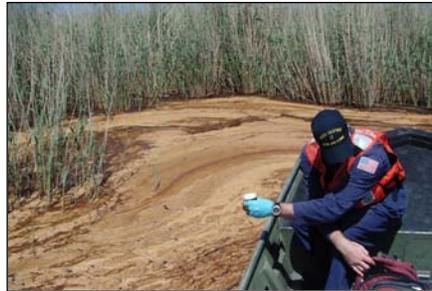


BIOREMEDIATION AND COASTAL WETLANDS

A REPORT FROM A TECHNICAL SYMPOSIUM
ON BIOREMEDIATION OF COASTAL WETLANDS
FOLLOWING AN OIL SPILL



ABOUT THE SYMPOSIUM

On August 31, 2010 a group of 37 experts and policy makers from academia, industry, government and oceanographic institutions came together in Pensacola Beach, Florida with the goal to discuss oil spill bioremediation – methods, effectiveness, issues and concerns – to determine possible applications and further research needed in the Gulf region. This symposium was sponsored by the Florida Sea Grant College Program, The University of Florida Institute of Food and Agricultural Sciences and The University of Cincinnati. The Scotts Miracle-Gro Company provided funding for the event.

The content of the day included presentations of background material on the current situation in the Gulf of Mexico, and conclusions from experiments with bioremediation and biostimulation in various sites over the past 20 years. Following discussion of the background material, the experts in this field were divided into teams to address key questions regarding the specific applicability of bioremediation methods for Gulf of Mexico habitats and conditions.

The day culminated in summaries of the team discussions, and determining shared themes, conclusions and recommendations from the participants.

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On the cover: Photos courtesy U.S. Coast Guard. Clockwise from top: South Pass, La., spill sampling, photo by Jesse Kavanaugh; St. Mary's Point, La., shoreline cleanup assessment, photo by Matthew Schofield; Plaquemines Parish, La., coastal marshes, photo by Timothy Tamargo; Bay Jimmy, La., skimming efforts, photo by Zac Crawford.



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PLENARY PRESENTATIONS

The Effects of the Gulf Oil Spill **Dr. George Crozier, Dauphin Island Sea Lab**

On April 22, 2010, the semi-submersible drill platform Deepwater Horizon sank in nearly 1,500 m of water in the northern Gulf of Mexico. After several attempts to close a failed blowout preventer valve, it became clear that estimates of 600 m³ (and other unofficial estimates as high as 4,000 m³) of oil were being released each day. Pre-approval of chemical dispersants (Corexit™) was made based on knowledge of the dispersant application over limited areas and for limited time *at the sea surface*. However, in this case, an unprecedented volume of dispersant has been applied both at the surface and through direct injection into the wellhead leak at 1,200 m depth. While (primarily) Federal agencies (NOAA, EPA, USCG/DHS) are seeking rapid answers to the toxicities of the dispersants used at these concentrations, duration and depth, the emphasis is on how dispersants may impact finfish, shellfish, turtle and bird safety and health. Moreover, reactive dispersants are held as trade secrets, though generally these have both solvent and surfactant fractions known to carry toxicity. To date there is no plan for understanding functional ecosystem shifts as a consequence of this magnitude of application of dispersants or the resulting re-distribution of oil or released compounds within the water column in addition to the shoreline habitats.

The confusion associated with what became the worst aquatic oil spill in the nation's history and the complexity of the crude oil all contributed to the problem in various habitats of the Gulf. Originally the concerns were greatest for the extraordinarily productive areas of submerged aquatic grasses, the emergent marshlands, and the oyster reefs of the estuaries. Human emotion and the accompanying economic benefits of the beaches elevated their plight despite the fact that they were possibly the most easily cleaned of shoreline habitats. Mechanical cleaning is currently being emphasized over possible biological action, perhaps to the detriment of the beaches considering the high probability of erosive events.

The shoreline damage was much worse in Louisiana and diminished with time and distance as the toxic elements "disappeared" as a result of dissolution (in the water column), evaporation (in the atmosphere), photodegradation and particularly microbial conversion of the carbon from crude oil components into the biosphere and remineralized to carbon dioxide. Impacted areas to the east showed decreasing impact with the "possible" issue of upwelling through the DeSoto Canyon east of Pensacola. This may prove to be a transport corridor for the dispersant-created subsurface "weathered" oil.

