivity), presumably

due to the production of nitrate from ammonia by nitrification.



1.

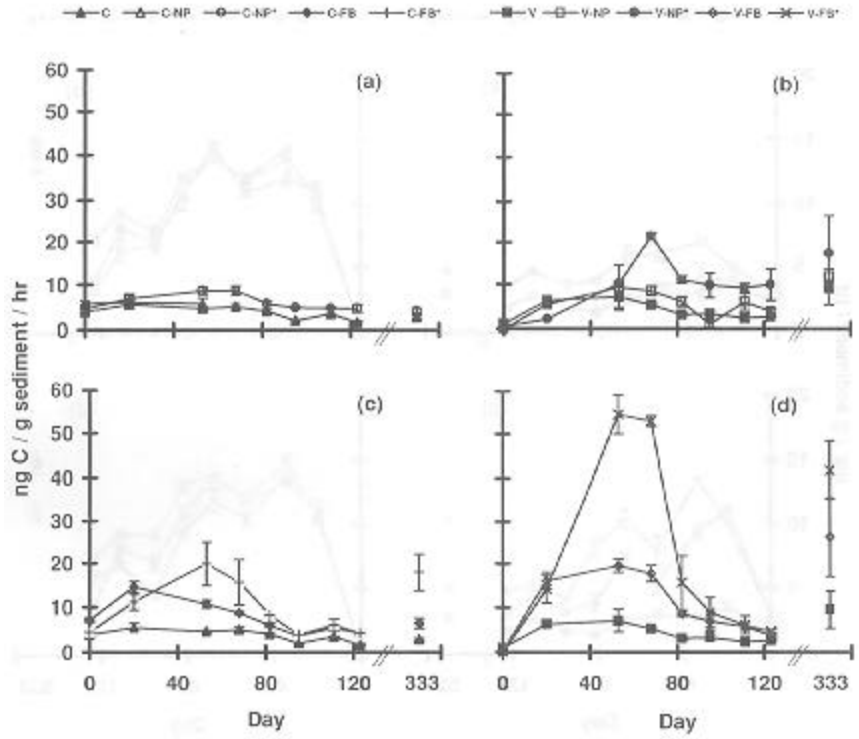
*Potential nutrient release in response to tidal exposure - V = Venture condensate 3% (w/v); N = Ammonium nitrate 33-0-0 (N:P:K); P = triple superphosphate 0-46-0 (N:P:K); FB = fish bonemeal 6-10-1 (N:P:K); * = periodic applications*

Organic material in sediments is decomposed by facultative aerobic microorganisms using dissolved oxygen within the interstitial water. As part of the slow nutrient release characteristic of the organic fertilizer, nitrogen within the fish bonemeal (as NH_2 groups in proteins, for example), is converted to ammonia by deamination reactions. In aerobic environments some of this ammonia is oxidized by nitrifying bacteria and ultimately converted to nitrate. However, as the oxygen is consumed and the sediments become anaerobic, nitrification cannot occur, and ammonia may accumulate. Release of ammonia mediated by microbial degradation of the fish bonemeal and rapid accumulation (corresponding to the decline in nitrate production), was detected within the enclosures treated with periodic additions of the fish bonemeal (Figure 1c). This chemical evidence was also confirmed by direct field observations (day 34 - sand treated with periodic additions of the organic fertilizer was beginning to turn black and emitted a faint "anoxic" odour). In a previous study, low ammonia and phosphate concentrations were observed for only 4 to 5 days within the subsur- face sediments of a cobble beach following treatment with a fertilizer solution.[20] The observed persistence of ammonia and phosphate the current study may be attributed to fine sediment (mean grain size diameter: 250 Mm) and

low-energy conditions at the study site.

Microbiological data.

