

# FIELD-TESTING BIOREMEDIATION TREATING AGENTS: LESSONS FROM AN EXPERIMENTAL SHORELINE OIL SPILL

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**ABSTRACT:** A 14-week, large-scale field study in Delaware demonstrated that, on a moderately exposed sandy beach, nutrients, not oil-degrading microorganisms, were the primary factors limiting biodegradation. The results are reviewed in the context of lessons and guidelines for a full-scale bioremediation response.

Bioremediation is “the act of adding materials to contaminated environments to cause an acceleration of the natural biodegradation processes” (U.S. Congress Office of Technology Assessment, 1991). There is considerable controversy about the effectiveness and effects of bioremediating oil stranded on coastal shorelines. With notable exceptions, laboratory and bench-top screening studies clearly demonstrate that the addition of nutrients—and, in some cases, oil-degrading microorganisms—enhances the rates at which components of oil are degraded or lost (Venosa *et al.*, 1991; Swannell *et al.*, 1996). In confined situations (terrestrial sites), various combinations of tilling, nutrient addition, and possibly inoculation of degrading organisms have enhanced the degradation of petroleum hydrocarbons. However, successful demonstrations of the bioremediation of oil stranded on shorelines have been elusive and controversial, in part because pilot and demonstration projects have been poorly designed or controlled and in part because trials at “spills of opportunity” are fraught with unpredictability (Swannell *et al.*, 1996).

To overcome these problems and to resolve uncertainties about the effectiveness of nutrients versus microbial inoculation, the U.S. Environmental Protection Agency’s National Risk Management Research Laboratory funded and conducted a full-scale field experiment on a sandy beach in Delaware during the summer and fall of 1994. The field experiment provided not only an opportunity to compare the effectiveness of several treatment strategies but also the opportunity to experience the logistical challenges and constraints of a full-scale operational response and monitoring program. In addition, it offered an opportunity to evaluate the safety and effects of oiling and treatment on marine resources.

Although the results of several parts of the study have been published in the scientific literature (Venosa *et al.*, 1996; Mearns *et al.*, 1995; Wrenn *et al.*, 1995), they have not yet been put in the context of an oil spill response. This paper summarizes the methods and results of that study, provides some new operational guidelines for bioremediating an oiled sand beach, and offers suggestions for future field tests of these and other oil spill-treating agents. For a full account of the Delaware study, the reader is urged to consult the scientific papers (Venosa *et al.*, 1996; Mearns *et al.*, 1995; Venosa *et al.*, 1995; Swannell *et al.*, 1996).

## The Delaware experiment: Synopsis

**Objectives, strategy, and methods.** The field study was initiated on July 1, 1994, at Fowler Beach, a medium- to coarse-grained sand beach (environmental sensitivity index = 4) 1 mile south of Slaughter Beach, Delaware Bay, Delaware. The objective was to obtain credible statistical evidence to determine if bioremediation with inorganic mineral nutrients and/or microbial inoculation enhances the removal of crude oil, to compute intrinsic (natural) and enhanced biodegradation rates, and to document toxicity trends.

A randomized complete block design was used to assess treatment effectiveness and effects. Twenty 4 × 9 m (36-m<sup>2</sup>) plots were laid out in five blocks each containing one of four treatments in random order. The four treatments evaluated were a no oil control; a no nutrient addition oiled control; the addition of water-soluble nutrients to oiled plots; and the addition of water-soluble nutrients to the oil plots supplemented with a natural microbial inoculum from the site. Thus there were five replicate plots per treatment. The loss of surface oil from each of the oiled plots was controlled using skirted boom. The longest dimension of the plot (9 m) was placed perpendicular to the shore; it thus covered the intertidal region from low (more than 11 hr/d submersion) to high (less than 2 hr/d submersion) intertidal zones.

To monitor oil release, caged oysters were placed several meters seaward of each block and at several points up-coast and down-coast of the study zone.

Crude oil was intentionally released onto 15 plots on July 1, 1994. Each oiled plot was evenly sprayed with 136 L of weathered Nigerian Bonny Light (API gravity 35.3) to achieve a nominal concentration of 5000 mg/kg dw total oil in the upper 20 cm. Beginning on July 5, 1994, commercial lawn sprinklers were used to deliver water, nutrients, and/or inocula (oil-degrading bacteria) to each plot during one low tide each day. Elevated sediment interstitial water nitrate concentrations were maintained at average levels up to 8 times (range about 2 to 10) background (0.8 mg nitrate-N/L) by daily treatment with 2 kg of technical grade sodium nitrate. Also included in the daily nutrient treatment was 128 g of sodium tripolyphosphate (not monitored). The total treatment for each plot was 196 kg of sodium nitrate and 12.4 kg of tripolyphosphate.