Anecdotal reports indicate that even possibly heavily oiled emergent marshes in Louisiana are currently showing remarkable and underestimated resilience. At this time there are estimates of only 1-4 square miles of marsh lost in a state that historically has lost 25 square miles per year. It is important to note however that this is actually several hundred miles of fringing marsh, therefore potentially affecting the important "edge" issues. In fact, the bottom line for the entire incident may be that the natural ecosystem is remarkably competent, due in large part to the thousands, if not millions, of years of the Gulf dealing with natural seeps of crude oil.

Field Experience in Oil Spill Bioremediation

Dr. Makram T. Suidan, University of Cincinnati

This presentation summarizes 20 years of field experimental research dealing with bioremediation, and a laboratory study using sediment from the site of the 1989 Exxon Valdez oil spill. Field experiments were conducted in Delaware, Quebec and Nova Scotia, and they examined the efficacy (in regard to hydrocarbon degradation) of different methods of bioremediation, as well as bio-augmentation (adding microbes).

The first experiment was conducted on a coarse sandy beach in Delaware. It examined bioremediation with nutrients, nitrogen (N) and phosphorus (P), vs. bio-augmentation with bacteria. Natural attenuation of hydrocarbons also was quantified with control plots. Natural attenuation of oil on the control plots was quite fast, perhaps because of the high level of N in the pore water (0.8 mg/L). Even so, increasing the pore water concentration of N to the 3-6 mg/L range enhanced the rate of hydrocarbon degradation. Inoculating the soil with bacteria did not significantly affect the hydrocarbon loss rate, and is not recommended as a general practice for bioremediation of oil. Even though bioremediation with nutrients had some effect, the natural level was high enough that it would be difficult to justify a full-scale bioremediation in this ecosystem in the event of a real spill, unless there were other extenuating circumstances.

The second experiment was conducted in a freshwater wetland associated with the St. Lawrence River in Ste. Croix, Quebec. In this case, the study examined whether bioremediation with nutrients could effectively stimulate degradation of hydrocarbons if the oil had penetrated below the soil surface. To simulate penetration, oil was raked into the sub-surface with a steel rake after it was applied to experimental plots. Treatments included oiling and no nutrients, no oiling with nutrients added (N and P), oiling with nutrients and plants removed, and a final treatment using a different form of N (NaNO_3) than in other treatments (NH_4NO_3). There was very little degradation of oil in any of the treatments, and it was concluded that bioremediation with nutrients may not be effective when oil has penetrated into the soil. Oxygen may be a limiting factor under these circumstances.

The third experiment was conducted in a salt marsh in Nova Scotia. The objective was to examine efficacy of bioremediation with nutrients, with vs. without the presence of the marsh plants. Oil was applied to the soil surface and treatments included controls and amendment with N and P. Hydrocarbon degradation, both with and without adding nutrients, was much more rapid than in the freshwater marsh in Quebec, likely because of greater oxygen availability at the surface vs. the earlier study that had sub-surface oiling. There were some treatment effects for alkane degradation (nutrient addition > controls), but not for degradation of polycyclic aromatic hydrocarbons.

Examination of the site of the Exxon Valdez Oil Spill (1989) conducted in 2001 and 2004 indicated that there was oil lingering in the pore water, and that roughly 11 hectares of shoreline was contaminated with 56,000 kg of subsurface oil. Degradation rates were thought to be limited by a lack of nutrients, low temperature, or low mass transfer rates of oil from the subsurface to the sediment-water interface. At a conference held in 2007, researchers concluded that further attempts to remediate under the site conditions would be futile. In 2010 my colleagues and I conducted controlled laboratory experiments using intact cores of oil-contaminated sediments from the site, subjected to amendments with nutrients and/or oxygen. We found that that oil remaining at the Exxon Valdez site was quite degradable despite its high degree of weathering. There was significant hydrocarbon degradation as long as oxygen was supplied. In this system, addition of nutrients would not likely lead to enhanced recovery, but physical perturbation of sediments, to expose more of the oil to an oxygenated environment (and greater microbial activity) might be effective – though the research did not examine economic or environmental feasibility.

**Field Environmental Conditions for the Persistence
Of the Exxon Valdez Oil in Prince William Sound, Alaska**

Dr. Michel C. Boufadel, Temple University

Dr. Makram T. Suidan, University of Cincinnati

Dr. Brian A. Wrenn, Temple University.

