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OBSERVATIONS OF AN OIL SPILL BIOREMEDIATION ACTIVITY IN GALVESTON BAY, TEXAS

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ABSTRACT

Bioremediation treatment and monitoring were observed at an oiled marsh in upper Galveston Bay, Texas, August 5-8, 1990, during response to the oil spill created by the collision of three Apex barges and the T/V SHNOUSSA. Samples of oil from treated and untreated sites were collected and independently analyzed for evidence of biodegradation. Required monitoring protocols for water and sediment quality and acquisition of samples for chemical analysis were expertly adhered to. Visual observations made within 24 hours after treatment indicated that the treated oil experienced color changes. However, after several days there were no significant visual differences in oil appearance between treated and untreated plots. Chemical analyses from samples collected by observers (independent of the required monitoring program) indicated that there were also no apparent chemical differences in petroleum hydrocarbon patterns between treated and untreated plots. Water from one or both of two treated sites was toxic to mysid shrimp, and it is possible that micronutrients (trace elements) in the nutrient mix may have contributed to that toxicity.

Data available to date do not indicate that the bioremediation treatment significantly degraded oil in the test plots at Marrow Marsh. Recommendations are offered for improving oversight, application, field safety and monitoring so that effectiveness of bioremediation can be better assessed in future applications.

INTRODUCTION

Bioremediation is a technology that attempts to accelerate microbial degradation of oil or other substances. This involves the application of nutrients or microbial products to contaminated environments. The goal is to enhance the natural process of chemical degradation. This report summarizes observations on the application and monitoring of a bioremediation activity in oiled marshes of Galveston Bay, Texas in August 1990.

Microorganisms can naturally degrade toxic substances by metabolizing them to less toxic by-products. However, in confined situations, such as at some hazardous waste sites, this natural process can be accelerated by adding some combination of energy (i.e., mixing), nutrients (such as nitrogen), or microorganisms. There is considerable interest, but little experience, in the use of bioremediation products to degrade spilled oil in open environments. For example, in 1988 there had been no open-environment applications in the United States. By December 1990, there were several open-environment applications of microbial agents or nutrients on open water or shoreline oil spills in Alaska (Prince et al., 1990;

Pritchard et al., 1990; Venosa et al., 1990), Texas (Texas General Land Office, 1990), California (Goodbread, 1990; USFWS, 1990), and New Jersey (DuPont Environmental Remediation Services, 1990; Levine, 1990).

There is a need for studies that document the effectiveness of bioremediation and document potential adverse impacts. Especially needed are field studies (as opposed to laboratory studies) which include appropriate controls (untreated sites) and which monitor environmental parameters before, during, and after the application. In the only study found in the published literature to date, marsh plants (*Sonneratia caseolaris*) suffered higher mortality and lower growth in nutrient-treated plots than in untreated oiled plots (Dutrieux et al., 1990). There is, therefore, great need for field validation of the effectiveness of bioremediation, including documentation of adverse effects using appropriate reference conditions (untreated sites) and monitoring.

During August 1990, an opportunity to use bioremediation arose as a part of the cleanup response to a major oil spill in Galveston Bay. After drifting in the center of the Bay for several days, oil was blown onto the northern shoreline, contaminating marsh areas that could not be cleaned by conventional mechanical methods, such as skimmers. The U.S. Coast Guard On-Scene Coordinator (OSC) directing the cleanup response was authorized by the Regional Response Team to use bioremediation to attempt removal of oil from the marshes. The bioremediation activity was to be carried out under a suite of specific criteria and protocols, including environmental monitoring. An additional condition was that the application and monitoring were to be done under oversight from a team of observers from the U.S. Environmental Protection Agency (EPA); the U.S. Coast Guard (USCG); and the National Oceanic and Atmospheric Administration (NOAA), whose Scientific Support Coordinators (SSC) and scientific staff advise the Coast Guard on technical issues during spill response. This report summarizes the observations made by the NOAA scientist who was requested by the SSC to oversee the application and monitoring program. NOAA's primary mission in this role was to determine the extent to which application and monitoring criteria were met and to offer an independent assessment of the overall effectiveness of the bioremediation.

With this oversight responsibility, the scientist observed and participated in all bioremediation applications and in all monitoring events conducted during the course of the remaining official response. Oversight was to include written and photographic records of activities, collection of oil samples for independent

verification of effectiveness, and a written assessment of the bioremediation experience.

There were numerous unplanned and unexpected logistical activities that interfered with, or distracted from, successful application and assessment of the bioremediation treatment. These included varying wind conditions, incursions into treated and untreated plots by boats, landing of aircraft, interruption of activity by a personnel emergency, failure of containment booms, and excessive foot traffic in the marsh. Finally, large areas were treated that were not monitored according to protocols and there was a general treatment of the area following the period of these observations.

BACKGROUND AND TREATMENT HISTORY

The Tanker Vessel SHNOUSSA collided with three Apex barges near buoy 58 in Galveston Bay during the afternoon of July 28, 1990, spilling approximately 700,000 gallons of catalytic feed stock, a partially refined oil (Henry, 1990). Some of the oil eventually moved onshore at points along the western side of the lower Galveston Bay during the first several days of the spill. Then, on the evening of August 3 (seven days after the accident), the oil moved north, contaminating shoreline and marshes at various locations along the western portion of the northern shore of Galveston Bay (Figure 1).

On July 31, 1990, the Chairman of the Texas Water Commission (TWC) requested the OSC to allow application of a bacterial bioremediation agent, Alpha BioSea. The OSC, in turn, referred the request to the Regional Response Team (RRT, Region VI). On August 1, a multi-agency meeting was held in Galveston to provide recommendations to the TWC regarding effectiveness, water quality monitoring, and toxicity of the proposed bioremediation treatment to the potentially affected habitats. Application sites were also considered. Approval by the RRT was granted on August 1 with specific restrictions and guidelines (Appendix).