Beginning on July 5, 1994, sediment and/or pore water in each plot was monitored biweekly or more frequently for several chemical and microbial end points (Venosa *et al.*, 1996):

Pore water:

Nitrate-nitrogen

Sediment cores:

Dichloromethane (DCM) extractable organic material (EOM)

Individual analytes, including:

28 alkanes (C10 to C36) plus pristane and phytane

27 polycyclic aromatic hydrocarbons (PAHs)

Hopane, a nonbiodegradable biomarker

Most probable number (MPN) of alkane-degrading bacteria (Wrenn and Venosa, 1996)

Most probable number (MPN) of PAH-degrading bacteria (Wrenn and Venosa, 1996)

Sediment and pore water in each plot were also sampled at 6-week intervals for several toxicity tests (Mearns *et al.*, 1995):

Pore water acute tests:

Sea urchin embryo bioassay

Microtox bioassay

Sediment acute test:

Elutriate Microtox bioassay

Sediment chronic test:

10-day amphipod survival

In addition, pore water was also sampled from two plots of each treatment at weeks 0, 1, 2, 4, 6, 8, 12, and 14 for chronic toxicity to grass shrimp embryos.

Biodegradation was tracked by GC/MS analysis of selected components in composited paired sediment cores taken randomly in each of four intertidal zones from each plot, and the measured concentrations were corrected for abiotic removal by normalizing to the non-biodegradable biomarker hopane. Plots were monitored for up to 14 weeks for changes in petroleum hydrocarbons, nutrients, oil-degrading bacteria, and toxicity.

Several additional auxiliary studies were done. To estimate maximum degradation rates, a laboratory study was done in closed laboratory flasks. To document the loss of oil from the study area and to determine how the overall oiling may have impacted offshore resources, oysters were placed in cages north, south, and offshore of the study area and sampled four times during the first month for oil contamination (PAHs).

## Results

The primary data are summarized on a common time scale in Figure 1. In brief: nominal initial oil concentrations (about 5000 mg/kg as total extractable organic matter [EOM, a rough measure of total petroleum]) were achieved in each plot; oil concentrations decreased exponentially over the next 14 weeks due to both physical processes (evaporation and dissolution by tidal immersion and wave action) and to biodegradation; the addition of oil-degrading microbes was ineffective beyond the addition of nutrients; and treatments neither enhanced nor depressed the toxicity of the oil. Measurement of hopane, a conservative biomarker, was the key factor in successfully separating physical removal (washout) from removal due to biodegradation. Following are the essential results.

Treatments increased the rate of degradation of alkanes and PAHs. The hopane half-life (half the remaining hopane) was 28 days with or

without any treatment; the half-life as measured by EOM was 20 days with or without treatment (see Figure 1A and B). The hopane reduction was due to nonbiological processes (tidal immersion, wave action, etc.), and the EOM reduction was caused by both physical and biodegradation processes: the difference is attributable to biodegradation, but significant differences due to treatment could not be resolved. Daily treatment with dissolved nutrients significantly accelerated natural degradation of alkanes twofold (from a half-life of about 28 days to about 14 days; see Figure 1C) and significantly increased the rate of PAH degradation by about 50% (see Figure 1D). Enhanced degradation of alkanes was apparent by day 14 and of PAHs by day 28. Addition of oil-degrading bacteria did not accelerate degradation.

Regardless of treatment, concentrations of alkane- and aromatic-degrading bacteria were significantly higher, by at least two orders of magnitude, in oiled plots than in unoiled plots throughout the course of the experiment (see Figure 1E and F). Bacterial concentrations in treated plots were always slightly greater (half-log) than in untreated oiled plots, although this difference was not statistically significant ( $p > 0.05$ ).

Treatments significantly enhanced the biodegradation of alkanes and PAHs in the uppermost intertidal zone (submerged 0.5 hr/day; data not shown; see Venosa *et al.*, 1996). Although trends suggest that treatment also enhanced degradation in middle and low elevations (submerged 2 to 11 hr/d), the differences were not significant.

The biodegradation rates observed in the field were about one-tenth those in comparable laboratory experiments (Venosa *et al.*, 1996). Comparable rates were not achieved because of the physical washout of the degrading microbial populations due to breaking waves and tidal flushing. However, the ratios of the degradation rates of alkyl-substituted and unsubstituted PAHs were consistent between the field and the lab, indicating that the hopane-normalized losses measured in the field were due to biodegradation, not washout.

Acute toxicity of beach pore water was neither statistically enhanced nor reduced by treatment type. Sea urchin egg fertilization (see Figure 1G) was the least sensitive test, showing slight initial toxicity to the oil and then no effect. Using Microtox, pore water samples from all oiled plots were clearly acutely toxic during the first week, but the toxicity decreased to 0 by day 42 (see Figure 1H).