Oil from the Exxon Valdez spill in 1989 persists in the subsurface of gravel beaches in Prince William Sound and contains considerable chemicals harmful to fauna. Remediation of these beaches was stopped in 1992 because it was assumed that the disappearance rate of oil was large enough to ensure complete removal of oil within a few years. Based on field experiments and numerical simulations, we show that oil persistence is due to a small freshwater recharge into a two-layered beach (a high-permeability upper layer underlain by a low-permeability layer). The upper layer provided the oil a temporary storage for its slow, continuous filling of the lower layer wherever the water table dropped below the interface of the two layers as a result of small freshwater recharge from inland. Once the oil entered the lower layer, it became entrapped by capillary forces and persisted there due to nearly anoxic conditions resulting from the tidal hydraulics in the two-layered beaches. In addition, beach hydraulics results in a unique configuration for oxygen and nutrients. Dr. Boufadel discussed the biodegradation of oil in the beaches of the Gulf of Mexico and how to predict its persistence.

FURTHER READING ON BIOREMEDIATION RESEARCH

Michel C. Boufadel, Youness Sharifi, Benoit Van Aken, Brian A. Wrenn, Kenneth Lee. Nutrient and Oxygen Concentrations within the Sediments of an Alaskan Beach Polluted with the Exxon Valdez Oil Spill, *Environ. Sci. Technol., Article ASAP*, September 2010.

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Xueqing Zhu, Albert D. Venosa, Makram T. Suidan, and Kenneth Lee. *Guidelines for the bioremediation of marine shorelines and freshwater wetlands*, September, 2001. Retrieved from <http://www.epa.gov/oem/docs/oil/edu/bioremed.pdf>

BREAKOUT GROUPS

The breakout groups all recognized that there is natural degradation of hydrocarbons by microbes in the Gulf of Mexico, and that the focus of their discussions is on accelerating this process by various types of interventions, referred to here as “bioremediation.” We have generally chosen not to explore the highly technical biochemistry of crude oil and its breakdown into inert substances, although this is an important component of understanding the process. In the same manner we have paid only passing attention to the complex community of bacteria, fungi and protozoa that occurs in marine sediments and elsewhere, which by its complex ecological relationships and activities, has the capacity to degrade crude oil.

Group 1. Applicability of Bioremediation in the Gulf of Mexico

Participants: Charles Sidman, Facilitator; Ann Aquillo (Scotts), David Tsao (BP), Terril Nell (UF), George Henderson (FWC), Brian Wrenn, Chris Verlinde (UF), Jeff Mullahey (UF), Henry Norris (FWC)

Breakout Group 1 discussed bioremediation as applicable to the Gulf of Mexico Deepwater Horizon oil spill. Its report covers the definition of recovery, evaluation of recovery, the factors that limit bioremediation, barriers to bioremediation and the importance of public policy issues. The group agreed that Gulf of Mexico ecosystems will recover by natural means, but bioremediation may potentially accelerate the rate of recovery. The type of oil and some favorable physical factors may support remediation, particularly applied to beaches. However there may be long-term negative consequences that must be balanced against the short-term gains.

Recovery of Gulf ecosystems can be defined in ecological terms or in terms of human perception, and these are equally important. The public and commercial sectors are preoccupied with issues of economic effects, food safety and the perception that oil spill effects have been reversed. Ecologically, recovery might be defined as survival of intact fauna and flora and return to full ecological function. The time period after which a system might be considered ‘recovered’ is dependent on how recovery is defined and measured, and indicates the need for on-going monitoring to establish these parameters.

The ability of an ecosystem to recover from imposed oil depends upon a variety of factors including:

- The composition of the oil, including chemical and physical attributes
- Site-specific characteristics
- Pre-spill conditions at each site, and specifically the underlying health of the system

Factors limiting both natural recovery and bioremediation include:

- Oxygen
- Oil-water interfacial area
- Physical state of the oil, e.g., tar ball, slick, emulsion ‘mousse,’ dissolved microparticles
- Toxicity of the oil to fauna, flora and hydrocarbon degrading microbes
- Nutrients available to microbes that degrade oil

Barriers to bioremediation include both the characteristics of the oil itself and of the oiled habitat. These factors have a strong influence on the technological capacity to enhance remediation and the operational difficulty and cost of doing so. Logistical constraints include the availability of appropriate technology and materials and the resources and skills to apply them.

