Summary of the Literature on the Use of Commercial Bioremediation Agents for Cleanup of Oil-Contaminated Environments

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Objective and Scope of This Study

- To conduct a comprehensive review of the use of commercial oil spill bioremediation (bio) products in all environments.
  - Literature assessed includes peer-reviewed articles, company reports, government reports, and reports by cleanup contractors
  - Many reports on the use of bio products are non-peer reviewed or “gray” literature.
  - Note: all the reports collected were evaluated comprehensively for their scientific merit, and only those judged appropriate and scientifically sound are earmarked for inclusion in this document.

Objective and Scope of This Study

- Review is useful for oil spill responders (e.g., on-scene coordinators and response contractors) to better understand the feasibility of bio technology and as an aid in selecting bioremediation products.

Introduction and Background

- Bioremediation: “the act of adding materials to contaminated environments to cause an acceleration of the natural biodegradation processes”
- U.S. EPA has defined bioremediation agents as “microbiological cultures, enzyme additives, or nutrient additives that significantly increase the rate of biodegradation to mitigate the effects of the [oil] discharge”

Introduction and Background

- Bioremediation has emerged as a promising technology as secondary treatment option for oil cleanup
  - technology is based on the premise that a large percentage of oil components are readily biodegradable in nature
- Bioremediation has several potential advantages:
  - less costly
  - less intrusive to the contaminated site
  - more environmentally benign end products.

Success of Oil Spill Bioremediation

- Depends on one's ability to establish and maintain conditions that favor enhanced oil biodegradation rates in the contaminated environment with:
  - *Bioaugmentation,* in which known oil-degrading bacteria are added to supplement the existing microbial population
  - *Biostimulation*, in which the growth of indigenous oil degraders is stimulated by the addition of nutrients or other growth-limiting co-substrates.

Assessment of Bioremediation Products in the Field: Peer-Reviewed Literature

- Field studies can provide the most convincing demonstration of the effectiveness of oil bioremediation since laboratory studies are not always able to account for numerous real world conditions

Peer-Reviewed Literature

- Swannell *et al.* (1996) conducted the most extensive literature review available on field evaluations of oil bioremediation in marine environments.
- Venosa (1998) presented an in-depth critical review of research studies emphasizing extensive inadequacies in the experimental design and control of published field tests.

Peer-Reviewed Literature

- However, none of the existing reviews focused on the field performance of commercial bio agents.
- They did not distinguish bioremediation due to addition of commercial products from bioremediation due to application of common agricultural fertilizers/nutrient solutions or non-commercial microbial strains.

Peer-Reviewed Literature

- Few tests have been carried out to evaluate the effectiveness of bioremediation products in the field
  - difficult and expensive to conduct.
- Oil contaminated sites are often highly heterogeneous
- Physical and chemical weathering may significantly affect the composition and concentration of oil contamination.
- Variability can be so high it can interfere with one's ability to discern significant treatment differences.
- The efficacy of bioremediation in the field can be verified through well-designed monitoring programs and proper data interpretation.

Peer-Reviewed Literature

- Evidence for the effectiveness of oil bioremediation should include:
  - faster disappearance of oil in treated areas than in untreated areas
  - a demonstration that biodegradation was the main reason for the increased rate of oil disappearance
  - non-biodegradable or slowly biodegradable components in oil - biomarkers - have been used successfully to distinguish between biodegradation and the physical or chemical loss of oil

Application of Bioaugmentation Products

- Rationale for this approach
- Indigenous microbial populations may not be capable of degrading the wide range of potential substrates present in complex mixtures such as petroleum
- They may be in a stressed state as a result of the recent exposure to the spill.

Application of Bioaugmentation Products

- Venosa *et al*. (1992) conducted a field test in Prince William Sound following the *Exxon Valdez (EVOS)* spill to investigate the effectiveness of two commercial microbial products vis-à-vis natural attenuation and nutrient addition alone.
- Trial failed to demonstrate enhanced oil biodegradation by these products.
  - No biostimulation occurred in the nutrient control plots. No significant differences between any of the treatment and control plots during the 27-day trial period.

