Bioresidementiation in Oil Spill Response

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Outline of Presentation

- Background and purpose
  - Definition of bioremediation
  - Process of biodegradation
- Biostimulation vs. Bioaugmentation
- Bioremediation on shorelines, land, and water: summary of research findings
- Product testing
- Guidance on implementation in the field
What is bioremediation?

- The exploitation of living microorganisms to convert organic contaminants into biomass and innocuous end products.

  - Innocuous end products include CO$_2$, water, and incompletely oxidized organics
Composition of Living Matter

- Among other trace substances, the primary components are:
  - Carbon
  - Nitrogen
  - Phosphorus
Uses of Carbon, Nitrogen, and Phosphorus

- **Carbon**: basic structural component of protoplasm and biomolecules
- **Nitrogen**: used in formation of amino acids for protein synthesis
- **Phosphorus**: used in formation of genetic material, high energy bonds, and membranes
What is the goal of a microorganism?
To make another microorganism!
Process of Biodegradation

- 1st step is addition of OH-group to alkane or PAH ring forming an alcohol

- Progressive oxidation to aldehyde, then to acid leading to chain length reduction

- In case of PAH, ring fission, then chain length reduction

- Eventually to water, CO$_2$, and biomass
Process of Biodegradation (cont’d)

- As oxygen added to hydrocarbons, compounds become more polar, more water soluble
- Polar compounds less toxic, more biodegradable
What are the requirements for successful bioremediation?
Requirements for Success

- Bacteria with **metabolic capabilities** must be present
- Rates of growth can be maximized by ensuring that:
  - Adequate supplies of oxygen and nutrients are present
  - pH between 6 and 9
Other Factors Affecting Success

- **Weight** of oil (lighter oils more biodegradable): asphaltenes inhibit diffusion

- Oil **surface area** important because biodegradation occurs mostly at the oil-water interface
Where are oil degraders found?

- Hydrocarbons are found everywhere
- Hydrocarbon degraders are also found everywhere
An oil spill causes huge influx of carbon (food). How does an organism cope?

- To convert carbon to biomass, bacteria must have nitrogen and phosphorus and oxygen.
- Bacteria will grow slowly in presence of usual amounts of N and P in nature.
- To maximize rate, give more N and P.
Biostimulation

The addition of factors (usually nutrients) that limit the ability of the degrading populations to grow at their maximum rate.
Bioaugmentation

The addition of highly competent microbial cultures to an impacted site to accelerate the biodegradation process.
Assumptions for Successful Bioaugmentation

- Culture must be able to grow rapidly on oil
  - Can be determined in lab
- Culture must be able to colonize the environment rapidly and out-compete the natural communities
  - Difficult to accomplish
- Culture must be able to grow to maximum carrying capacity of site
  - Success depends on washout rate
Fact #1: Microorganisms need N and P to convert oil to biomass

Fact #2: Oil degraders are everywhere

QUESTION 1: How necessary is it to add more oil degraders to an oil spill?
Answer: it is probably not necessary!
QUESTION 2: Is this true in all cases?

- For oil spills, probably yes
- For certain types of hazardous waste sites or environments, bioaugmentation could play a role; examples:
  - MtBE in groundwater
  - DNAPL (chlorinated organics)
Biodegradability of Oil

- **Gasoline**: bioremediation usually not needed (volatile)
- **Easily degradable**: diesel, heating oils, light and medium crude oils
- **More difficult to degrade oils**: heavy crudes, No. 6 Fuel Oil, bunker C
- **Vegetable oils/animal fats**: unsaturated oils oxidize and polymerize
Biodegradable Components

- Alkanes (saturates) \( (C_nH_{2n+2}) \): easy
- Polycyclic Aromatic Hydrocarbons (PAHs): moderate (depends on no. of rings)
- Heavy polar compounds (NSO): difficult
- Asphaltenes: very difficult
Saturates (Examples)

- **Straight Chain Alkanes**: easy to degrade
  Example: **HEPTADECANE** (17 carbons)

- **Branched Alkanes**: harder to degrade
  Example: **PRISTANE** (2,6,10,14-tetra-methyl-pentadecane)
PAHs (Examples):
Naphthalene (easy to degrade)
PAHs (Examples):
Methyl-Naphthalene (harder to degrade)
Nutrient Application