Microbial responses were monitored over the sampling period to determine the efficacy of fertilizer addition. The addition of Venture condensate to the sediments appeared to have a minimal effect on total bacterial productivity as indicated by the measurement of ^{3}H-thymidine incorporation into bacterial biomass. In both the unoiled (Figure 2a) and oiled (Figure 2b) enclosures, periodic additions of the inorganic fertilizers (NP*) stimulated bacterial activity rates above that found in the unfertilized enclosures, while a single application had little effect. In contrast, single and periodic additions of the fish bonemeal (FB and FB*), resulted in sustained elevated bacterial production rates within the unoiled intertidal sediment (Figure 2c). In the oiled enclosures, periodic addition of the fish bonemeal increased bacterial growth rates by a factor of 3 over the single application (Figure 2d). Despite the stimulatory effect of the organic fertilizer, bacterial productivity rates declined with the seasonal decrease in seawater temperatures.



2.

*Change in total bacterial productivity in response to Venture condensate and nutrient additions - C = unoiled control; V = Venture condensate 3% (w/v); N = Ammonium nitrate 33-0-0 (N:P:K); P = triple superphosphate 0-46-0 (N:P:K); FB = fish bonemeal 6-10-1 (N:P:K); * = periodic applications*

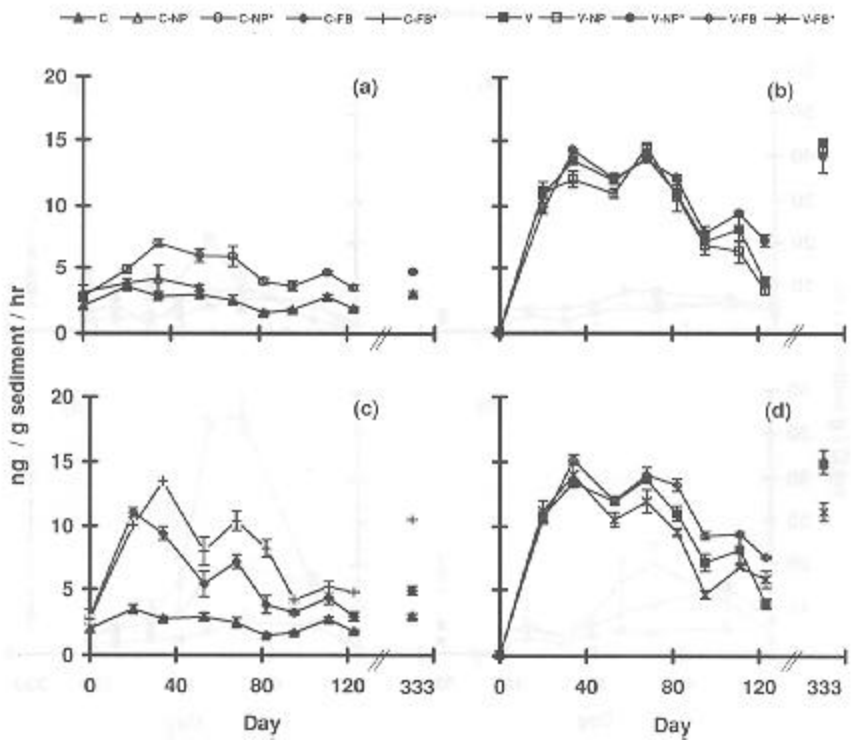
Relative heterotrophic activity rates within the sediments of the experimental enclosures were measured by monitoring the uptake and respiration rates of ^{14}C-glutamic acid. Periodic additions of the inorganic fertilizers (NP*) stimulated activity in the unoiled sediments (Figure 3a). Except for the last

two sample periods in 1992, the addition of the inorganic fertilizers appeared to have little or no effect on relative heterotrophic activity rates within the oiled enclosures (Figure 3b). Both single and periodic additions of the fish bonemeal (FB and FB*) significantly increased and sustained heterotrophic activity rates until ambient seasonal temperatures decreased significantly (Figure 3c,d). Consistent with observations in previous studies, heterotrophic activity was enhanced significantly by the addition of oil alone.[13] While periodic additions of the fish bonemeal retarded relative heterotrophic activity rates, a single application had a stimulatory effect (Figure 3d).