Field trials that claimed success in demonstrating the effectiveness of oil bioaugmentation

- Alpha BioSeaTM (Alpha Environmental, Inc.) to treat the Angolan Palanca crude oil spilled from *Mega Borg* off Texas coast (Mauro and Wynne, 1990; Swannell *et al*., 1996)
- TerraZymeTM (Oppenheimer Biotechnology) in enhancing biodegradation of a heavy oil spilled from *Nakhodka* in Japan (Tsutsumi *et al*., 2000).
- However, the success of these studies was based on either visual observation (i.e. the *Mega Borg* study) or digital photographic image analysis (i.e., the *Nakhodka* study).
- **No comprehensive monitoring program was used to verify the oil was indeed removed through enhanced biodegradation.**

Application of Biostimulation Products

- Most shoreline ecosystems heavily contaminated with hydrocarbons
  - Nutrients are likely the limiting factors in oil biodegradation.
  - One exception is wetlands
    - If oil has penetrated wetland or marsh sediment to any appreciable extent, the impact zone is anoxic or anaerobic
    - Oxygen limitation will be the predominant mechanism precluding effective treatment.

Application of Biostimulation Products

- Commonly used water-soluble nutrient products include mineral nutrient salts (e.g. KNO3, NaNO3, NH4NO3, K2HPO4, MgNH4PO4)
- This approach has been effective in enhancing oil biodegradation in many field trials (Swannell *et al*., 1996; Venosa *et al*., 1996)

Application of Biostimulation Products

- 1999 and 2000 - field study was conducted on the shoreline of the St. Lawrence River (Garcia-Blanco *et al.*, 2001; Venosa *et al.*, 2002)

- The authors concluded that nutrient amendment of an oil-contaminated freshwater wetland *where significant penetration of oil has taken place into the sediment* has limited potential for enhanced cleanup

Application of Biostimulation Products

- All these results suggest that the success of biostimulation is case specific:
  - oil properties
  - the nature of the nutrient products
  - the characteristics of the contaminated environments
  - when oxygen is not a limiting factor
  - keys for the success of oil biostimulation is to maintain an optimal nutrient level in the interstitial pore water

Application of Biostimulation Products

- Use of slow release fertilizers is one of the approaches used to overcome washout problems and provide continuous sources of nutrients to contaminated areas
- There are also readily available nutrient products normally in solid forms that consist of either:
  - relatively insoluble nutrients or water-soluble nutrients coated with hydrophobic materials such as paraffin or vegetable oils

Application of Biostimulation Products

- EVOS: Customblen (Sierra Chemical Co.), a slow-release granular fertilizer chosen to apply over 120 km of the oil-contaminated shorelines during 1989 and 1990
- This fertilizer consists of vegetable oil coated calcium phosphate, ammonium phosphate, and ammonium nitrate (N:P:K ratio 28-8-0)
- Results, product performed well on some of the shorelines of Prince William Sound

Application of Biostimulation Products

- Inipol EAP22 (Societe, CECA S.A., France) is currently listed on the NCP Product Schedule as a nutrient additive.
- EVOS: chosen as one of the nutrient products to use in the cleanup
- Sveum *et al.* (1994) indicated that this oleophilic fertilizer appeared to be more effective than water-soluble fertilizers when the spilled oil resided in the intertidal zone.
  - But advantages in enhancing oil biodegradation in the supratidal zone where water transport is limited.

Application of Biostimulation Products Conclusion

- Peer-reviewed literature on the use of
- bioremediation products has clearly
- indicated that biostimulation, if used
- properly, could be a cost-effective
- treatment tool for cleaning certain oil-
- contaminated environments.

Assessment of Oil Bioremediation Products: Non-Peer Reviewed Literature

- A thorough search of non-peer-reviewed and ‘gray’ literature for response to oil spills in inland, estuarine, and marine environments
- A comprehensive review of this information is presented in the full version of this paper.