Pore water chronic toxicity, as measured frequently by a 14-day grass shrimp embryo hatching success test, was also neither statistically enhanced nor reduced by treatment type; however, since only two plots from each treatment were monitored, there was high variance (see Figure 1I). One bacteria-treated plot continued to show chronic toxicity for 60 days, thus contributing to the high variability.

The direct contact sediment bioassays were more sensitive than the pore water bioassays. Regardless of treatment, sediments remained toxic to the Microtox test and to benthic amphipods well beyond day 42 (see Figure 1J and K). Despite substantial oil loss and biodegradation, sediments remained chronically toxic to amphipods for 14 weeks. However, because of poor control and reference sediment survival (triangle and square symbols; see Figure 1K), amphipod data are somewhat suspect.

The study resulted in the release of oil that contaminated nearby oysters for several weeks (see Figure 1L). Background tissue concentrations of total PAHs were well below 10  $\mu\text{g}/\text{kg}$  wet weight (ww). Concentrations in oysters placed immediately adjacent to oiled plots increased within a few hours to an average of about 120  $\mu\text{g}/\text{kg}$  ww and then dropped quickly to about 75  $\mu\text{g}/\text{kg}$  ww the next day. From this point concentrations slowly decreased to about 20  $\mu\text{g}/\text{kg}$  ww over the next 2 weeks. However, oysters placed north and south of the study zone were contaminated to about half these levels only for a few days and lost their contamination quickly.

In summary, this field experiment demonstrated that oil was lost naturally because of both physical and chemical processes and biodegradation, that degradation of oil alkanes and PAHs in upper intertidal sandy sediments could be enhanced with the continuous addition of dissolved nutrients, that treatment with oil-degrading bacteria provided no additional benefit, and that treatment neither enhanced nor reduced the toxicity of the oil.

## Implications for responses on sand beaches

Oil can penetrate 10 to 25 cm on medium- to coarse-grained sand beaches; light oils penetrate more deeply than heavy oils, and there is the potential for deeper burial as clean sediments are deposited on top