- Marine shorelines
- Wetlands
- Soil
- Water
Marine Shorelines

- Contamination occurs in upper intertidal zone—washout may be rapid
  - More so at spring than neap tide

- Design of effective nutrient delivery system requires understanding of transport phenomena
Nutrient Transport Driven by:

- Flow of freshwater from coastal aquifers
- Tides
- Waves (most important)
Wetland Environments

- Oil spills most likely to affect coastal marshes and wetlands
- Only research data available are ORD-funded studies in Quebec and Nova Scotia
Properties of Wetland Environments

- Usually oxygen limited, very quiescent (little wave action)
  - Become anaerobic a few mm below ground surface

- Likely not nutrient-limited
  - However, plants easily out-compete for available nutrients
How does one apply bioremediation to wetlands?

- Depends on goal:
  - If oil removal is main goal, natural attenuation may be the favored strategy, especially if penetration took place.
    - Biostimulation might still be appropriate if significant penetration has not occurred.
  - If ecosystem recovery is main goal, nutrient addition may speed restoration substantially.
Land Bioremediation – to be discussed by Kerry Sublette
Bioremediation on Water

- To be successful, all amendments must stay with the slick and not disperse

  - This is extremely unlikely, even with oleophilic fertilizers
  - Therefore, bioremediation on water not considered viable
Protocol for Testing Bioremediation Agents for Listing on the NCP Product Schedule
Existing Protocol

- Natural seawater
- Triplicate flasks
  - No-nutrient control
  - Nutrient control (no product)
  - Product with or without nutrients
    - Depends on vendor preference
- Incubate on shaker for 28 days
- Sacrifice flasks on days 0, 7, 28
- Extract and analyze by GC/MS
  - Also measure gravimetric weight of oil
  - Quantify hydrocarbon degraders by MPN
- Statistical analysis of results using ANOVA
Proposed Revisions

- Two environmental conditions
  - Seawater
  - Freshwater
- Replace natural waters with artificial
  - Sterilized
  - Standard inoculum provided by EPA
- Eliminate gravimetric analysis
- Eliminate MPN analysis
- Eliminate the 7-day sampling
- Eliminate the nutrient control
- Simplify the statistical analysis
Purpose of Standard Inoculum

- For living products (bacteria, etc.)
  - Inoculum used as a positive control to qualify the testing lab
- For non-living products (nutrients, etc.)
  - Product must support the inoculum in its biodegradation of the oil
Decision Rule

- To be listed, a product must demonstrate a statistically significant difference ($p < 0.05$) from the control in BOTH alkanes and PAHs after 28 days as determined by ANOVA.

- No need to pass both freshwater and saltwater tests unless vendor wants its product to be used in both places.
Guidance for Implementation of Bioremediation in the Field
Decision Tree for Selection and Application of Bioremediation

Step 1: Pretreatment Assessment

- Oil Type & Concentration
- Background Nutrient Content
- Shoreline Type
- Other Site Characteristics
If bioremediation selected:

Step 2: Bioremediation Planning

- Nutrient Products
- Nutrient Application Strategy
- Sampling and Monitoring Plan
Step 3: Implementation, Assessment, and Termination

- Analysis of Biodegradation and Physical Loss
- Toxicological and Ecological Analysis
Oil Type

- Higher API gravity (> 30°) oils easier to degrade

- Order of sensitivity:
  - $n$-alkanes > branched alkanes > low MW PAHs > cyclic alkanes > high MW PAHs > resins/asphaltenes
Step 1: Pretreatment Assessment

- **Oil Concentration**
  - **Low** (10s of mg/kg): less likely to be limited by N and P; thus, natural attenuation may be appropriate
  - **Intermediate** (1-60 g/kg): likely to be limited by N and P, may or may not need nutrient addition
  - **High** (> 80 g/kg or higher): may be inhibitory or toxic
Step 1: Pretreatment Assessment

- Background nutrient content
  - Prince William Sound: 1.5 mg N/L accelerated biodegradation rates
  - Brest, France: 1.4 mg N/L in background caused rapid biodegradation
  - Delaware: 0.8 mg N/L led to moderately rapid biodegradation of hydrocarbons