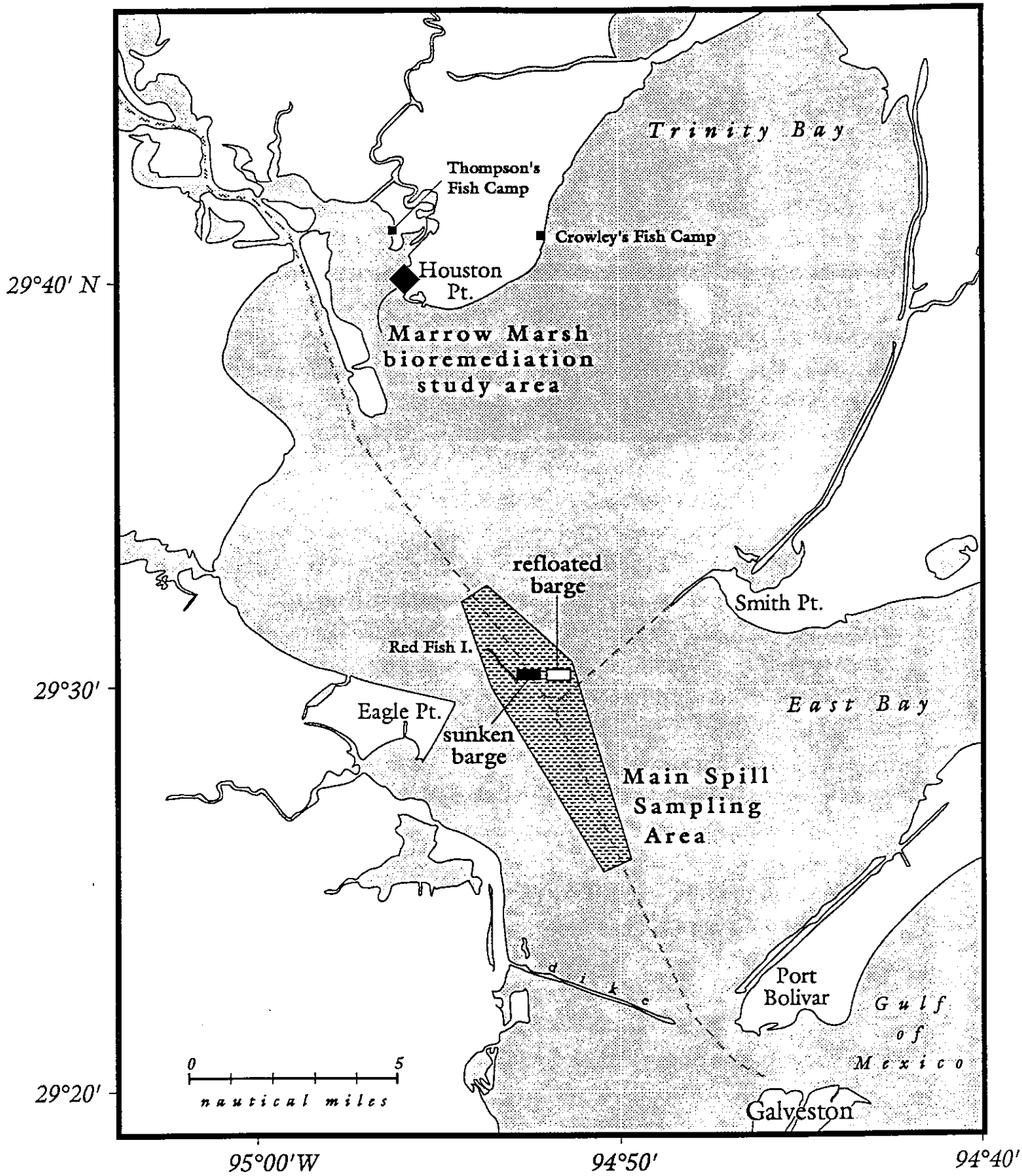

Figure 1. Location of the spill and marsh areas of northern Galveston Bay where bioremediation treatment occurred.

METHODS

Marrow Marsh was the primary area selected for application and monitoring of the bioremediation product. The marsh is a 1.0 by 0.2 km stretch of shallow channels and marsh islets running northwest to southeast along the western shoreline north of Houston Point and immediately adjacent to, and south of, the entrance to Cedar Bayou (Figure 2). In addition, unmonitored applications were subsequently made in Pasture Cove, a small inlet about 0.5 km southeast of Marrow Marsh and in Swan Marsh, about 2-3 km southeast of Marrow Marsh (Roques, 1990; Texas Water Commission, 1990; Figure 2).

Study Area

The marsh was mainly composed of 1-1.5 m tall marsh grass erroneously reported as "*Spartina*"; it was not *Spartina*, but possibly a species of the marsh grass, *Sciripus*. Fiddler crab (*Uca*) and marsh crab holes were common (5-10 per square foot). During the study period, it was popularly believed that oil had killed all of the crabs in the marsh. However, at sunset on the first day, monitoring personnel observed many live fiddler and marsh crabs in all study areas. Littorine snails were also observed on the outer, more exposed edges of the marsh, but not inside. A specimen of the horse mussel (*Modiolus* sp.) was collected in mud at the outer edge of the marsh. Small fish, possibly juvenile mullet, were abundant on the north and east (unoiled) sides of the northern marsh block, but appeared only occasionally in the oiled area used for the treatment.

Prior to treatment, oil was observed inside marsh channels and throughout the marsh islets.

Experimental Design

The major objective of monitoring was to determine whether treatment accelerated degradation of oil without additional environmental impacts: did chemical, physical, and biological conditions differ between the test sites before and after treatment? Testing was to be done in four well-marked areas: two treated with a microbial solution, Alpha BioSea, and two left untreated (controls). The bioremediation contractor claimed that there would be significant improvement in oil at the treated areas compared to the untreated areas within a few minutes to a few hours after application. Accordingly, a monitoring activity was planned to make

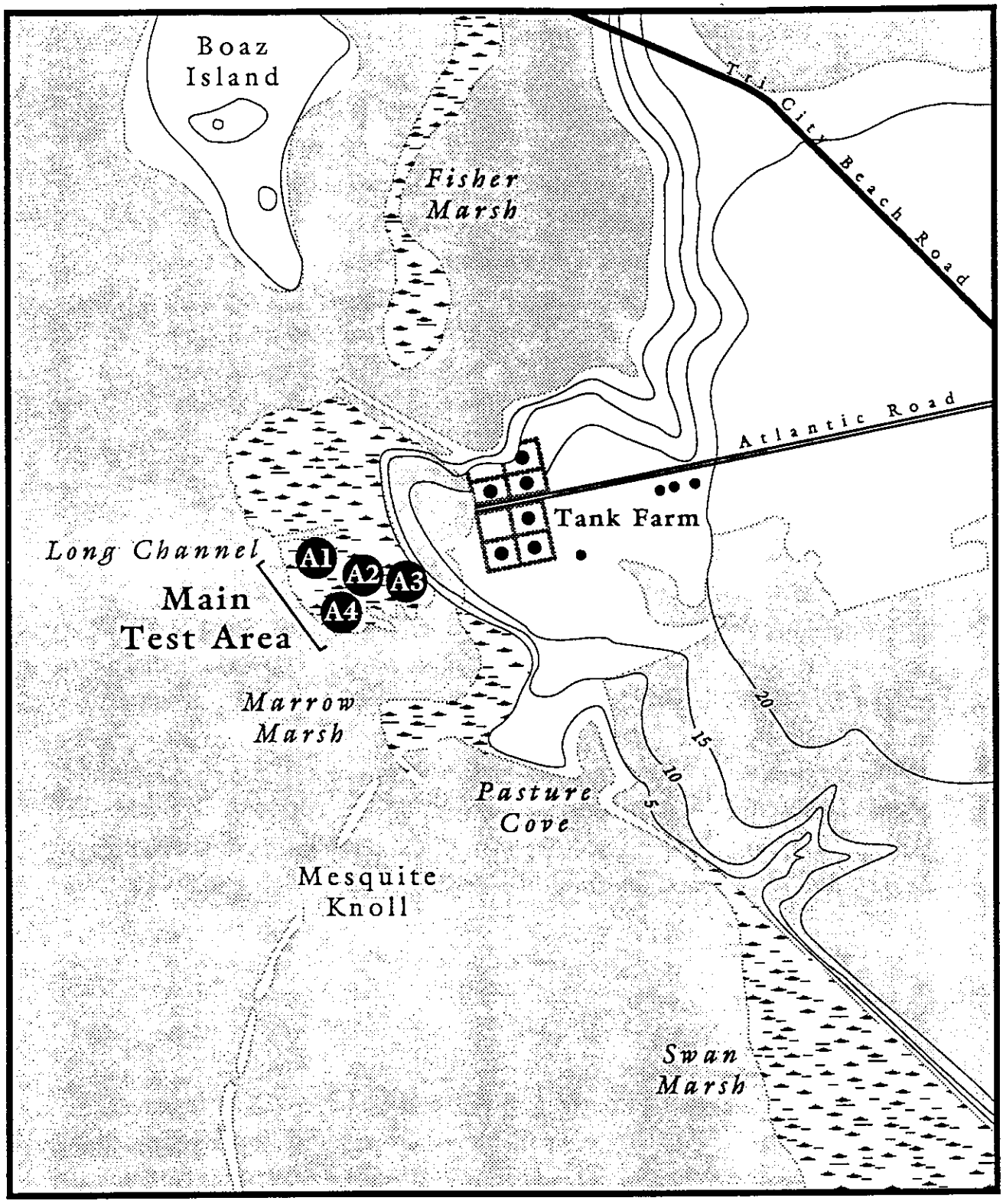

Figure 2. Marrow Marsh and Pasture Cove area in northern Galveston Bay near the entrance to the Houston Ship Channel showing bioremediation test plots (A1 through A4).

visual observations and collect samples just before application and within a few hours of application on the day of application (Day 0), and then for each of several days after application (24, 48, and 96 hours).

Four test plots were identified and set up on the morning of August 5, 1990. Plots to be treated were identified as A1 and A2; control plots were A3 and A4 (Figure 2). Surface material and non-movable material within a test plot were somewhat isolated by deployment of containment booms around each of the test plots. Boom teams also deployed an absorbent boom and a non-absorbent boom around the western edge (water side) of the marsh complex. Thus, any oil or other floating material outside the test plots, but within the marsh test area, were generally contained within the marsh.