The implementation of remediation activities poses significant challenges in the public policy arena. Regulatory requirements (for instance, permits to manipulate nutrients in wetlands) could be time-consuming and complex.

The changing perceptions of the public and media – and the effects of these on decision makers – are a significant factor, and therefore effective communication on intentions, expectations and decisions will be needed. Remediation will be conducted in an environment in which the public and decision makers are not well informed about the process and there is a significant knowledge gap of appropriate science on which to base remediation efforts. Initial efforts could be to assess which locations and situations would best support remediation efforts and which are less amenable to intervention, either because of the technical constraints or the severity of collateral consequences.

Group 2. Methodology for Effective Bioremediation

Participants: Perran Ross, Facilitator; Leigh Ann Winn (Scotts), John Pardue (LSU), Concepción Calvo Sainz (Univ of Granada), Ron Out (Scotts), Andrew Zimmerman (UF), Makram Suidan (UC), Michel Boufadel (Temple), Grady Harmon (IMT)

Breakout Group 2 discussed the methodology of bioremediation as it might be applied in the Gulf of Mexico. The group's discussion covered the biological, biochemical and physical requirements for enhanced bioremediation, the different limiting factors in different coastal habitats, the actual activities that might be undertaken, special characteristics of the Gulf affecting remediation, the feasibility of remediation in different situations and the socio-political factors affecting implementation. Enhancement of natural remediation by addition of bacteria, nutrients and micronutrients, and manipulation of physical conditions is complex but technically feasible in some habitat types and situations. However, effectiveness and constraints will be both site-specific conditions and external factors such as permits, public perceptions, costs and the evaluation of the severity of unintended consequences.

The microbes that can degrade hydrocarbons occur naturally in diverse communities in which different kinds of bacteria have different capacities to degrade hydrocarbons. These communities change through time in response to the availability and characteristics of oil. It is possible to collect 'native' microbe communities from remediation sites, artificially grow these to produce larger quantities and high concentrations, and release these 'enhanced' bacteria back into the environment. However the quantity of bacteria may not be the most limiting factor. Access to oxygen, major nutrients (N and P in different forms and relative amounts) and micronutrients (e.g., Fe, Zn, Mg) are important. Other factors include: sediment particle size, pore and interstitial space features, the presence or absence of surfactants and oxidizing agents, and the form and chemical composition of the oil. Terrestrial soil and subsurface aquifers have been treated with grown bacteria, nutrients and complex mixtures of additional additives. Application in marine and tidal systems may pose significant difficulties of application and management of the distribution of applied remediation materials (e.g., loss to tidal flushing).

Factors affecting bioremediation

- Type of bacteria (aerobic/anaerobic)
- Numbers of bacteria, growth and succession
- Individual capacity of bacteria
- O₂ (probably not limiting in seawater)
- Nutrients N (in different forms NH₃, NO_x) PO₄
- Micronutrients – e.g., Zn, Mg, Fe, K
 - May be present in oil or sufficient in seawater. It is best not to rely on micronutrients within the oil. The presence in seawater is likely. However, iron might precipitate in the marshes and would need to be supplied.
- Oxidizer
- Surfactant
- Temperature (GoM surface warm)/depth cold
- Energy to mix (tide, wave, rain)

- Surface/particle size, particle/tide/beach/location
- Substrate, sand/mud/peat
- Methods of application, surface/injection
- Efficiency/cost

The group considered that it was unlikely that these factors could be applied or manipulated on a sufficient scale to be effective in the open ocean. Application on beaches, coastal marshes and possibly in shallow, inshore waters and embayments might be feasible.

To actually implement bioremediation the following activities could be conducted:

- Collect and study bacteria/type/amount
- Grow indigenous bacteria for re-release ¹
- Measure nutrients and evaluate need for additions and which ones
- Add nutrients²
- Manipulate O₂
 - Oxidizers, e.g., peroxide ²
 - Physical
 - Pump Water
- Add particles, adsorbents or absorbents, such as charcoal or activated carbon (*note: if added to enhance bioremediation, then needs to be 100% removed. Could also be added to protect wildlife from contacting oiled plants and marshes*).
- Change nature of oil (emulsion/Bio aid) (*note: surfactants, dispersants, and/or surface-washing agents need to be NCP-listed and need to lift and float the oil rather than disperse*).
- Add Micronutrients²

Note ¹: The re-released microbes would need to be listed on the National Contingency Plan Product (NCP) schedule prior to being released in marshes and waters. This entails specific tests to be conducted and processed for listing.