Potential hydrocarbon degradation rates in the sand within the enclosures were determined by measuring the respiration rate of added ^{14}C labeled hexadecane.[5,29] Figure 4a illustrates the changes in hexadecane turnover time in response to treatment within the oiled enclosures between day 53 and day 123. The results showed that prior to day 82, addition of nutrients had little effect on the biodegradation rates of hexadecane. After this period, as hexadecane biodegradation rates declined (higher turnover time) concurrently with seasonal temperatures, the addition of both inorganic and organic nutrients had a significant stimulatory effect (Figure 4a,b). The hexadecane turnover time data showed that periodic addition of the inorganic fertilizer was the most effective bioremediation strategy. A lag phase prior to the onset of hexadecane utilization, observed in the radio-tracer analysis, was not evident in the chemical results (Figure 5). However, both data sets show that oil degradation was stimulated to the greatest extent by the addition of the inorganic fertilizers.

Chemical Analysis.

To account for variability in the oil loading of the sediment on day 0, relative concentrations of oil were used to compare the time-series changes in the composition of the crude oil in different enclosures. Calculation of relative oil concentrations was based on the concentration of individual components as the percentage of total n-alkanes, normalized to the concentration within the same enclosure on day 0.



3.

*Change in relative heterotrophic activity in response to Venture condensate and nutrient additions - C = unoiled control; V = Venture condensate 3% (w/v); N = Ammonium nitrate 33-0-0 (N:P:K); P = triple superphosphate 0-46-0 (N:P:K); FB = fish bonemeal 6-10-1 (N:P:K); * = periodic applications*

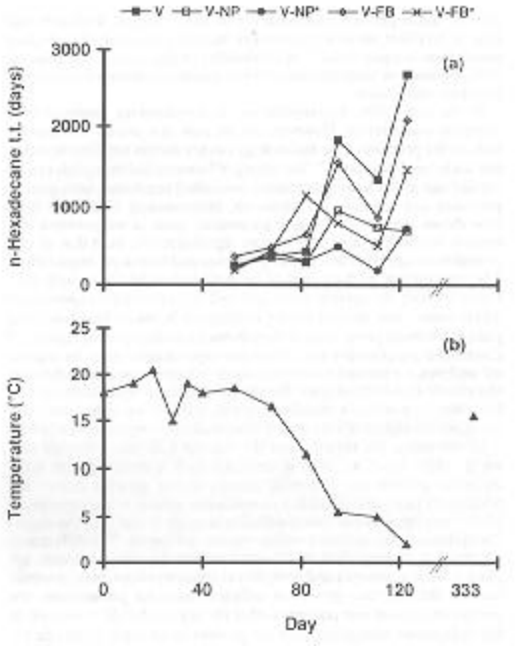
While microbiological measurements suggested that fertilizers stimulated the growth of hydrocarbon degraders, conclusive evidence of enhanced oil biodegradation from nutrient treatment was determined from the results of gas chromatographic analyses. Changes in the ratio of *n*-heptadecane to pristane were used as an indicator of biodegradation based on the assumption that the branched alkane (pristane) was slower to biodegrade than *n*-heptadecane. While there has been some concern in recent studies that these compounds may degrade at similar rates,[4,19] our use of the *n*-heptadecane:pristane ratio as a biodegradation indicator has been validated by GC/MS analysis quantifying the conserved internal biomarker 17A(H),21B(H)-hopane within the residual oils of our experimental enclosures. Observations of the ratios of *n*-heptadecane to pristane confirmed that the differences between the treated enclosures were attributable to biodegradation and not bulk oil loss associated with physical dispersion (Figure 6). Changes in the composition and concentration of the *n*-alkane fraction of the residual oil (n-C_{12} to n-C_{35}) illustrated the effect of nutrient treatment on biodegradation (Figure 7). The results on day 123 and day 333 showed that periodic additions of the fish bonemeal had a negative effect as an oil bioremediation agent. A single addition of the fish bonemeal had no effect during the experimental period. In contrast, the application of the inorganic fertilizer mixture significantly enhanced the biodegradation rates of aliphatic components up to n-C_{26}. Periodic applications of the inorganic fertilizer failed to offer substantial improvement, suggesting that optimal

nutrient concentrations were achieved and maintained during the study period by a single application of the inorganic fertilizer.