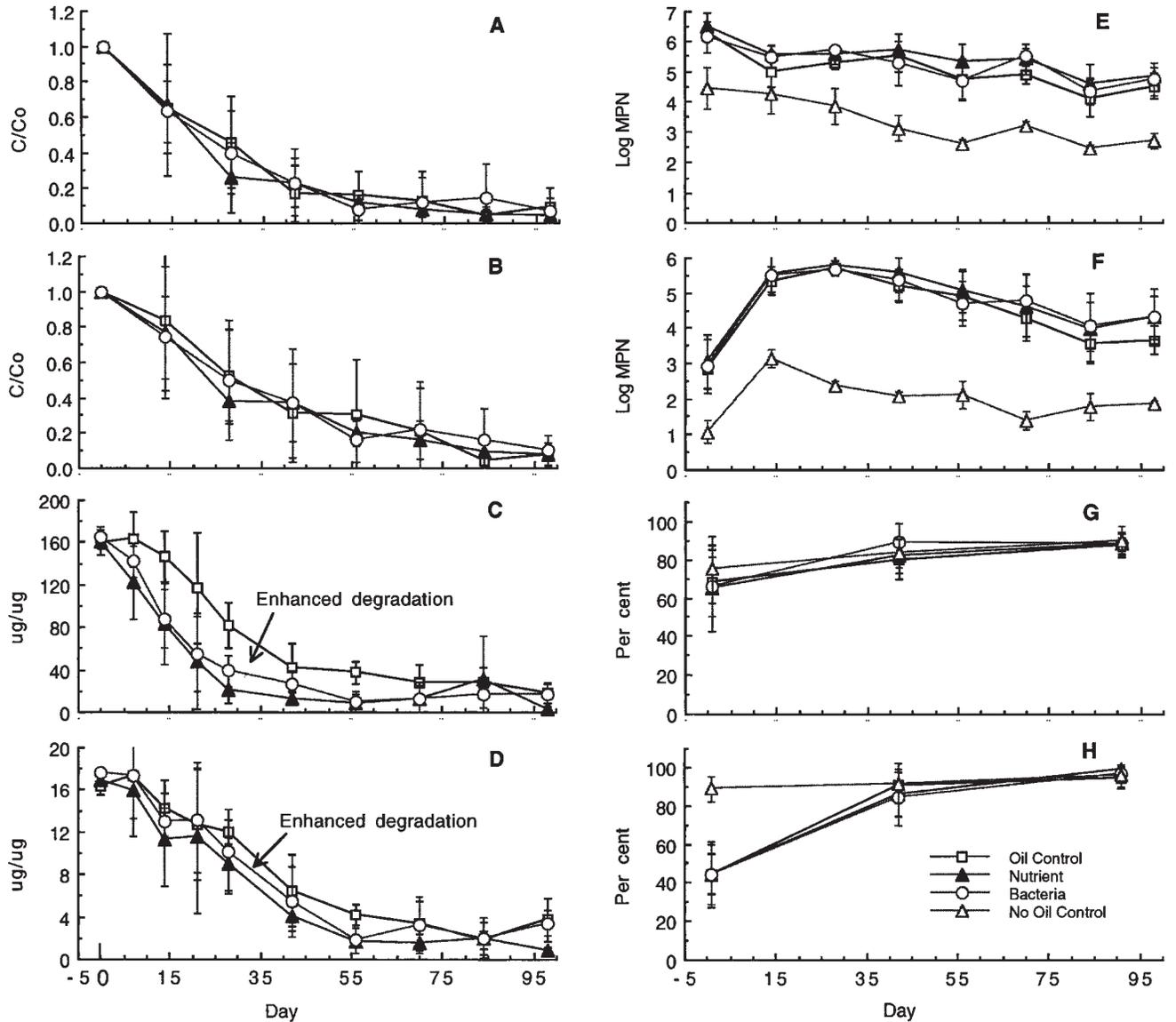


Figure 1. Comparisons on a common time scale (100 days, about 14 weeks) of mean ( $\pm 1$  sd) of sediment chemical, microbial, and toxicological conditions in replicate ( $n = 5$ ) plots treated with weathered Bonny Light Nigerian crude oil (oil control, *open squares*), oil plus continual application of dissolved nutrients (nutrient, *closed triangles*), oil treated with both dissolved nutrients and cultured bacteria (bacteria, *open circles*), and no oil and no treatment (no oil control, *open triangles*) on a sandy beach in Delaware, July–October 1994. There were no significant differences among the three oiled treatments for loss rates of extractable organic material (EOM, a rough measure of TPH, total petroleum hydrocarbons) (A) or hopane, a recalcitrant (nondegradable) hydrocarbon (B). All measurements of EOM and hopane are referenced to initial concentrations ( $C/C_0$ ;  $C_0$  about 5000 mg/kg dw for EOM and about 200  $\mu\text{g}/\text{kg}$  dw for hopane; background EOM was about 10 to 80 mg/kg dw and background hopane was about 0.007  $\mu\text{g}/\text{kg}$  dw). However, relative to untreated oil controls, analyte-to-hopane ratios demonstrated that nutrient and nutrient + bacteria applications equally doubled the loss rates of C<sub>10</sub>–C<sub>35</sub> alkanes (C) and increased by 20% to 50% the loss rates of the total of 27 2–4-ring polycyclic aromatic hydrocarbons (D). Concentrations of sediment alkane-degrading bacteria (E) and PAH-degrading bacteria (F) increased rapidly in response to oil exposure and then slowly declined. Relative to unoiled controls, beach pore water samples taken at 42-day (6-week) intervals from oiled plots slightly inhibited sea urchin egg fertilization initially (G) but significantly depressed light production of photoluminescent *Microtox* bacteria (H) and hatchability of grass shrimp embryos (I,  $n = 2$  per treatment per sampling event); toxicity was lost at different rates but with no significant difference among oil treatment types. Bulk sediment elutriate was initially highly toxic to *Microtox* organisms (J), but toxicity declined. Bulk sediment from oiled plots also highly depressed 10-day survival of benthic amphipods (K) throughout the entire monitoring period, and there was some toxicity in the unoiled control plots themselves. Oil lost from the 1-km beach study zone was initially accumulated (as PAHs) in oysters placed in cages several meters seaward of the study blocks (central), upstream of the study site (south), and downstream of the study site (north)(L); in all cases depuration was nearly complete in 28 days. (*continued on next page*)