Government Agency Reports

- Several field studies or applications on the
- use of commercial bioaugmentation
- agents have been published in
- government agency reports with mixed
- results
  - Mearns (1991), bioaugmentation field test of an oiled marsh in an estuary environment of upper Galveston Bay, Texas

Government Agency Reports

- August 1990, collision between three Apex Barges and the Greek tanker Shinoussa.
- Four plots were used in selected areas of Marrow Marsh:
  - 2 treated with a commercial bioaugmentation product (Alpha BioSeaTM, Alpha Env. Inc.)
  - two left untreated as controls

Government Agency Reports

- Oil constituents were determined using GC/MS and the extent of biodegradation was measured by the decline in the n-C18/phytane ratio in this study.
- Mearns later summarized lessons learned from this experiment:
  - bioremediation is not a rapid response tool
  - experimental design should meet basic statistical requirements
  - more comprehensive monitoring is needed to demonstrate the efficacy of treatment

Government Agency Reports

- Alaska Department of Environmental Conservation, Athey et al. (2001)
  - Published a manual on treating oil spills in tundra environments which compiled dozens of case studies
  - Five involved the use of bioremedial approaches, mostly biostimulation with agricultural fertilizers

Government Agency Reports Conclusion

- Several articles that cover bioremediation experience following the EVOS (Bragg *et al*., 1992; Pritchard *et al*., 1991; Venosa *et al*., 1990)
  - Field studies on the use of nutrient products have produced mixed results.
- For a detailed description of proper protocol for oil bioremediation field studies and evaluation, refer to EPA's *Guidelines for the Bioremediation of Marine Shorelines and Freshwater Wetlands* (Zhu *et al*., 2001)

Information Collection from Product Companies 2002

- 70 vendors of bio agents listed on the NCP Schedule or on *20th International Oil Spill Control Directory* (Oil Spill Intelligence Report) were contacted in regard to their interest in participating in EPA's case study review

- A total of eight vendors were willing to participate and submitted at least some the information requested

Information Collection from Product Companies 2002

- The amount and quality of the information submitted by vendors was highly variable
- Case study information mostly ranged from a few sentences to two pages
  - a few detailed technical reports of up to 50 pages
- Impossible to give a comprehensive review of each case based on this limited information

Summary of Vendor Reports

- Bio products have been applied to clean up oil contamination in various ecosystems and under a wide range of environmental conditions
- Applications include:
  - *in-situ* remediation of hydrocarbon contaminated marine shorelines
  - surface water, groundwater, and bilge water
  - *ex-situ* treatment of hydrocarbon contaminated soil (*e.g.*, using a land treatment unit) and water (*e.g.*, in a bioreactor)

Summary of Vendor Reports

- Major limitation of vendor case studies
  - due to the confounding of different effects, it is impossible to determine whether the claimed enhanced oil biodegradation, if true, resulted mainly from the addition of microbial cultures, nutrients, enzymes, oxygen, or any combination of above
- The technical merit of these company reports was generally not sound in terms of providing strong or even suggesting moderate scientific evidence for demonstrating the effectiveness of bio products.

Summary of Vendor Reports

- According to the peer-reviewed literature, bioaugmentation appears to have little benefit for the treatment of spilled oil in an open environment
  - Microbial addition has not shown to work better than nutrient addition alone in many field trials
- Biostimulation has been proven to be a promising tool to treat certain aerobic oil-contaminated marine shorelines

CONCLUSIONS

- Different nutrient products have shown variable effectiveness:
  - depending on oil properties
  - the nature of the nutrient products
  - the characteristics of the contaminated environments

CONCLUSIONS

- Bioremedial approaches may have a role in treating hydrocarbon contamination for non-point sources

- Limited information appears to suggest that application of bio agents could show promise for the treatment of hydrocarbon contamination in storm water

CONCLUSIONS

- The extreme uncertainty associated with the efficacy of bio agents is due to the poorly designed field tests that have been conducted to demonstrate efficacy
- Much of the reported literature lacked proper controls and treatment randomization and replication, or the data were incorrectly analyzed

- If there is any hope for advancement of commercial bioremediation, field experiments based on sound scientific principles are needed.

- THANK YOU
- Questions?