Discussion

The objective of this experiment was to assess the ability of inorganic and organic fertilizers to provide sufficient assimilable nitrogen and phosphorus to promote the growth and activity of indigenous oil-degrading microorganisms. Previous studies have demonstrated the effectiveness of inorganic fertilizer additions to stimulate the biodegradation rates of oil stranded within sandy beach sediments.[12,14,15] In comparison to such inorganic nutrients, the organic fertilizer used in this study may offer additional benefits due to its slow nutrient release characteristics, its ability to provide trace elements and other growth factors, and greater public acceptability for a natural product.

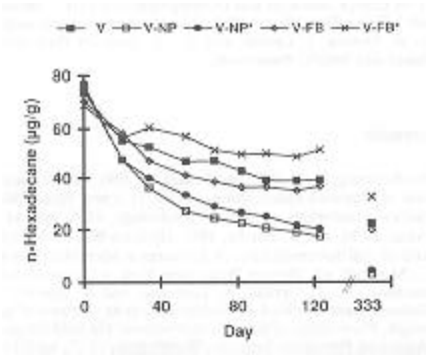
Application of the fish bonemeal to the oiled enclosures stimulated the highest rates of microbial growth and activity. However, the turnover time for hexadecane was reduced to a greater extent (higher rates of degradation) by both single and periodic additions of the inorganic fertilizers (ammonium nitrate and super triplephosphate). Since microbiological measurements derived from the uptake and mineralization rates of specific radio-labeled substrates only provide an index of bacterial activity and not a measure of oil degradation,[12] bioremediation activity was subsequently confirmed by detailed chemical analysis. In the current study, evidence of bioremediation in the enclosures treated with the inorganic fertilizers included chemical changes in the ratio of *n*-heptadecane to pristane, and the distribution and concentration of the aliphatic hydrocarbons. No difference was observed in the *n*-heptadecane:pristane ratio between the oiled control and organic fertilizer treated enclosures. Observed alterations in the composition of the aliphatic hydrocarbons suggested that periodic additions of the fish bonemeal actually suppressed oil degradation.



4.

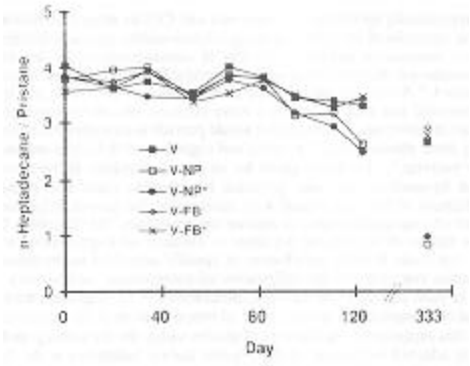
*Effect of nutrient treatment on the turnover time for hexadecane in oiled sediment and seasonal temperature data for the intertidal overlying waters at the study site - V = Venture condensate 3% (w/v); N = Ammonium nitrate 33-0-0 (N:P:K); P = triple superphosphate 0-46-0 (N:P:K); FB = fish bonemeal 6-10-1 (N:P:K); * = periodic applications*

The results obtained with the application of organic fertilizers in the present study were not expected. Previous bioremediation field studies have demonstrated the advantage to adding the oleophilic nutrient Inipol EAP 22, a microemulsion containing organic nitrogen (urea) and phosphorus [tri(Laureth-4)-phosphate] encapsulated within oleic acid, as a starter to induce rapid microbial growth.[10,25] Urea (H_2N-CO-NH_2), has been applied routinely to agricultural soils where it is enzymatically hydrolyzed to ammonia and CO_2 by microbial activity. The operational advantage of using sulphur-coated urea as a fertilizer over ammonium nitrate (at similar N concentrations) for the bioremediation of oil stranded within intertidal beaches has been demonstrated.[15] At similar nitrogen and phosphorus concentrations, the fish bonemeal was expected to be a more effective bioremediation agent than the inorganic fertilizers as it would provide macronutrients including trace elements (such as iron) and organic growth factors required by bacteria.[6,19] Further support for an organic fertilizer derived from fish by-products was also provided by a recent study that proved additions of fish and animal meal accelerated the growth of bacteria and oil-degradation rates in marine environments.[3] Explanations for the failure of the organic fertilizer to enhance oil degradation rates in our study include enrichment of specific microbial communities; diauxic responses of the indigenous microorganisms, and toxicity.