Shortly after booming, the marsh was entered by the monitoring and observation team to take pre-treatment monitoring samples. Test plots were accessed by a 14- to 16-foot skiff; however, direct entry into the shallow, boomed plots required use of a very small (2.5-ft by 7-ft) aluminum pram (pirogue) which accommodated no more than two people. Whether aboard the skiff or pram, the NOAA scientist was nearly always within sight of the sampling activity.

Pre-treatment monitoring samples were collected in the following sequence:

- visual observations
- surface material (including visual description)
- subsurface water
- sediments and physical measurements

Required sampling methods are described in the Appendix.

Following pretreatment sampling of the first two plots, the application contractor was called in. The liquid bioremediation product was premixed in a large plastic barrel set in the center of a 14-foot aluminum outboard boat. A bacteria mix—a lyophilized (freeze-dried) microbial agent (Alpha BioSea, a light-colored powder prepackaged in plastic bags) in a corn starch carrier—and a blue-colored nutrient/micronutrient mix were added to ambient brackish water in the barrel. The blue nutrient mix was described by a TWC official as the commercial plant food, Miracle-Gro, but a spokesman for Alpha indicated that it was not Miracle-Gro, but a special nutrient/micronutrient mix. The specific content of the microbial and nutrient mixes was not reported. Each batch or stock solution contained 10 pounds of microbe/corn starch mix and either one pound (Alpha spokesman) or two pounds

(EPA spokesman) of nutrient/micronutrient mix. The specific content of the microbial and nutrient mixes was not determined. The resulting mix produced a turbid solution with an overlying brown foam.

The content of the barrel—the stock solution—was fed through a short hose to a high pressure pump operated by a gas-powered engine. An overboard intake hose delivered ambient water to the pump for diluting the stock bioremediation solution during spraying. Dilution was either 3 percent (33.3 parts ambient water to 1 part stock) or 6 per cent (16.6:1). Diluted stock solution was sprayed on the marsh through a fire hose nozzle operated by hand in long, slow, sweeping motions. The product was delivered to a reach of up to 30 to 50 feet.

Material was sprayed on each of the two test plots, A1 and A2, and on 30 to 60 m of marsh in between and around the treated plots, covering a width of at least fifty feet inside the water line.

Site treatment occurred between 1600 and 1800 on August 5, leaving only 2 to 3 hours of light to complete the first round of post-treatment sampling. Therefore, only the two treated sites were sampled 1 to 2 hours after treatment. However, all sites (treated and untreated) were revisited at approximately 24, 48, and 96 hours following treatment. Sampling at the two treated plots 1 to 2 hours after treatment, and at all plots 24 and 48 hours post-treatment, was completed as described above for the pre-treatment sampling and in more detail in the Appendix.

All required monitoring was completed by staff of the Texas Water Commission.

Oversight Observations

In addition to the required monitoring, NOAA made independent visual observations of oil and collected additional samples of oil, water, and sediment at treated and untreated sites during the course of the response activity. Samples were collected in methylene chloride-cleaned glass jars, put on ice on shore, and transported on ice to the Louisiana State University Institute for Environmental Studies, Baton Rouge, within several hours of collection. These oversight samples were characterized by a gas chromatography-mass spectroscopy (GC/MS) method developed to target detailed changes in the molecular composition of the oil. Relative concentrations of 30 different specific chemical parameters were determined for all of the oversight samples that were analyzed as part of the bioremediation study. These include the normal paraffin components, common polynuclear aromatic hydrocarbons such as phenanthrene and its related homologs, and resistive

biomarkers such as the hopanes. Also examined were the nC-18/phytane ratios which would be expected to decrease substantially as a result of microbial degradation (Henry, 1990). To maximize detection of degradation, ratios from the bioremediation samples were compared with ratios in fresh oil collected at the spill site.

RESULTS

The principal objective of monitoring and oversight was to document the effectiveness of bioremediation. The treatment area containing test plots A1, A2, A3, and A4 was the subject of required monitoring and the results of data available to NOAA at the time of observation form the focus of this report. In addition, samples and observations made directly by NOAA at the first and third treatment areas are also described below. No observations were made by the scientist at the fourth treatment site, Swan Marsh.

Changes in the Oil

Assessment of change in the oil was based on visual observations and chemical analyses of surface material, sub-surface water, and sediments.

Visual Changes. Immediately following treatment, it was difficult to determine visual differences among the four plots. However, on the first full day following treatment, light tan or golden, flock-like material was observed in various percentages in all four experimental plots (Table 1). On the second day after application, the amount of this yellowish material, relative to total surface material, was approximately 75 to 80% in each of the two treated plots and 20 and 60%, in untreated plots A3 and A4. Oil was present in sediments at all four plots.

It was not possible to determine visually how the amount of oil in each plot changed over time, but the monitoring team did visually estimate and compare the amount of oil among the plots. The total amount of oil in each plot was ranked (1, most to 4, least) with an order that ranged from most (1) in plot A3 (untreated) to least (4) in plot A4 (untreated). The two treated plots contained the next most (2; A1) and next least (3; A2) amount of total oil (surface material, Table 2).

Table 1.
Percent of light-colored oil in test plots

Date	Treated A1	Treated A2	Control A3	Control A4
8/6/90	NR	NR	10[1]	30-40[1]
8/7/90	75-80	75-80	20[2]	60

NR = not recorded
1 = yellow material
2 = stringy material

Table 2.
Ranking of test plots by relative amount of oil on different sampling days: 1 = most; 4 = least

Date	Treated A1	Treated A2	Control A3	Control A4
8/5/90	2	3	1	4
8/6/90	2	3	1	4
8/7/90	NR	NR	NR	4

NR = not recorded

In the late afternoon on August 5, following treatment, yellowish material was also observed in streaks on darker oil along the edge of the long channel which had been treated earlier in the day. In Pasture Cove, the third treatment area, oil pooled in hoofprints of cattle exhibited scattered, 1-cm round, yellow- and cream-colored spots or pustules several hours after treatment (Figure 16). These were subject to considerable media attention and videotaping.

Since the kind of physical or visual changes to expect following treatment were not known, the NOAA scientist and other observers did not make as careful

observations pre-treatment as were made post-treatment. Later examination of some pre-treatment photographs revealed yellowish material in oil but it was difficult to quantify the amount or attribute it to a specific site and time.

Chemical Changes. Through Day 2 (48-hour post application) three replicate sets reportedly totalling 132 samples of surface material, subsurface water, and surface sediment were successfully collected by the TWC monitoring team for petroleum hydrocarbon analysis from the four test plots within the second treatment area of Marrow marsh. These samples were stored on ice and subsequently shipped to a U.S. Environmental Protection Agency (U.S. EPA). laboratory for chemical analysis. After response termination, on Day 4 post application, an additional set of replicated samples were reportedly collected (Texas Water Commission, 1990), and were included with the samples sent to EPA.

During the day of treatment, and the following 2 days, and USCG observers collected an additional set of 36 water, oil, mousse and sediment samples from all three treatment areas. These included 12 samples from the four test plots, A1 through A4 in the second application area and 16 samples from the first application area. Additional samples were collected from the third area.

Figure 3 compares Total Ion Chromatographs (TICs) of oil/water mix from a treated site (A1) sample to a non-treated site sample (A3) collected 48 hours after application. These represent the most extreme differences between sites in the collections. There was no obvious difference in these spectra.

A plot of nC-18/phytane ratios for 10 analyzed samples from treated plots five from untreated plots and three from fresher oil collected at Redfish Island 3 to 5 days earlier, hints at a possible degradation in samples from treated plots; but, the differences are not significant and no significant differences in the slopes (rate of degradation of the ratio) noted over the the 2 to 3 day study period coupled with the 3 to 5 day pre-study samples (Figure 4).

Examination of these data indicate there were not any noteworthy changes in the relative abundances of specific compounds in the oil before and after treatment or between treated and untreated sites for the 48 hour period represented by samples from the test plots.

A more definitive statistical assessment may be possible when the data are available from the replicated samples taken by the TWC and being analyzed by the EPA.