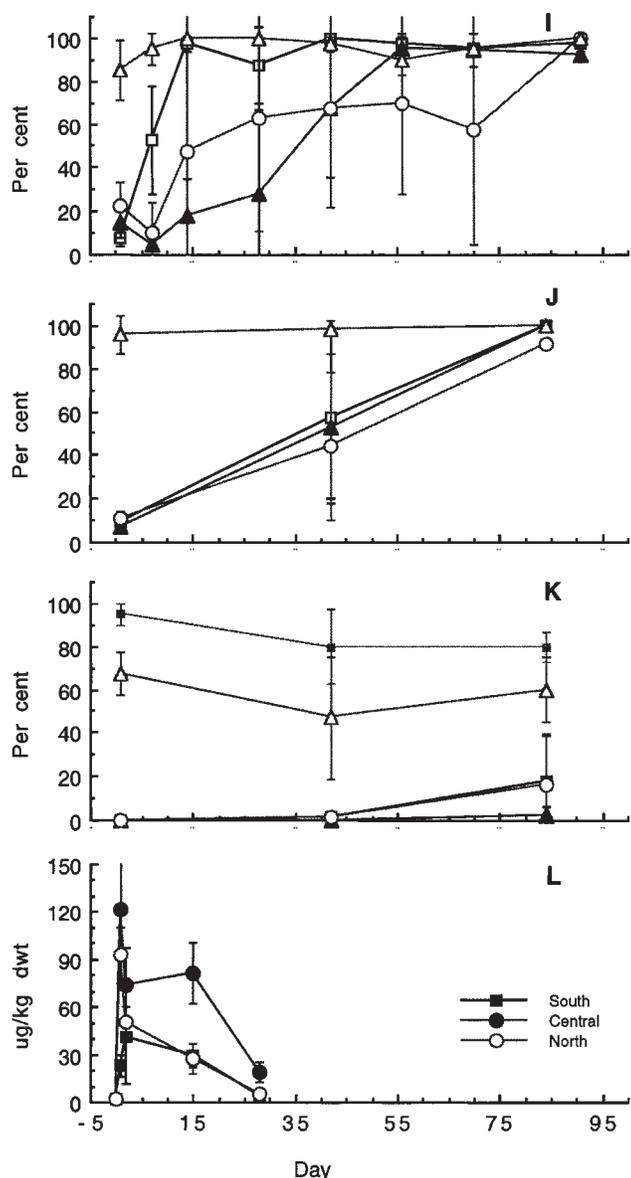


Figure 1. (continued)

of the oiled layer. Resources at risk include intertidal invertebrates that provide food for resident and especially migratory shorebirds, benthic fish that feed in the intertidal zone, commercial fish (capelin) that breed in the intertidal zone, and, in subtropical and tropical areas, turtle eggs. Once oil is stranded on a shoreline, there several response options: (1) take no action—allow natural attenuation to proceed; (2) attempt to remove, treat, and replace some or all of the oiled sand; or (3) treat the oil in place to reduce its impact or accelerate natural removal. The second option involves the use of heavy equipment, generates considerable traffic, and may significantly alter beach accretion and erosion processes. Thus, if it is appropriate for the site, bioremediation is a desired, but longer-term, option.

The decision to bioremediate a sandy shoreline should be made in light of the specific situation and in the context of other treatment and removal options. In our experience, the steps in a sandy shore oil spill bioremediation response action should include the following:

1. Pretreatment assessment
2. Treatment planning and monitoring

3. Acquisition and deployment of personnel and resources
4. Termination of treatment

It should be noted that these steps can be evaluated for many regions long before a spill event, not just during it.

**Pretreatment assessment.** The purpose of a pretreatment assessment is to determine whether or not bioremediation is a viable response alternative. A primary question is, How soon must the oil be removed or degraded? If a quick response is needed (within several days), in-situ bioremediation is not an option. Because of initial oil toxicity (Lee and Levy, 1989), bioremediation has a several-day "start-up" time (Lee and Levy, 1989; Venosa *et al.*, 1992) and, based on field work in Delaware (Venosa *et al.*, 1996), can reduce the half-life of degradable oil components from many weeks to about several weeks, depending of the levels of background nutrients.

If these limitations are acceptable, the next question is, What factors are going to limit natural degradation? These factors include: (1) the thickness of the surface oil; (2) exposure conditions (wave action, tidal immersion); and (3) the availability of nutrients, degrading microorganisms, and oxygen.

In the Delaware study the light oil easily penetrated into the sandy sediments: we did not have to deal with thick surface deposits of tar, tar balls, or asphalt, which would not easily degrade and which would interfere with degradation of the oil in the sand. In a real response, thick deposits of bulk oil should be removed prior to bioremediation treatment.

The Delaware study site beach was moderately energetic, exposed to two equal daily tides and to wind waves (up to 2 feet) and swell. These were the primary factors causing physical removal of bulk oil from the beach. Thus it is possible that less energetic shorelines, such as recently studied by Lee *et al.* (1997, these proceedings), would benefit considerably more.

In most beaches contaminated with crude oil, the primary limiting factor will be low nutrient supply. Usually the abundance of degrading microorganisms will not be limiting. In the Delaware experiment, the abundance of alkane- and PAH-degrading bacteria increased several orders of magnitude within 4 days simply because of exposure to oil. Because of the moderately high exposure conditions, dissolved oxygen concentrations in the pore water were always high in all plots. Therefore, we recommend that, in the event of a catastrophic oil spill impacting a sand beach, the first task should be to measure the natural nutrient concentrations in that environment to determine if they are already high enough to sustain significant intrinsic biodegradation. Continuously renewable concentrations approaching 1 to 2 mg nitrogen per liter interstitial pore water should support near optimum hydrocarbon biodegradation activity. If concentrations are well below this, then the planning process (see text following) must include an estimation of the nitrogen loading rates necessary to achieve this concentration.