5.

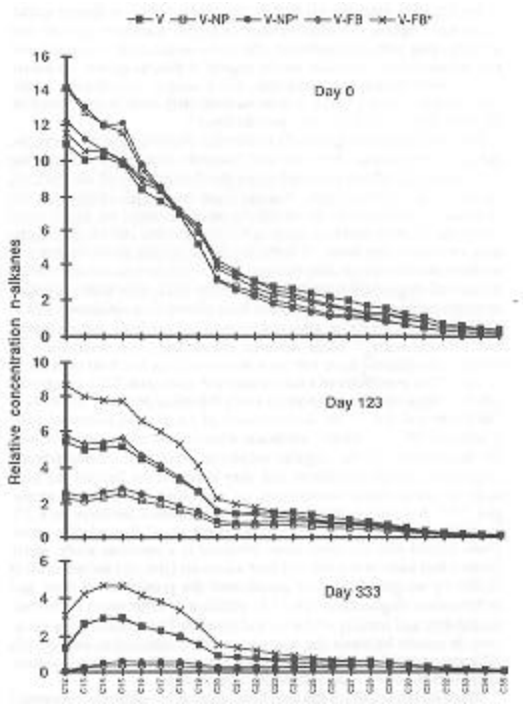
*Change in the n-hexadecane in response to nutrient enrichment - V = Venture condensate 3% (w/v); N = Ammonium nitrate 33-0-0 (N:P:K); P = triple superphosphate 0-46-0 (N:P:K); FB = fish bonemeal 6-10-1 (N:P:K); * = periodic applications*



6.

*Change in the n-heptadecane/pristane ratio in response to nutrient enrichment - V = Venture condensate 3% (w/v); N = Ammonium nitrate 33-0-0 (N:P:K); P = triple superphosphate 0-46-0 (N:P:K); FB = fish bonemeal 6-10-1 (N:P:K); * = periodic applications*

In most aquatic environments, enrichments of oil-degrading microbial communities occur soon after oil contamination of the shorelines. In this experiment, the increase of species within the microbial population adapted to the use of the organic carbon substrates in the fish bonemeal may have been so dramatic (as indicated by anoxia in the enclosures fertilized periodically) that other microorganisms, including the oil-degraders, did not have a chance to get established. As a result, similar or slower rates of oil degradation were observed following fish bonemeal additions. Unfortunately, this hypothesis was not validated by taxonomic studies of the bacterial populations within the nutrient-treated enclosures. To counter this problem caused by rapid development of heterotrophic bacterial populations without oil-degrading capabilities, a recent study has suggested a novel approach based on the use of a bioremediation agent composed of a polymerized-urea fertilizer together with oil-degrading bacterial strains already adapted to the nutrient formulation.[22]



7.

*Time-series change in the relative concentration of n-alkanes (numbered by chain length) in response to nutrient treatment - V = Venture condensate 3% (w/v); N = Ammonium nitrate 33-0-0 (N:P:K); P = triple superphosphate 0-46-0 (N:P:K); FB = fish bonemeal 6-10-1 (N:P:K); * = periodic applications*

Preferential use of components within the organic fertilizer by indigenous bacteria over the oil within the experimental sediment enclosures may explain the observations of higher microbial growth and activity rates without significant effects on residual oil concentrations and composition. Examples on the impact of diauxic growth on microbial oil degradation are numerous. For example, hexadecane biodegradation has been found not to occur until fatty acids in the oleophilic nutrient Inipol EAP 22 were metabolized.[21]