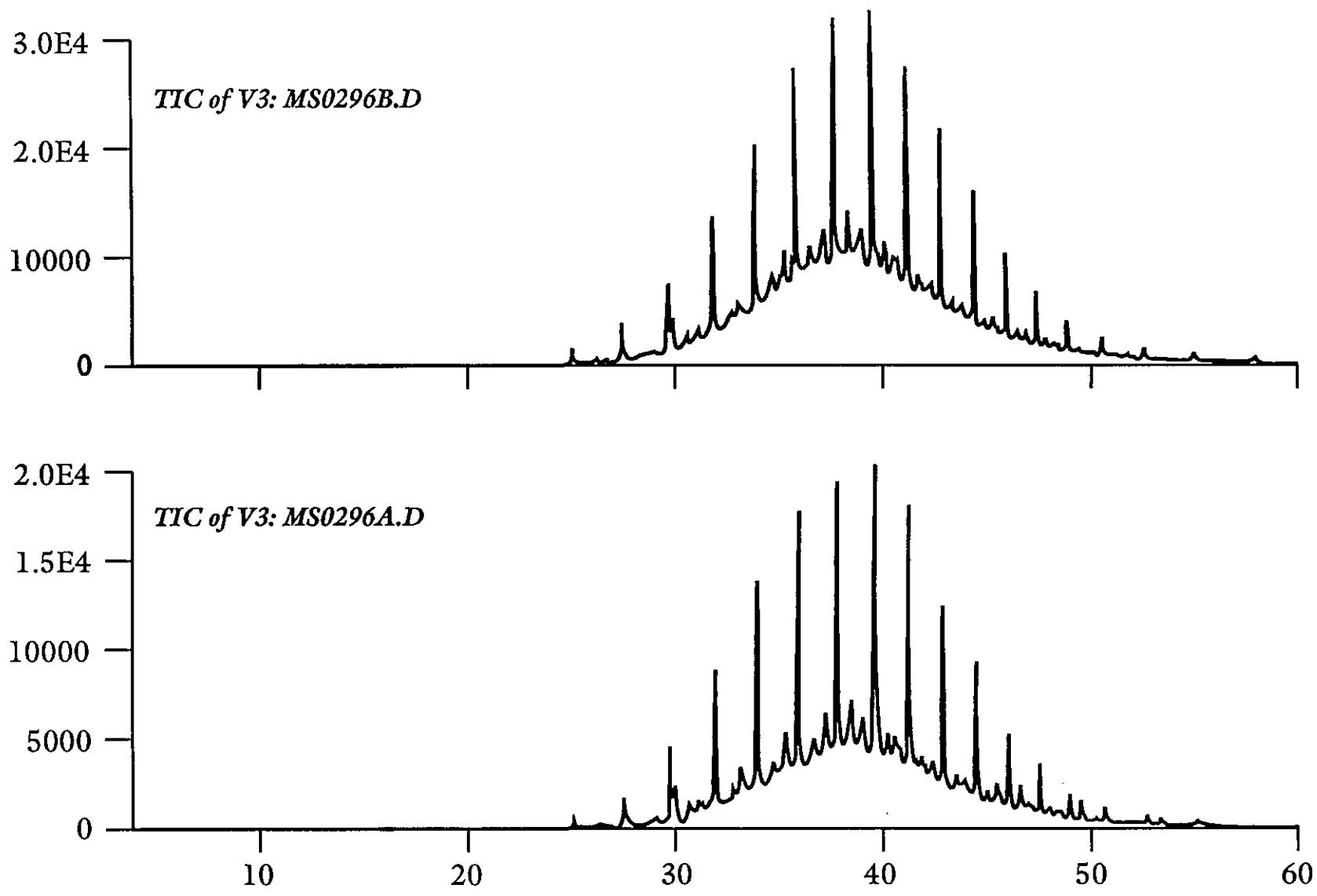

Figure 3. Comparison of GC/MS TICs of treated oil versus non-treated oil from Marrow Marsh. Both samples were collected 48 hours after application.

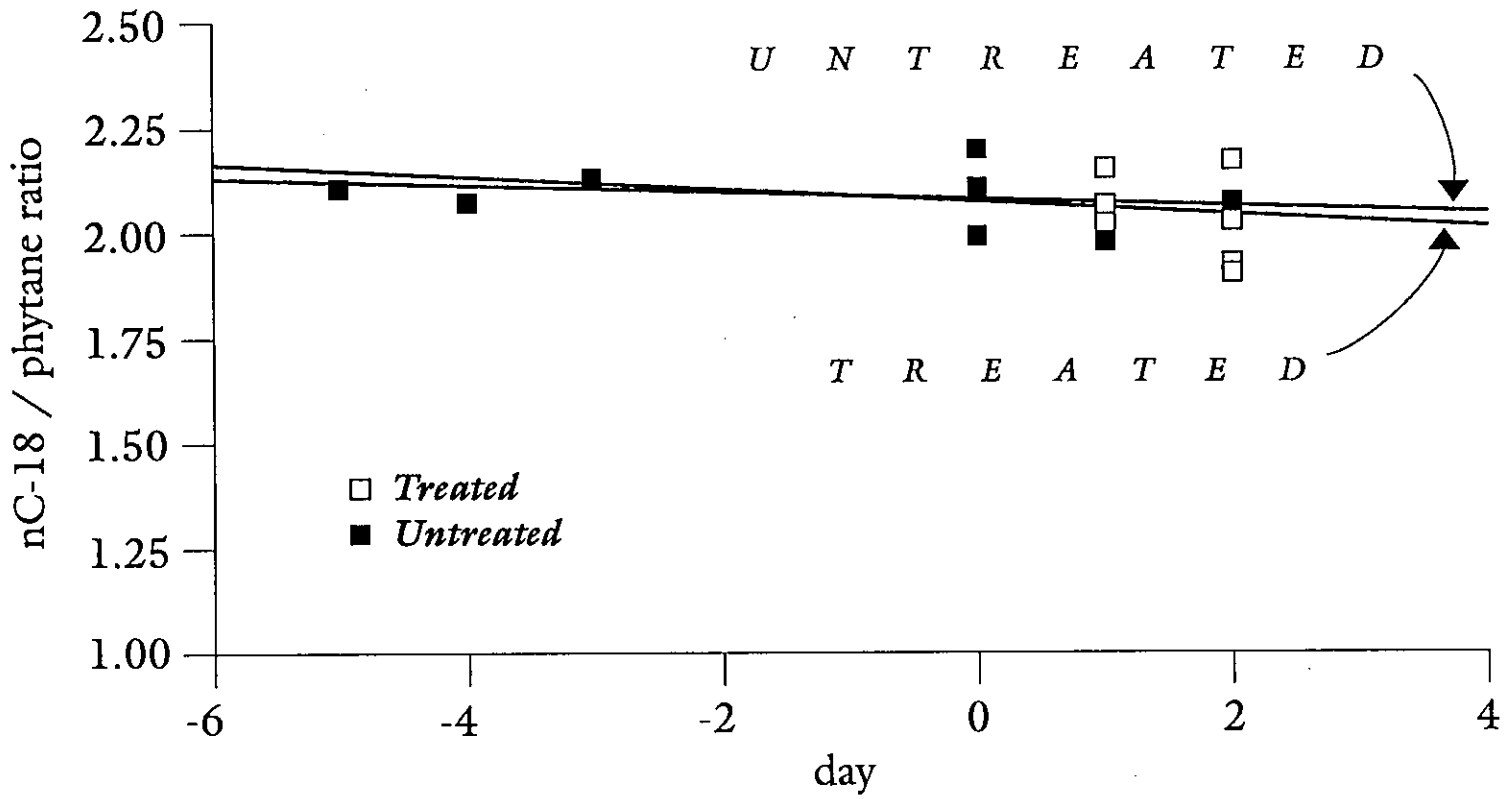

Figure 4. Summary of plot of the nC-18/phytane index ratios for the treated and untreated areas. The plotted lines represent the slope of the mean change in the index over time.