If there is any question or uncertainty about the level of oil degraders naturally present at a site, degrader densities and activity should be confirmed as part of the pretreatment assessment (see Venosa *et al.*, 1996, for methods).

**Treatment planning and monitoring.** Once it has been determined that bioremediation is a viable option because of time constraints, non-interference from heavy oiling, and identified limiting factors, a response can be planned and executed. The focus of planning should be to determine (1) the bioremediation treating agent (nutrient) loading rate(s), (2) an estimate of the treatment duration, and (3) monitoring needs (including the establishment of untreated areas or "set asides").

Generally, nitrogen is the limiting nutrient in temperate marine coastal situations, and phosphorus is the limiting nutrient in freshwater and some tropical situations. The pretreatment assessment should determine this specifically. This determination will dictate what nutrient ratios will be needed.

Assuming that nutrients are the limiting factors, loading rates can be estimated by measuring or estimating beach interstitial water-flushing rates using methods such as described in Wrenn *et al.* (1997). The loading rates are then used to estimate the amount and kind of product (fertilizer) to purchase or stockpile. The Delaware study confirmed that soluble forms of nitrogen and phosphorus fertilizers were sufficient to enhance degradation. It is possible that pelletized or other slow-release materials would also be suitable (Swannell *et al.*, 1996). In any case, the merits and costs of each form need to be reviewed and discussed by knowledgeable experts.

Treatment should be terminated if or when (1) it is not effective or (2) the oil has degraded to acceptable concentrations. Monitoring (see text following) will provide the feedback to answer both questions. Estimating the treatment duration, and therefore the response termination, requires agreeing on criteria for deciding how clean is clean. For example, for protection and restoration of sediment-dwelling marine life, where a primary concern is the sediment concentration of PAHs, one may wish to consider sediment quality guidelines as criteria. For example, Long and Morgan (1991) found that the toxicity effects range for total parent PAHs in sediments was 35 mg/kg dw (median) and 4 mg/kg (low). Alternatively, acceptable sediment toxicity reduction may be the agreed end point for termination. In either case, operational monitoring is required.

The objectives of operational monitoring are (1) to determine if the treatment is effective and to terminate it if it is not; (2) if effective, to terminate the response once the concentration of oil components are at acceptable concentrations; and (3) to ensure that there are no detrimental effects on the biota (toxicity added by treatment). Operational monitoring must include frequent nutrient measurements and efficacy tests (accelerated degradation); it must include effects (toxicity, biodiversity, environmental impact assessments, etc.) for safety insurance. To determine effectiveness—that is, if treatment is accelerating degradation—monitoring requires comparison of specific chemicals (analytes) among treated and untreated areas. Accordingly, planning must include the selection of specific chemical analytes and also specific areas to be left untreated (set asides).

The Delaware study convincingly demonstrated oil degradation in sediments only when specific analytes were measured and normalized to a nondegrading chemical, such as hopane. Only when this was done was it also apparent that treatment accelerated loss of total oil (as measured by EOM). Therefore, monitoring degradation effectiveness must be done using specific analytes analyzed by chromatographic techniques and then only when analytes are normalized to a recalcitrant compound such as hopane (as in this study) or alkylated chrysenes (Lee *et al.*, 1997, these proceedings). Analyte-to-recalcitrant (nonbiodegradable) compound ratios have the added benefit that they take into account variability in the distribution of concentrations of the residual oil stranded on the beach. We discourage the use of bulk oil concentration estimators (TPH or EOM) to monitor the effectiveness of bioremediation. In addition to high variability, these bulk oil measurements also assay for other natural compounds and are biased toward the recalcitrant compounds, which may have little or no biological significance.

When planning a bioremediation response monitoring program, one must consider sampling replication and independence, sample frequency, and information turn-around time. Until the Delaware study, no studies were available for evaluating replication and frequency needs. In the Delaware study, power curves confirmed that five replicate plots were essential to demonstrate effectiveness for that site; had only four replicates been used, the effectiveness of treatment on degrading total alkanes and total PAHs would not have been detected (Venosa *et al.*, 1996). Five replicates were needed to detect significant treatment differences because the background nutrient concentrations at Fowler Beach were high enough to sustain a high natural attenuation rate. Had those concentrations been lower, the number of replicates needed to detect a treatment difference would likely have been much lower. Therefore, decisions on the effectiveness or noneffectiveness of a treatment program should be based on the variance expected in the testing program and the treatment differences expected to be detectable. If background nutrients are high, treatment differences will be low, thus requiring more replicates.