Note ²: Needs to be NCP-listed prior to adding to marshes and waters.

The Gulf of Mexico coast has a number of factors that affect bioremediation. Among these are the relatively warm surface water and substrate temperatures (45-85F/10-30C). Shoreline profiles range from moderate (beaches and coastal barrier dunes) to extremely flat (marshes). The daily tidal range is relatively small (approx 1m), restricting daily mixing but extending horizontally over large distances in flat marshes. Major mixing and energy input results from seasonal storms (summer hurricanes, winter storm fronts). The physical shape of the marsh is a highly complex fractal distribution of channels, bars and grass flats. For these reasons bioremediation is likely most practical in beach situations, less so but possible in selected marsh habitats and difficult in any submerged inshore situation.

As with Group 1, this group considered the socio-political factors to be of significant importance in determining the application of remediation. These factors include:

- Permits for manipulations
- Legal and liability considerations
- Unintended consequences
 - Addition of N and P may cause hypoxia and eutrophication
 - Toxicity (heavy metals, oxidizers) on other plants and animals
 - Physical effects of remediation (equipment, substrate disturbance)

The group weighed the logistical constraints and difficulties and possible unintended consequences against the effect of doing nothing (taking no action) except monitoring the progress of natural remediation and recovery.

Group 3. Monitoring Ecological and Water Quality Effects

Participants: Karl Havens, Facilitator; Jackie Whitehouse (UF), George Crozier (DISL), Leon Terlingen (Scotts), Tom Frazer (UF), Brian Silliman (webcast), Jan Valentic (Scotts), Carrie Stevenson (UF), Debbie Miller (UF), Gil McRae (FWC), David Morres (DEP)

Breakout Group 3 discussed the ecological concerns and monitoring needs of bioremediation. The group discussion covered: (1) whether natural processes are sufficient to allow recovery before taking remedial action; (2) how to initiate small-scale pilot projects to evaluate effects; and (3) how to use multiple endpoints (e.g., PAH reduction, ecological diversity and function) to measure success. In general, the Gulf region suffers from a lack of long-term data necessary to characterize the structure and function of its ecosystem. Experimental work is needed to determine the capacity of the system to deal with disturbance, as well as the natural rate of biodegradation sufficient to remove oil. The magnitude and timing of disturbance matter, and small-scale experiments are a logical first step. Initial experimental manipulations of nutrients and oxygen are needed, and the influence of prescribed and natural fire needs to be considered. Experiments should be evaluated using a variety of response measures, including reduction of toxic and bio-accumulated hydrocarbons, as well as responses of key groups of organisms.

The group proposed four key biological groups for monitoring that might reveal ecosystem response.

- Above- and Below-ground Productivity of Plants
- Larval and Juvenile Fish Abundance, Reproduction, and other Traits
- Microbial Community
 - Species Composition
 - Relative Abundance of Hydrocarbon Degrading Taxa
- Invertebrate Community Structure and Function

These organisms may respond to nutrient enhancement in an undesired manner if nutrient inputs exceed a threshold for effective recycling, so this kind of monitoring is critical whenever there is a plan to add nutrients to Gulf waters.

Natural coral reef and sea grass systems adjacent to treated beaches and marshes are likely to be highly sensitive to unintended consequences of nutrient enrichment.

GENERAL DISCUSSIONS, SHARED THEMES

Uncertainty

All participants concluded that caution must be exercised in moving forward with bioremediation in the Gulf of Mexico. This caution is based partially on uncertainties about the application of the process in particular Gulf coastal habitats, and concerns about the balance of benefit and cost in effects on coastal ecosystems. Available experimental evidence indicates relatively modest increases in oil degradation under some conditions. The basic questions always present are:

Will natural degradation provide adequate recovery?

Will the economic and ecological costs of enhanced bioremediation result in significant increases in recovery rate?

Directly following from these concerns is the need to test and inquire whether interventions such as nutrient enrichment are sufficiently effective to be worth both expense and the ecological consequences. This inquiry needs to occur before any interventions are initiated.