Water and Sediment Quality

Measurements were made in each plot at each sampling event for dissolved oxygen, temperature, and salinity. All data were recorded by the TWC, and as time permitted, were recorded by the scientist (Table 3). In general, water temperatures were extremely high, ranging from 32.5 to 41.0°C. Dissolved oxygen concentration was also very high, on the order of 8 to 9 mg/L. Since all measurements were made during daylight hours, it is not possible to say if dissolved oxygen levels dropped at night due to respiration. On several occasions during daylight, we observed benthic algal or bacterial mats expressing tiny bubbles (presumably oxygen) and occasionally breaking away and floating. Salinity was low. Some values recorded in the field on August 6 ranged from 5.3 to 6.8 o/oo. Subsurface water samples analyzed by EPA (1990) ranged from 9 - 10 o/oo.

Table 3.

Selected water quality measurements in test plots at Marrow Marsh, Galveston Bay, Texas, August 5-7, 1990

Date	Time	Site	Temperature C	Dissolved Oxygen (ppm)	Salinity o/oo
8/5/90	1240	A1	35.0	-	-
	1415	A2	34.0	-	-
	2009	A2	32.5	-	-
8/6/90	1430	A1	37.5	8.8	-
	1550	A1	41.0[1]	8.8	-
	1620	A2	40.0	-	6.8
	1650	A3	-	-	-
	1830	A4	36.0	-	5.3
8/7/90	1515	A4	34.0	-	-

[1] =Inside boom: water outside was 40°C.

Water clarity was low on most visits, at most 10 to 15 cm. However, during the early evening hours, when boats, vehicles, and people had left the marsh, visibility appeared to increase, and the bottoms of some channels were visible.

Surface sediments were composed of a fine greyish-brown silt impregnated with dark organic matter and roots of marsh plants (Figure 14). Along the intertidal marsh edges were numerous holes of fiddler crabs, many of which emerged live at sunset. No attempt was made to estimate densities of holes or per cent with live crabs. Examination of grabs indicated the presence of oil in the form of droplets or sheen. By the third day, oil appeared to form a thin dark subsurface layer overlain by a very thin light-colored layer. This may have represented resettled sediments suspended during periods of active boat activity earlier in the day.

Toxicity

Post-treatment water from the A1 + A2 composite water samples was acutely toxic to mysids; the LC50 was 67% (confidence limits, 55 to 81%; EPA et al., 1990). There was no acute toxicity in the pre-treatment composited water from these sites. Undiluted water from the control plots A3 + A4 was slightly toxic to mysids (45% mortality; EPA et al., 1990). None of the samples were toxic to silversides.

This information suggests that at the Marrow Marsh site, toxicity of ambient water increased several hours after application of bioremediation material. More samples were needed to support or reject this conclusion.

Included in the tests done by EPA (1990) was a composite of two "beach pretreatment" samples, the origin of which is not known to this writer, but may have been from a Pelican Island site remediated as a test prior to oil impacting Marrow Marsh. The composite was very toxic to mysids with and LC50 of 26% (dilution; confidence limits 17 to 40%).

Operational Observations

Material was sprayed on each of two test plots and on 30 to 60 m of marsh in between and around the treated plots, covering a width of at least 50 feet inside the water line. During the approximately 2-hour application period in mid-afternoon, the wind shifted from south to north, and spray was observed blowing back toward the applicators and observers and downwind toward areas designated as untreated area, where pre-treatment sampling was underway. In addition, open water spraying of some material was observed between test plots in the second application area Figure 10).

Several additional observations were made of conditions and activities that may have compromised the operation or disturbed the marsh. Hundreds of 2 x 2-

foot white absorbent pads, apparently remaining from previous cleanup activity, were left adrift in the boomed marsh area outside the four test plots (Figure 5). Six to ten cleanup personnel were observed mopping up oil while walking along the edge of the first area just several hours prior to treatment. This area was 10 to 20 meters north of the actual test area; this resulted in smashing marsh plants and pushing oil into the mud.

During pre-treatment set-up activities, skiffs occasionally became stuck in the soft mud of shallow marsh channels. One resuspended a considerable amount of sediment as noted by a muddy "roostertail" as it attempted extraction at high engine speed. An airboat powered by an airplane propellor entered the experimental area to make observations and was subsequently called upon to undertake a successful rescue of a cleanup contractor staff member who sank deeply into mud while traversing the area on foot. Later on the next day the air boat passed alongside control plot A4, depressing the marsh grass as it passed. This could have moved oil in or out of the area as well as impacted the marsh grass.

At least one cow, from a small herd on a ranch adjacent to the marsh, entered the marsh 10 to 20 m from the northern test area for about an hour, suggesting the marsh was frequented by the herd.

The bioremediation application was a media event. Several times during the first and second day, news or video camera teams approached the area by boat, helicopter, and from shore . During sampling of the third plot the monitoring party was hailed by a video team attempting to enter the marsh from the nearby pasture. The team was waved away. A news helicopter landed briefly on a large plot of marsh about 100 meters north of the test area (Figure 11), but departed soon after being waved away by observers.

Operations were temporarily delayed while two officials were put ashore on a marsh islet adjacent to one test plot to capture an apparently oiled duck to be removed to a cleaning station. After a 10- to 15-minute chase across the islet, the duck escaped and experimental operations resumed. On the second day of monitoring, an automatic cannon was set up inshore of untreated site A-3 to keep birds from landing in the marsh. The cannon went off at about 5-minute intervals and provided an occasional surprise to the monitoring team. One person commented that a water moccasin (poisonous aquatic snake) had been seen in the study area during set-up operations.

DISCUSSION

Effectiveness of Treatment

An implicit assumption about the use of bioremediation is that it works. That is, the application of microorganisms or fertilizers to oiled environments in fact results in reduction of oil or oil compounds at rates significantly faster than would occur in the absence of the application. Further, assumptions resulting from recent applications are that this treatment technology is logistically feasible, enhances the recovery of damaged resources, and does not result in additional risks to natural resources, health or safety, beyond those already incurred in the absence of bioremediation activity.

In both treated and untreated test plots there were definitely changes in the color and consistency of oil within a few minutes to hours following application of bioremediation material. These were most noticeable in the third treatment area at Pasture Cove where small pools of oil remained relatively undisturbed in cattle hoof prints. Characteristically, 1-cm yellow or cream colored spots developed on the surfaces of wet treated oil within a few hours of spraying. However, as noted above, yellowish material was also observed in both the treated and untreated test plots in the second application area.

It is not yet possible to conclude there was a significant difference between treated (A1 and A2) and untreated (A3 and A4) plots in terms of oil color change or degradation of specific chemicals. It does appear that the change to yellowish-light tan flocky material may depend on the original amount of material (oil) present; i.e.., the least amount of yellowish-light tan material (5%) was in an untreated plot which was also the most oiled plot; whereas, a lot of yellowish-light tan material (60% of total surface material) was in the second untreated plot which also was the least oiled plot. Oil amount or volume may need to be taken into account as a variable in evaluating the success of the treatment in terms of producing visual change.

There was no apparent increased degradation within 48 hours of treatment of potentially labile compounds (such as phytane) in samples from treated versus untreated areas subjected to chemical analysis. There are several possible explanations for failure to document biodegradation of the oil.

- *Already a degraded product*? The composition of oil from any site in the Marrow Marsh area was nearly identical to the composition of oil from the barges and from Redfish Island and all were similar to oil in which the most degradable components were already removed as part of the production of

this unusual product. It is possible that the oil is a composition that was extremely reticent to further biological or biochemical degradation under the field conditions experienced during the spill. The scans of original (fresh) oil and Marrow Marsh oil before or after treatment look like degraded oil as described by Kennicutt (1988).

SSC observers also examined oil at other sites in Galveston Bay including at Redfish Island near the wrecked and leaking barges. The samples collected at Redfish Island demonstrated similar color changes (appearance of yellowish material) seen in the Marrow Marsh and Pasture Cove area. It could not be determined if the amount of such material was any greater or less among the different sites. A common parameter to monitor for microbial mediated degradation is the change in the nC18/phytane ratio (Kennicutt, 1988). These two compounds have similar vapor pressures and water solubilities and loss due to volatilization or dissolution would be similar. But the isoprenoid phytane is a branched alkane and biologically degraded at slower rates so that a decrease in the ratio supports microbial degradation. The lack of significant differences among samples from throughout Galveston Bay during the spill supports the possibility that the oil was already in a form that had been degraded.