Bioremediation has generally received a positive response from the public. Nevertheless, there are still concerns about potential adverse environmental effects resulting from the fertilization of oil-contaminated marine environments. Among these are the possibility that the addition of fertilizers of metabolic by-products from oil degradation could cause eutrophication, leading to algal blooms and oxygen depletion, or that components of fertilizer formulations could be toxic to sensitive marine species and humans.[8,9,27] A primary factor controlling natural oil-degradation rates is the ratio of interstitial water nitrogen concentrations to oil.[4] Field studies have shown that optimum rates of oil degradation could be achieved by maintaining high, but nontoxic, levels of nutrients.[14] While adverse effects from bioremediation by nutrient enrichment have not been observed in actual field operations to date,[19] the possibility of a future incident does exist. Oil biodegradation was suppressed in the present study following periodic additions of fish bonemeal due to the development of anoxic and potential toxic

conditions (that is, excess ammonia concentrations) associated with the degradation of the organic substrate. Microbial oil-degradation rates under anoxic conditions are very slow and far beyond the time scale of biotechnical restoration or microbial remediation strategies.[1,14,17] A single application of the fish bonemeal fertilizer (at 6.7% by weight of the condensate) had no effect on oil degradation rates. These results differed from those obtained in a previous study, which showed that natural organic nutrient additions (fish and animal meal at 10.0% by weight of the oil) accelerated the growth of bacteria and hydrocarbon degradation rates.[3] In addition to differences in the bioavailability and toxicity of the oil and nutrient formulations, the variations in results between the studies may be attributed to site-specific differences in tidal exchange rates, sediment grain size, temperature, oxygen availability, and microflora composition.

Based on the results of the gas chromatographic analyses it appeared that a single application of the inorganic fertilizer sustained optimal oil-degradation rates. While the nutrient concentrations within the interstitial water of the in-situ sediment enclosures were unknown, the nutrient release experiments showed the rapid lost of nitrate from the enclosures. This is consistent with the high water-solubility values for nitrate. Since periodic reapplication of the inorganic fertilizers had little or no effect, we now hypothesize that the microbial oil-degrading consortium became limited by availability of degradable components in the oil and low temperatures after its initial stimulation by inorganic fertilizer application.

In the late 1980s, bioremediation as a technology received wide attention and interest. However, we are now in a period of reevaluation, as the promise of the technology has not always been borne out in use under real situations.[8] The testing of bioremediation agents can be carried out in the laboratory under controlled conditions with greater precision and accuracy.[11,28] However, experimental field trials have now shown that environmental parameters such as temperature and oxygen availability may play a more significant role than that of bioremediation agents in determining the eventual rate of oil degradation. The inconsistent performance of bioremediation has been well illustrated by both the current study and spill-of-opportunity experiments where some sites showed strong differences between bioremediated plots and control plots, while others showed less distinct differences.[8,19] Controlled experiments using replicate experimental plots for statistical analyses are needed to advance the development and acceptance of bioremediation technologies. For the production of operational guidelines based on sound scientific decisions, there is an immediate need for experimental protocols to test bioremediation agents in the field.[16]

In summary, the results from the current field study showed that while additions of an organic fertilizer (fish bonemeal) stimulated microbial growth and metabolic activity to the greatest extent, the addition of inorganic fertilizers (ammonium nitrate, triple superphosphate) was the superior bioremediation strategy to enhance the degradation rates of oil stranded within coastal sediments. The differences observed in bioremediation efficacy between the two fertilizers, applied at similar nitrogen and phosphorus concentrations, may be attributed to the selective growth of different bacterial populations, the preferential use of components within the organic fertilizer over oil by the indigenous microflora, and the production of toxic metabolic by-products from the degradation of the organic fertilizer. These results and our previous studies have demonstrated that each polluted environment

(including the same site at different times) is likely to respond to bioremediation treatment differently.[12,13,15] Successful bioremediation programs will require application methodologies (form and type of fertilizer, type and frequency of application) specifically tailored to the environmental parameters (including oil characteristics) at each contaminated site. While bioremediation is the most environmentally friendly of the oil spill countermeasures under development, it should not be used without adequate knowledge of the product and the environment in which it is to be employed.

Acknowledgments

We are grateful for the financial support from the Interdepartmental Panel on Energy Research and Development (PERD), Canada, and Canada's Green Plan. We would also like to express our gratitude to D. Guay, R. Dumas, J. Lavoie, and D. A. Steel for their technical assistance and helpful discussions.