The Delaware study also reconfirmed that half-lives of various oil components are on the order of several days to several weeks where background nutrient levels are high, and that nutrient treatment can shorten these half-lives considerably. Therefore, monitoring should occur at intervals and frequencies sufficient to describe these kinds of decay patterns. Given half-lives on the order of several weeks, sample frequencies should be weekly during the first month and perhaps biweekly thereafter. If shorter half-lives are anticipated, sampling intervals should be shortened.

A qualified laboratory should be able to analyze 25 GC/MS sediment samples in about a week. Considering the long-term course of a bioremediation response (weeks), about 4 to 5 weeks of monitoring should be sufficient to decide if termination of an ineffective treatment is appropriate.

If termination of treatment is predicated on achieving toxicity criteria, then chemical monitoring should be accompanied by toxicity monitoring. There are many types of sediment and pore water toxicity tests, five of which were used in the Delaware study (Mearns *et al.*, 1995). We recommend using the solid-phase Microtox (see also Lee *et al.*, 1997, these proceedings) and at least one chronic test such as a grass shrimp embryo test or a 10-day sediment amphipod bioassay (if the beach matrix is appropriate for amphipod survival).

**Acquisition and deployment of treating agent(s), delivery system, and personnel.** The fertilizers used in the Delaware study were readily available from a local agriculture supply house. Fertilizers are commercially available throughout the United States. Thus, depending on the conclusions of the pretreatment assessment, it is possible that the selected treating agents may be readily available without any special or difficult logistics or extensive costs.

In the Delaware study, portable lawn sprinklers were used to pump dissolved nutrients from header tanks to the fifteen 36-m<sup>2</sup> oiled plots. This implies that a large-scale response using dissolved nutrients will require either large mixing tanks or ambient-water dilution of smaller concentrates, plus a temporary sprinkler system. Alternatively, we note that special slow-release treating agents were customized for the *Exxon Valdez* oil spill bioremediation response. These were delivered by hand-held sprayers (for oleophilic fertilizer) or hand-held lawn-food applicators (granular or pelletized fertilizer).

Although the nutrient addition used here accelerated biodegradation, the incremental increase (twofold for alkanes, 50% for PAHs) might not be high enough to warrant initiation of a major and perhaps costly bioremediation action. See Lee *et al.* (1997, these proceedings) for comparative information. Obviously, the training and experience of implementing personnel must be commensurate with both the treatment systems employed and the monitoring requirements.

**Termination of treatment.** As indicated in text preceding, treatment should be terminated when (1) it is not effective, (2) the oil has degraded to acceptable concentrations, and/or (3) toxicity is increasing. Monitoring several untreated but oiled shoreline segments (i.e., treatment-free “set asides”) provides essential reference data for determining the effectiveness of the full-scale treatment. For example, if treatment is not significantly (at least statistically) accelerating degradation beyond that occurring naturally, it should be terminated. Finally, treatment and toxicity monitoring can be terminated when concentrations of analytes reach acceptable (i.e., previously agreed upon) concentrations or when toxicity has been reduced to acceptable levels.

## Implications for future studies

The Delaware study left open one important question about bioremediation of an oiled sandy beach and also provided considerable impetus for conducting additional studies of bioremediation and other treating agents.

As noted by Venosa *et al.* (1996), treatment accelerated degradation of alkanes and PAHs more in the upper than in the lower intertidal zone because the upper intertidal zone was submerged for a shorter time period (i.e., degrading organisms were exposed to nutrients and oxygen for longer time periods at higher elevations). Therefore, there remains a need to determine conclusively if continuous nutrient treatment can indeed accelerate oil degradation at lower tidal elevations. This is particularly important to document for more protected, less energetic beaches.

This review and the response recommendations that followed apply specifically to sand beaches, not necessarily to other environments such as marshes or protected rocky shores. There is great need to conduct well-designed trials on these shorelines. Many of the methods used at the Delaware site can be modified for other shorelines.

There appears to be no need to add oil-degrading microorganisms to an oiled sand beach (Venosa *et al.*, 1996; Lee *et al.*, 1997; Lee and Levy, 1987; Swannell *et al.*, 1996). Considerable justification will have to be developed to persuade sponsors to continue field-testing inocula. Positive experiences at contained terrestrial waste sites cannot be taken as evidence of success on a wave-swept sandy beach. The abiotic loss mechanisms that act upon petroleum, nutrients, and microorganisms are substantially different on a beach than on a terrestrial soil environment.