- *Not enough time allowed for degradation.* In EPA flask experiments (Venosa et al., 1990), various microbial products did not begin expressing signs of degradation activity (oxygen uptake) until after a 3 to 5-day lag period. Actual degradation of alkanes did not become obvious until 10 to 20-days. These observations support the suggestion that the monitoring conducted at Marrow Marsh was too short in duration to document degradation, which may not have become significant until long after the response was over.

- *The natural rate of biodegradation non-limiting.* Marshes are extremely productive, nutrient-rich environments. Marrow Marsh lies adjacent to the Houston Ship Canal, which has experienced numerous oil spills throughout its history. It is possible that a high nutrient recycling capability coupled with high natural concentrations of oil-degrading microorganisms is already present in the marsh ecosystem and that any additions are of minor consequence in this habitat (Lee and Levy, 1989). Most of the natural

organisms would be expected to reside on sediments (under the oil), whereas those applied during bioremediation are added to the surface of the oil as an aqueous oil interface, thus speeding surface degradation. It should be noted again that cattle were present on pasturage above the marsh, observed walking in the marsh, and their foot traffic observed in Pasture Cove. Through runoff and direct use, excrement and urine from these cattle could be a significant chronic source of microorganisms, nutrients, and organically rich material to the marsh. Finally, it should be noted that significant freshwater runoff occurred during the previous very wet spring and early summer and would contribute nutrients and microorganisms to the Bay environment.

- *Physical transport of oil out of system.* Upon arrival in the test area on the second day, reddish-yellow oil was observed streaming out of at least one test plot containment boom (Figure 8). An early morning overflight (USCG Videotape, August 8, 1990) recorded a massive amount of oil streaming out of Pasture Cove (under the boom) 2 days after application of bioremediation agents. Shifting winds and leaking containment booms may have worked to move oil both in and out of test areas.

- *Insufficient bioremediation agent.* It is not known what final concentrations of bacteria and nutrients were reached or if they were indeed adequate for degrading existing oil. No measurements were made of the microbial concentrations in test or control plots.

Effects of Treatment

Until all data from the required monitoring are available it is premature to conclude what, if any, effects the bioremediation agent had on water and sediment quality. The data reported above represent only a snapshot of what was obtained and merely serve to indicate the general environmental conditions existing during the first few days of the activity.

However, to my knowledge, all the toxicity data from monitoring are available as reported by EPA (1990). It is probably premature to conclude that the bioremediation agent itself contributed to the toxicity of Marrow Marsh water to mysids, but the null hypothesis, that it was not toxic, cannot be ruled out. To my

knowledge, there is no currently available published literature on the toxicity of microbially based bioremediation agents. However, toxicity data is available from EPA on nutrient applications (Prince et al., 1990). It is unlikely microorganisms in the mix contributed toxic substances to the water, but that cannot be ruled out. However, it is not unlikely that the nutrient mix may have contained potentially toxic materials including ammonia and trace elements.

This point is worth considering further. Although there is disagreement between the purveyor company and other observers regarding whether the nutrient mix was a commercial product (Miracle-Gro) or a mix of proprietary composition, both are highly soluble and contain "micronutrients". The following discussion is hypothetical only, but provides a basis for concerns that nutrient mixes containing micronutrients may be highly toxic to aquatic invertebrates such as crustaceans. According to the label, Miracle-Gro, is highly soluble and contains extremely high levels of copper (0.07% or 70,000 ppb dw), zinc (0.06% or 600,000 ppb dw), molybdenum (0.0005% or 5,000 ppb dw), manganese (0.15% or 1,500,000 ppb dw) and soluble iron (0.15% or 1,500,000 ppb dw). If the nutrient used was Miracle-Gro, and the amount per 55 gallon (212 L) batch was 2 pounds (.91 kg), the resulting concentration of copper in the stock solution would be 300 ppb. Diluted 16:1, the final sprayed effluent would contain copper at a concentration of 18 ppb. Landing undiluted on the marsh sediment surface could result in copper concentrations substantially higher than the EPA chronic water quality criteria of 2.9 ppb. Only in watery areas where it was quickly diluted by a factor of 6 or more would the quality criteria be met. Thus, the possibility exists that toxic concentrations of trace metals such as copper can be achieved in bioremediation treatments using nutrient/micronutrient formulations, and the hypothesis cannot be ruled out that a trace element micronutrient contributed to the observed toxicity of Marrow Marsh water to mysids in the EPA toxicity test performed by EPA (1990).

A similar case might be made concerning the possible toxicity of ammonia released as part of a bioremediation treatment. However, determination of safe and toxic concentrations of ammonia requires site-specific information on pH, salinity and temperature.

Elsewhere, nutrient and micronutrient applications are of concern and these other cases should be evaluated as part of the assessment of the Marrow Marsh application. For example, Miracle-Gro was expressly used as a nutrient amendment in a bioremediation application in a marsh in Seal Beach, California, impacted by a

50 bbl crude oil blowout (Goodbread, 1990). Toxicity tests have been done on a number of other products as part of an EPA screening study (Venosa et al., 1990).

The origin of toxicity in the "beach pretreatment" sample cannot be speculated until it is known exactly when and where it was collected.

Operational Disturbances

While microbial bioremediation materials in themselves may be potentially ecologically safe (whereas some nutrient mixes may not be) the operations and logistics surrounding and supporting a bioremediation treatment during a response can also cause considerable collateral damage to living resources and impact the results of monitoring. We were able to observe directly damage to marsh grass caused by foot traffic from cleanup and deployment teams, bioremediation applicators and others entering the marsh to capture water fowl or film the activities. We were unable to document environmental effects of airboat and helicopter traffic or stuck outboard motor propellors. Impacts from human and mechanical activities must be considered as part of the bioremediation exercise and effort made to limit and document it.

CONCLUSION AND RECOMMENDATIONS

This represents a preliminary report of one bioremediation application and monitoring experiment. Limited data indicate that there was no enhancement of oil biodegradation as a result of the treatment. As more data becomes available, additional reports of this activity should be prepared and disseminated so that others interested in bioremediation of oil spills will have the full benefit of this event. Especially valuable would be publication of the full results of the required monitoring data collected by the Texas Water Commission and the results of samples analyzed for petroleum hydrocarbons by the EPA.

Meanwhile, based on information available to date, a number of things can be recommended to improve our ability to document the benefits and effects of bioremediation during real oil spill response activities.

Use in Oil Spill Response

The preliminary results of this application confirm what has been learned to date about bioremediation - it is not yet a tool for rapid response. Accordingly,

proposals to use bioremediation as a rapid response tool should be discouraged and certainly not be allowed to interfere with, or draw resources from, other known rapid response activities. This may include the "no rapid response" alternative especially where there is a chance that increased human activity associated with bioremediation may cause more ecological damage than if the oiled area were left to recover naturally.

Composition, Content, and Application of Bioremediation Products

While microbes themselves may be harmless or non-toxic, the complete composition of all products applied during a bioremediation activity must be known. Pathogens, or microbes related to known pathogens (such as species of *Vibrio* and *Klebsiella*) may be in some microbial mixes. But, of equal importance, nutrient mixes applied by themselves, or together with microbial formulations, may contain materials toxic to sensitive animals. Care must be taken to avoid or mitigate use of nutrient mixes that also contain "micro-nutrients" such as highly soluble forms of copper, zinc and other trace metals. Regardless of the composition, final application concentrations of materials of potential concern need to be known in advance so that adjustments can be made to minimize toxic effects.

Toxicity of Bioremediation Agents

Since the potential clearly exits that bioremediation products may contain toxic substances, toxicity information should be obtained in advance of application.

Monitoring

Based on this exercise in Galveston Bay, it is clear that until we understand how to use bioremediation safely and effectively, monitoring is an essential requirement of bioremediation activities. The experience in Galveston Bay demonstrates that with appropriate resources and a competent monitoring team, the required monitoring is feasible and effective. Accordingly, the costs of monitoring must be included in the cost of bioremediation. Monitoring may, in fact, cost more than the application itself. The remaining recommendations focus on monitoring.