References

1. Aeckersberg, F., F. Bak, and F. Widdel, 1991. Anaerobic oxidation of saturated hydrocarbons to CO_2 by a new type of sulfate-reducing bacterium. *Archives Microbiology*, v156, pp5 - 14

2. Atlas, R. M. and R. Bartha, 1992. Hydrocarbon biodegradation and oil spill bioremediation. *in* Advances in Microbial Ecology, K. C. Marshall, ed. Plenum Press, New York, v12, pp287 - 338

3. Basseres, A., P. Eyraud, A. Ladousse, and B. Tramier, 1993. Enhancement of spilled oil biodegradation by nutrients of natural origin. *Proceedings of the 1993 International Oil Spill Conference*, American Petroleum Institute, Washington, D.C., pp495 - 501

4. Bragg, J. R., R. C. Prince, E. J. Harner, and R. M. Atlas, 1994. Effectiveness of bioremediation for the *Exxon Valdez* oil spill. *Nature*, v368, pp413 - 418

5. Caparello, D. M. and P. A. LaRock, 1975. A radioisotope assay for the quantification of hydrocarbon potential in environmental samples. *Microbial Ecology*, v2, pp28 - 42

6. Dibble, J. T. and R. Bartha, 1976. Effect of iron on the biodegradation of petroleum in sea water. *Applied Environmental Microbiology*, v31, pp544 - 550

7. Hansen, H. P. and K. Grasshoff, 1983. Automated chemical analysis. *in* Methods of Seawater Analysis, K. Grasshoff, M. Ehrhardt, and K. Kremling, eds. Verlag Chemie, Weinheim, pp347 - 379

8. Hoff, R., 1993. Bioremediation: an overview of its development and use for oil spill cleanup. *Marine Pollution Bulletin*, v26, n9, pp476 - 481

9. Holloway, M., 1991. Soiled shores. *Scientific American*, October 1991, pp103 - 116

10. Ladousse, A. and B. Tramier, 1991. Results of 12 years of research in spilled oil remediation Inipol EAP 22. *Proceedings of the 1991 International Oil Spill Conference*, American Petroleum Institute, Washington,

D.C., pp577 - 581

11. Lee, K. and E. M. Levy, 1987. Enhanced biodegradation of a light crude oil in sandy beaches. *Proceedings of the 1987 Oil Spill Conference*, American Petroleum Institute, Washington, D.C., pp411 - 416

12. Lee, K. and E. M. Levy, 1989. Biodegradation of petroleum in the marine environment and its enhancement. *in* Aquatic Toxicology and Water Quality Management, J. O. Nriagu and J. S. S. Lakshminarayana, eds. John Wiley and Sons, Inc., New York, pp217 - 243

13. Lee, K. and E. M. Levy, 1989. Enhancement of the natural biodegradation of condensate and crude oils on beaches of Atlantic Canada. *Proceedings of the 1989 Oil Spill Conference*, American Petroleum Institute, Washington, D.C., pp479 - 486

14. Lee, K. and E. M. Levy, 1991. Bioremediation: waxy crude oils stranded on low-energy shorelines. *Proceedings of the 1991 International Oil Spill Conference*, American Petroleum Institute, Washington, D.C., pp541 - 547

15. Lee, K., Tremblay, G. H., and E. M. Levy, 1993. Bioremediation: application of slow-release fertilizers on low-energy shorelines. *Proceedings of the 1993 International Oil Spill Conference*, American Petroleum Institute, Washington, D.C., pp449 - 454

16. Merlin, F. X., K. Lee, R. P. J. Swannell, J. Oudot, A. Basseres, T. Reilly, C. Chaumery, C. Dalmazzone, and P. Sveum, 1994. Protocol for experimental assessment of bioremediation agents on a petroleum polluted shoreline. *Proceedings of the 17th Arctic and Marine Oil Spill Program (AMOP) Technical Seminar*, Environment Canada. Ottawa, Ontario, pp465 - 478