Site specificity

All three groups recognized that the application of bioremediation must be site-specific because each site has different characteristics, both of its inherent ecosystem and physical characteristics, and of any oil present. Responses and interventions will therefore have to be site-specific and matched to the needs and characteristics of each site. Available experimental evidence strongly supports site-specific remediation actions. Past interventions that had significant effects in one system had no effect in others.

Need to assess and monitor

All three groups and much subsequent discussion explored the need for detailed pretreatment assessment and post-treatment monitoring to evaluate the effect of any interventions. These include assessment of microbial hydrocarbon degraders at the site, nutrient and oxygen conditions, physical conditions of particle size, organic matter, existing flora and fauna and the nature of oil present. Post-treatment monitoring includes tracking the location, components of and degradation rates of oil, amounts and dispersal of nutrient and other additives within and away from the site and the consequences – both expected and otherwise – on numerous elements of the system.

Negative effects of treatment

Concerns were expressed by all groups that intervention and addition of nutrients, micronutrients, oxidizing agents and other additives might affect components of the system other than the target hydrocarbon degrading microbial community. Coastal systems are affected in many ways by excessive nutrient inputs. The most well-documented changes include increased macrophyte growth (sometimes of undesirable species such as cattail), blooms of harmful algae, reduced dissolved oxygen concentration, and other changes in system structure and function. Therefore, if the intention is to degrade oil by adding nutrients, what's needed is a careful assessment to determine that other undesired changes will not occur.

Socio-political issues

All three groups raised the concern that issues of public policy, regulation, public perception, media attention and economic drivers would strongly influence the decision to initiate bioremediation and affect how, where and when it was applied. These included both concerns that excessive caution and unjustified ecological concerns might inhibit a useful action, and that public and economic pressure might drive

remediation efforts without concern for ecological consequences. This concern received extended discussion in the general session. Participants familiar with the economic drivers for the tourism and seafood industries in the Gulf Coast suggested that the perception of oil contamination was as powerful a factor on local economies as the actual contamination and eventual effect. This created a strong social pressure for action to remove the perception of contamination, and bioremediation activity might fill that role, whether it was effective or not.

CONCLUSIONS AND RECOMMENDATIONS

The general conclusion that emerged in discussion was that bioremediation could be initiated on a site-specific, small-scale, experimental basis with strong monitoring to assess effects and accompanied by widespread and effective communication and outreach to ensure the best possible public and official understanding of the possible consequences, both positive and negative.

First steps to initiate such an approach would be to identify specific locations where suitable habitat and sufficient level of oil contamination combines with a location and scale that would allow well-controlled, pilot, experimental application of bioremediation techniques. The next step would be adequate pretreatment evaluation of sites, then the design of experimental approaches and monitoring programs to ensure adequate evaluation. Current developments in the federal BioChem Strike Team (BCST) might provide a vehicle to initiate these inquiries.

APPENDIX A: CONTENT/PROGRAM

- Welcome
 - Welcome from Event Sponsors – **Dr. Karl Havens**
 - Opening Remarks – Escambia County Commissioner, District 4 – **Grover Robinson**

- Background: Effects of the Gulf Oil Spill
 - Dr. George Crozier** - Executive Director, Dauphin Island Sea Lab (**p. 1**)

- Background: “Field Experience in Oil Spill Bioremediation – the Exxon Valdez 19 Years Later” (**p. 2**)
 - Dr. Makram Suidan** - Herman Schneider Professor of Environmental Engineering, University of Cincinnati

- Field Environmental Conditions for the Persistence of the Exxon Valdez Oil in Prince William Sound, Alaska (**p. 3**)
 - Drs. Michel C. Boufadel, Makram T. Suidan, Brian A. Wrenn** – Temple University, University of Cincinnati

- Breakout Group Discussions:
 - Lead Facilitator – **Dr. James Perran Ross** - Process and Results Expected
 - Three breakout groups with facilitators

 - Group 1: Applicability of Bioremediation for Rehabilitating Oiled Wetlands (or other areas) in the Gulf of Mexico (**pp. 4-5**)

 - Group 2: Methodology for Effective Bioremediation (**pp. 5-6**)

 - Group 3: Monitoring Ecological and Water Quality Effects (**p. 7**)

- Individual Group Reports and Discussion

- Total Group Discussion/Overall Summary of Recommendations (**pp. 8-9**)

- Further Reading (**p. 3**)

- Closing Remarks

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