Experimental Design

No hypothesis or suite of hypotheses were proposed in advance of treatment. And, there was no prior selection of one or more oil condition endpoints for determining effectiveness of the agent used in this application. Any experimental

study requires selection of one or more quantitative end points (criteria) for judging effectiveness of treatment. Observations of characteristics developing following treatment are interesting, but must be later tested as separate experiments.

In the future, monitoring should follow an experimental design focused on testing specific hypotheses about the degradation endpoints expected and the predicted impacts, or non-impacts, on water quality and the health, abundance, and diversity of marine life. The null hypotheses that are being tested by the application, must be discussed and stated in advance, including the endpoints to be measured and the amount and direction of change expected and the degree of certainty desired. If, for example, a color change is anticipated, agreement should be reached on the amount or percent of oiled surface that must be changed, relative to reference areas, that would satisfy a conclusion that there has been a change due to treatment. Similarly, the rate of change expected should be stated explicitly and in advance.

Replicates

Ideally, to meet basic statistical requirements, there should be a minimum of three control and three test (treatment) areas sampled in tandem over time. Under actual field operational conditions, it might be possible to arrive at some conclusions from two control and two treated plots, as was done in the second treatment area at Marrow Marsh. Use of untreated controls (also in replicate) is an absolute requirement so that effects of the treatment can be separated from natural changes.

Sampling Frequency and Duration

Sampling should be conducted at frequencies and over periods of time that encompass the expected duration of responses, including lag responses. A three-week activity should have been planned, not a four-day one. Monitoring for fertilizer-based bioremediation exercises in Alaska were planned over a 12 week period (Pritchard et al., 1990).

Ecological Monitoring

Missing from this program was an ecological assessment that assured that marine and aquatic organisms were not impacted by the activity, or, in fact, benefited from it. An exception was the water sampling for toxicity testing; but, this was completed many days after application and involved very few samples.

Rapid bioassessment techniques to evaluate the abundance and diversity of aquatic communities have been developed by some state and Federal agencies. These may be extremely useful tools to add to a bioremediation monitoring activity.

Amount of Oil

Oil amount or volume needs to be taken into account as a variable in evaluating the success of the treatment in terms of producing visual change.

Time and Monitoring Resource Needs

There is a need to fully understand the resources and time needed to make sure a bioremediation treatment is effective. Monitoring alone consumes many hours per event. An accounting must be made of the readiness of supporting laboratories, appropriate vehicles for access (i.e., pirogues and paddles in marshes), and logistics support in addition to that already provided for under the response activity.

Safety

No evidence was provided to field personnel that exposure to bioremediation products was or was not a health risk. The possible microbial composition of the agent used in Marrow Marsh was provided among the documents reviewed (Texas General Land Office, 1990), but it is not clear to the author that this was the specific composition of the product used in Marrow Marsh. This is of potential concern since the list included at least one species of *Vibrio* bacteria, a genus that includes pathogenic species. Future application activities must be explicit about the composition of materials, what to do if there is contact or inhalation and what not to worry about if there is no problem.

Unregulated aircraft approaches, air boat transits, air cannon firing, cattle, deep soft mud, bitting insects, oil fumes, heat, minimal sun protection, potential for thunderstorm lightening strikes, inappropriate footwear, and, possible risk of snake bite, provided, on reflection, additional and probably greater hazards to successful conduct of bioremediation testing and monitoring in the marsh and safety of monitoring personnel and observers in open boats. Proper monitoring required many long hours (to dusk) in small open boats in a remote area and required unanticipated caution and patience. The resources and information needed to support a safe and effective treatment and monitoring program needs to be anticipated well in advance.

Attempts to test the effectiveness of bioremediation should be encouraged, but, in the near term, only under scientifically credible monitoring and surveillance. An intermediate step of conducting treatments in replicated large-scale mesocosms would go a long way toward increasing our confidence in the utility of this potential treatment method. With solid data from successful applications, we will learn how to use this potential tool.

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APPENDIX

MONITORING METHODS

This section describes the required methods used by staff of the Texas Water Commission to monitor the effectiveness and effects of the bioremediation application to Marrow Marsh, Galveston Bay, August 5-13, 1990.

Approval and Guidelines

On July 31, 1990 (Day 2), the Federal On-Scene Coordinator (OSC) was asked by the Chairman of the TWC to approve one or more applications of a bacterial bioremediation agent, Alpha Systems. Approval by the RRT was granted to the OSC on August 1 (Day 5) with the following restrictions and guidelines:

1. Application should be in areas where mechanical recovery could not be used.
2. A technical working group would be set up to monitor the process of bioremediation.
3. A credible, scientifically sound monitoring program should be conducted.
4. Application techniques should be acceptable to the USCG On-Scene Coordinator.
5. The application should stay away from commercial oyster beds if possible. And,
6. Application of the bioremediation agents should not interfere with other recovery operations.

Study Area

Marrow Marsh is a shallow area measuring approximately 1 km long by 0.2 km wide, occupied by four groupings of marsh plants:

Area 1 - a solid, nearly square block of marsh plants extending 100 m south of the Cedar Bayou entrance and bounded on the south by a long, straight, deep channel that runs due east-west;

Area 2 - a block of scattered marsh islets separated by numerous channels and extending another 100-150 m south of the deep channel;

Area 3 - a relatively open deeper area generally free of marsh plants extending 100 to 200 m south of area 2; and,

Area 4 - another area of scattered marsh islets extending to a small bluff or headland.

Southeast of the headland is Pasture Cove, a small, lagoon-like, protected inlet surrounded by shell and hard-packed mud beach with numerous hoofprints from cattle. There are small blocks of marsh grass both within the inlet and at its head.

Setting up Test Plots

Four test sites or plots were identified and set up in Area 2 on the morning of August 5, 1990. Plots to be treated were identified as A1 and A2; control plots were A3 and A4. Surface material and non-moveable material within a test plot were isolated from offshore channel water by three barriers:

1. Containment booms around each of the test plots.
2. Absorbant boom around the entire marsh.
3. Containment boom around the entire marsh.

Thus, any oil or other floating material outside the test plots, but within the marsh test area, was trapped within the marsh. In addition, these served to prevent additional oil from entering the marsh during the course of the monitoring.

Boom teams deployed an absorbent boom and a non-absorbent boom around the entire marsh complex. The marsh was entered by the monitoring and observation teams in two 14- to 16-ft skiffs; but, approach to marsh islets for sampling required use of a very small (2.5-ft by 7-ft) aluminum pram (pirogue). A joint state-federal team identified suitable marsh islets which were then marked with four stakes. At each site, a contracted boom team of two to three people entered the area, surrounding each of four plots or islets with at least 100 feet of anchored plastic containment boom. Plots were located approximately 150 - 200 ft apart. Setup took most of the morning through early afternoon.

Pretreatment Sampling.

Following booming, one or two personnel entered a boomed plot to take pre-treatment monitoring samples. Start and stop times were recorded and samples were taken in the following order:

- visual observations
- surface material (including visual description)
- subsurface water
- sediments and physical measurements

At each site, triplicate surface material/water and subsurface water samples were taken with 1-L glass (1 quart mason) jars previously cleaned with methylene chloride. Surface material (200 - 700 ml water and 5 to 20 ml of floating oil) was allowed to flow slowly into a tilted immersed jar. Subsurface water for chemical analysis was collected by immersing a capped jar inverted inside a plastic ziplock bag. Once immersed, the plastic bag was removed, any adhering oil allowed to resurface, and then the lid removed and the jar tilted up, allowing only subsurface water to displace the air. The jar was then re-capped and brought through to the surface. During the first day, only (pre- and post treatment) single bulk subsurface water samples for toxicity testing were collected by filling by immersion a clean 4-L plastic cubitainer.

Triplicate sediment samples were collected using a small (0.04-square meter) box core attached to a long pipe handle. A patch of sediment at marsh-edge and within intertidal depth was identified. The core was slowly pushed into the sediment, closed, and slowly withdrawn. Contained sediment was then slowly deposited into a clean plastic tray. If surface material was considered undisturbed, a clean plastic scoop[was used to scrape off the entire top (2 cm) surface, which was placed in a methylene chloride-washed 1-L jar. If the surface was disturbed, the sample was rejected and a new one taken.

The triplicate samples for each medium were taken at points separated by 2 to 10 feet in a somewhat random fashion dictated by the geometry of marsh and open water.