Many agencies are skeptical and concerned about releasing large amounts of oil into clean environments for the purpose of testing oil spill countermeasures. The Delaware experience was a success in that it did not result in releases of oil that threatened resources either within or beyond the study site. Caged oysters were deployed seaward, up-coast, and down-coast of the treatment area to document possible loss of oil. Those located within a few meters of the study blocks accumulated oil briefly but also depurated rapidly. Caged shellfish and semipermeable membrane devices (SPMDs; Shigenaka and Henry, 1995) may be a useful tool for making sure that an intentional oiling experiment remains relatively confined and nonthreatening.

We urge that future field testing studies also monitor the status and trends of resident biota, such as intertidal infauna. This will allow direct measurement of the benefits or effects of treatment on native populations.

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### Biography

Alan Mearns is leader of the NOAA Hazardous Materials Response and Assessments Division's Biological Assessment Team and has conducted numerous studies on the effects and effectiveness of marine pollution control technologies.

### References

1. Lee, K., S. E. Cobanli, G. H. Tremblay, J. Gauthier, and M. Griffin, 1997. Bioaugmentation and biostimulation: A paradox between laboratory and field studies. *Proceedings of the 1997 International Oil Spill Conference*. American Petroleum Institute, Washington, D.C. (these proceedings)
2. 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
3. Lee, K. and E. M. Levy, 1989. Enhancement of the natural biodegradation of condensate and crude oil on beaches of Atlantic Canada. *Proceedings of the 1989 Oil Spill Conference*. American Petroleum Institute, Washington, D.C., pp479–486
4. Long, E. R. and L. G. Morgan, 1991. The potential for biological effects of sediment-sorbed contaminants tested in the National Status and Trends Program. NOAA Technical Memorandum NOS OMA 52. National Oceanic and Atmospheric Administration, Seattle, Washington
5. Mearns, A. J., K. Doe, W. Fisher, et al., 1995. Toxicity trends during an oil spill bioremediation experiment. *Proceedings of the 18th Arctic and Marine Oilspill Program (AMOP) Technical Seminar*. June 14–16, 1995, West Edmonton Mall Hotel, Edmonton, Alberta, Canada. Environment Canada, Ottawa, v2
6. Shigenaka, G. and C. B. Henry, Jr., 1995. Use of mussels and semipermeable membrane devices to assess bioavailability of residual polynuclear aromatic hydrocarbons three years after the *Exxon Valdez* oil spill. In P. G. Wells, J. N. Butler, and J. S. Huges (editors), *Exxon Valdez Oil Spill: Fate and Effects in Alaskan Waters*. STP 1219, ASTM Publication PCN 04-012190-16. American Society for Testing and Materials, Philadelphia, Pennsylvania, pp239–260
7. Swannell, R. P. J., K. Lee, and M. McDonagh, 1996. Field evaluations of marine oil spill bioremediation. *Microbiological Reviews*, June, pp342–365
8. U.S. Congress Office of Technology Assessment, 1991. Bioremediation of marine oil spills—background paper. OTA-BP-0-70. U.S. Government Printing Office, Washington, D.C.
9. Venosa, A. D., J. R. Haines, and D. M. Allen, 1992. Efficacy of commercial inocula in enhancing biodegradation of weathered crude oil contaminating a Prince William Sound beach. *Journal of Industrial Microbiology*, v10, pp1–11
10. Venosa, A. D., J. R. Haines, W. Nisamanepong, R. Govind, S. Pradhan, and B. Siddique, 1991. Screening of commercial inocula for efficacy in simulating oil biodegradation in closed laboratory system. *Journal of Hazardous Materials*, v28, pp131–144
11. Venosa, A. D., M. T. Suidan, B. A. Wrenn, et al., 1996. Bioremediation of an experimental oil spill on the shoreline of Delaware Bay. *Environmental Science and Technology*, v30, n5, pp1764–1775
12. Wrenn, B. A., M. T. Suidan, K. L. Strohmeier, B. L. Eberhardt, G. J. Wilson, and A. D. Venosa, 1995. Nutrient retention in the bioremediation zone of a sandy beach. *Proceedings of the 1995 International Oil Spill Conference*. American Petroleum Institute, Washington, D.C., pp896–897
13. Wrenn, B. A., M. T. Suidan, K. L. Strohmeier, B. L. Eberhart, G. J. Wilson, and A. D. Venosa, 1997. Nutrient transport during bioremediation of contaminated beaches: Evaluation with lithium as a conservative tracer. *Water Research* (in press)
14. Wrenn, B. A. and A. D. Venosa, 1996. Selective enumeration of aromatic and aliphatic hydrocarbon degrading bacteria by a most-probable-number procedure. *Canadian Journal of Microbiology*, v42, pp252–258