17. Michaelsen, M., R. Hulsch, T. Hopner, and L. Berthe-Corti, 1992. Hexadecane mineralization in oxygen-controlled sediment-seawater cultivations with autochthonous microorganisms. *Applied and Environmental Microbiology*, v58, pp3072 - 3077

18. National Academy of Sciences, 1985. Oil in the Sea: Inputs, Fates, and Effects. National Academy Press, Washington, D.C., pp270 - 368

19. Prince, R. C., 1993. Petroleum spill bioremediation in marine environments. *Critical Reviews in Microbiology*, v19, n4, pp217 - 242

20. Pritchard, P. H. and C. F. Costa, 1991. EPA's Alaska oil spill bioremediation project. *Environmental Science and Technology*, v25, pp372 - 379

21. Rivet, L., G. Mille, A. Basseres, A. Ladousse, C. Gerin, M. Acquaviva, and J.-C. Bertrand, 1993. *N*-alkane biodegradation by a marine bacterium in the presence of an oleophilic nutriment. *Biotechnology Letters*, v15, pp637 - 640

22. Rosenberg, E., R. Legmann, A. Kushmaro, R. Taube, E. Adler, and E. Z. Ron, 1992. Petroleum bioremediation - a multiphase problem. *Biodegradation*, v3, pp337 - 350

23. Shearer, K. D., A. Maage, J. Opstvedt, and H. Mundheim, 1992. Effects of high-ash diets on growth, feed

efficiency, and zinc status of juvenile Atlantic salmon (*Salmo salar*). *Aquaculture*, v106, pp345 - 355

24. Strain, P. M., 1986. The persistence and mobility of a light crude oil in a sandy beach. *Marine Environmental Research*, v19, pp49 - 76

25. Sveum, P. and A. Ladousse, 1989. Biodegradation of oil in the Arctic: enhancement by oil-soluble fertilizer application. *Proceedings of the 1989 International Oil Spill Conference*, American Petroleum Institute, Washington, D.C., pp439 - 446

26. Swannell, R. P. J. and I. M. Head, 1994. Bioremediation comes of age. *Nature*, v368, pp396 - 397

27. U.S. Congress, Office of Technology Assessment, 1991. Bioremediation for Marine Oil Spills - Background Paper OTA-BP-O-70. U.S. Government Printing Office, Washington, D.C., May, 1991. 31pp

28. Venosa, A. D., M. Kadkhodayan, D. W. King, B. A. Wrenn, J. R. Haines, T. Herrington, K. Strohmeier, and M. T. Suidan, 1993. Testing the efficacy of oil spill bioremediation products. *Proceedings of the 1993 International Oil Spill Conference*, American Petroleum Institute, Washington, D.C., pp487 - 493

29. Walker, J. D. and R. R. Colwell, 1976. Measuring potential activity of hydrocarbon degrading bacteria. *Applied and Environmental Microbiology*, v31, pp189 - 197

Author

Kenneth Lee is a Research Scientist for the Marine Environmental Sciences Branch at the Maurice Lamontagne Institute, Department of Fisheries and Oceans, Canada. He is responsible for research programs in chemical oceanography that deal with the microbial transformation of contaminants in estuarine and coastal environments.

Table 1. Composition of organic fertilizer (percent)

Total N	7.2
Available phosphoric acid (P_2O_5)	12.6
Soluble potash (K_2O)	1.1
Calcium (Ca)	11.2
Minimum organic matter	50

Table 2. Experimental treatment of in-situ sediment enclosures

	Fertilizer (mg/Lwet sediment)			Application
Enclosure	N	P	FB	Schedule
C	-	-	-	-
C - NP	316	373	-	single
C - NP	316	373	-	periodic
C - FB	-	-	1731	single
C - FB	-	-	1731	periodic

V	-	-	-	-
V - NP	316	373	-	single
V - NP	316	373	-	periodic
V - FB	-	-	1731	single
V - FB	-	-	1731	periodic

C = unoiled control
V = Venture condensate 3% (w/v)
N = Ammonium nitrate 33-0-0 (N:P:K)
P = Triple superphosphate 0-46-0 (N:P:K)
FB = Fish bonemeal 6-10-1 (N:P:K)