All water and sediment samples were stored on ice in plastic ice chests and kept cold or frozen until analyzed.

One sampling cycle took about 45 to 60 minutes per site or about 4 hours for the entire four-plot area.

Product Application

Following pretreatment sampling of the first two plots, the application contractor was called. The stock bioremediation solution was prepared by mixing ingredients in a 55-gallon barrel set in the center of a 14-foot outboard-powered boat. The bacteria mix, a lyophilized (freeze-dried) microbial agent (a white powder prepackaged in plastic bags) in corn starch (yellowish powder), and a blue-colored nutrient/micronutrient mix were added to ambient water in the barrel, with the solution brought to the top with ambient water. The blue nutrient mix was described as the commercial plant food, Miracle-Gro, by one official, but a spokesman for Alpha indicated that it was not Miracle-Gro, but a special nutrient/micronutrient mix. The specific content of the microbial and nutrient mixes was not determined. Each batch or stock solution contained 10 pounds of microbe/corn starch mix and either one pound (Alpha spokesman) or two pounds (EPA spokesman) of nutrient/micronutrient mix. The resulting mix produced a turbid solution with an overlying brown foam.

The content of the barrel, the stock solution, was fed through a short hose to a high-pressure pump operated by a gas-powered engine. An overboard intake hose delivered ambient water to the pump for diluting the stock bioremediation solution during spraying. Dilution was either 3 per cent (33.3 parts ambient water to 1 part stock) or 6 per cent (16.6 : 1). Diluted stock solution was sprayed on the marsh through a fire hose nozzle operated by hand in long, slow sweeping motions. Product was delivered to a reach of up to 30 to 50 feet.

In the agreed-upon test plot area, bioremediation material was sprayed on each of two test plots, A1 and A2. It was also applied on 30 to 60 m of marsh in between and around the treated plots, covering a width of at least fifty feet inside the water line. During the approximately 2-hour application period in mid-afternoon the wind shifted from south to north, and spray was observed blowing back toward the applicators and observers, and downwind toward areas designated as untreated, where pre-treatment sampling was underway.

Post-treatment Monitoring

Site treatment in Area 2, Plots A1 and A2, occurred between 1600 and 1800 on August 5, leaving only 2 to 3 hours of light to complete the first round of post-treatment sampling. Therefore, only the two treated sites were sampled within 1 to 2 hours of treatment. However, all sites (treated and untreated) were revisited at approximately 24, 48, and 96 hours post-treatment. Sampling at the two treated plots

1 to 2 hours after treatment, and at all plots 24 and 48 hours post-treatment was completed as described above for the pre-treatment sampling. Sampling thereafter was not observed by the scientist.

Distribution of Samples

The six 4-L subsurface water samples collected on the first day of sampling were shipped on ice in plastic chests to the U.S. Environmental Protection Agency laboratory in Gulf Breeze, Florida, for toxicity testing. Instructions were given to composite water from pre-treatment sites A1 with A2, post-treatment sites A1 with A2, and control sites A3 with A4. Water from each was of these three composites was diluted to 25%, 50% and 75% with filtered Santa Rosa Sound seawater and deionized water, and each tested in duplicate for 96 hour mortality to mysids (*Mysidopsis bahia*) and silversides (*Menidia beryllina*; EPA, 1990).

A total of at least 132 samples of surface material, subsurface water and surface sediment were collected, chilled and then frozen for subsequent chemical analysis. Samples were shipped to a U.S. Environmental Protection Agency laboratory in Edison, New Jersey, for analysis of petroleum hydrocarbons.

Additional Applications and Monitoring Chronology

Although the terminated observer oversight observations, treatment and monitoring at Marrow Marsh continued under TWC control. On August 9 (Day 13), monitoring was completed at the second application area (Area 2)in Marrow Marsh (96 hours). On August 13 (Day 16) the floating booms and remaining absorbent pads were removed and a second treatment was made throughout the entire Marrow Marsh (DuPont Environmental Remediation Services, 1990). Samples were collected by the TWC at six permanently marked sites before this treatment and at 24, 48, and 72 hours (August 14, 15 and 16) after treatment. Three sites were also sampled in an adjacent, less oil-impacted, and untreated marsh (Swan Marsh). Thus the test/control comparison in Area 2 of Marrow marsh was effectively terminated on August 13, or one week after the first treatment.

There were additional treatments. As noted previously, the northernmost block of Marrow Marsh, Area 1, was sprayed with bioremediation agent along the south edge on August 5. Samples of slick and sediment were collected by the scientist, other observers and the USCG but not, to the best of my knowledge, by the TWC.

Pasture Cove, 0.5 km southeast of the central part of Marrow Marsh, was treated with bioremediation agent on August 6, the material covering oil that had pooled in cattle hoofprints, depressions and grass in the hard-pan soil along the waterline. Sediment/oil samples were collected by a NOAA observer and by the TWC during treatment, but none were collected from a similar untreated control cove. Additional samples were collected by both groups on the following day, August 7.

On August 2, bioremediation agent was applied near sunset for approximately 10 minutes on a beach at Pelican Island, near Galveston. To the best of the knowledge of the there was no follow-up on the previous night's application.

Figure 5. Sampling test plot. Hundreds of oiled absorbant pads adrift in marsh channel.

Figure 6. Depressed marsh grass (*Sciripis* sp) in first treatment area.

Figure 7. Oil-soaked footprint and depressed marsh grass in first treatment area.

Figure 8. Oil leaking through opening in containment boom at a test site.

Figure 9. Application of bioremediation agent to marsh in second treatment area outside of test plot A1.

Figure 10. Open-water application of bioremediation agents near test site A1.

Figure 11. News helicopter landing in marsh near first treatment area.

Figure 12. Camera crew attempting to enter marsh near test site A3.

Figure 13. Sampling subsurface water at test site A1.

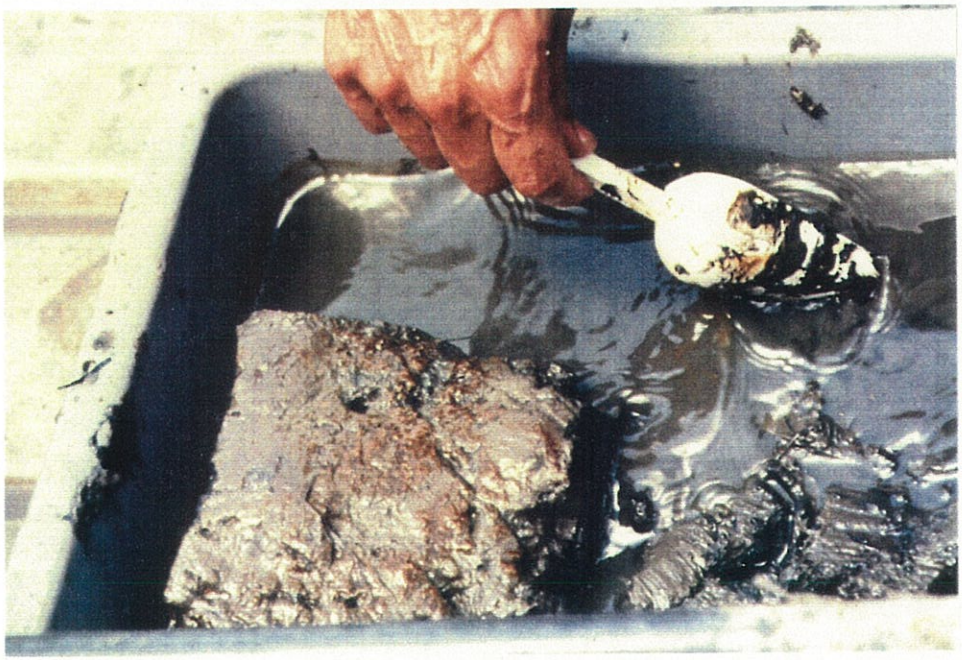

Figure 14. Sampling surface mud collected by grab at test site A1.

Figure 15. Pasture Cove, third application area. Aerial view before treatment, looking northeast.

Figure 16. Collecting sample of oil with yellow- and cream-colored spotty material several hours after treatment at Pasture Cove.