- Thus, if nutrient levels around 2 mg/L or less, natural attenuation is appropriate
Step 1: Pretreatment Assessment

- Types of shorelines
  - High energy not amenable: washout too rapid & waves scour organisms from substrate
  - Low energy favorable for nutrient application, must be aware of possible oxygen deficiency
  - Medium and coarse sandy beaches most favorable
  - Wetlands usually oxygen limited, not nutrient limited
    - If penetration not deep, may still be able to use bioremediation
Step 1: Pretreatment Assessment

- Other Factors
  - Climate: cold temperatures slow the process
    - Greater viscosity
    - Slower biodegradation due to slower metabolic rates
  - Prior exposure to oil: if none, lag or adaptation period greater
Step 2: Bioremediation Planning

- Nutrient Products
  - Nitrate- vs. Ammonium-based fertilizers
  - Environmental factors
    - Water soluble fertilizers
    - Slow-release fertilizers
    - Oleophilic fertilizers
Step 2: Bioremediation Planning

- Develop Application Strategy
  - Optimal nutrient concentration
  - Frequency of application
  - Methods of application
Step 2: Bioremediation Planning

- Optimal nutrient concentration
  - Microcosm studies
    - Continuous flow with $C_{17}$ on sand: 2.5 mg N/L supported maximal degradation
    - Continuous flow with crude oil on sand: 10 mg N/L supported maximal degradation
    - Tidal flow with crude oil on sand: 25 mg N/L supported maximal degradation
Optimal nutrient concentration

Field studies

- Prince William Sound: rates accelerated by 1.5 mg/L interstitial pore water nitrogen
- Brest France: rates no longer limiting at nitrogen concentrations > 1.4 mg/L
- Delaware: rates enhanced by maintenance of average 3-6 mg N/L in pore water

Thus, to enhance to near maximum rates, maintain 2-10 mg N/L in pore water
Frequency of nutrient addition
- Depends on tidal effects
  - Washout high at spring tides and high energy
  - Nutrient persistence longer at neap tides and low energy

Methods of nutrient addition
- 3 types of fertilizers:
  - Dry, granular (easy and flexible)
  - Liquid oleophilic (easy but expensive)
  - Water-soluble inorganic solutions (complicated equipment)
Resource Ratio Theory (first suggested in 1982 and confirmed in mid-1990s)

- Ratio of N:P affects community structure
  - Ratio of 10:1 is best for alkane degraders
  - For PAH degraders, P concentrations should be lower
- To better stimulate PAH degradation, use an N:P ratio of 100:1
Sampling and Monitoring Plan

- **Important variables**
  - Interstitial nutrients (very important)
  - Dissolved oxygen
  - Concentration of oil and its constituents (GC/MS and TPH)
  - Microbial activity (MPNs)
  - Environmental effects (ecotoxicity)
  - Others (temperature, pH)

- Samples should cover entire depth of oil penetration
- Statistical considerations
Step 3: Assessment and Termination

- Analysis of biodegradation vs. physical loss
- Ecosystem function analysis
Step 3: Assessment and Termination

How to confirm biodegradation is occurring

- Monitor changes in concentrations of individual oil constituents
- Normalize to a conservative internal marker
Physical vs. Biodegradative Loss

- **What are biomarkers?**
  - Complex organic compound(s) in oil composed of carbon and hydrogen
  - Derived from biomembranes
  - Known to be extremely resistant to biodegradation.

Step 3: Assessment and Termination
Assumptions for an Effective Biomarker

- Must be non-biodegradable
- Must have same or similar volatility and solubility as other oil components
- General classes of biomarkers:
  - Pristane and phytane (limited effectiveness)
  - 4-Ring alkylated PAHs (effective for months)
  - Hopanes and steranes (very effective)
Structure of $C_{30} - 17\alpha(H), 21\beta(H)$-Hopane ($C_{30}H_{52}$)
Other ways to assess progress

- Observe the relative rate of decline of alkanes
  - The higher the molecular weight, the slower the biodegradation
- Observe the rate of decline of parent PAHs to alkylated homologs
  - Alkylated homologs will biodegrade slower
Ecosystem Function Analysis

- Microbial response (MPN)
- Microtox (solid and liquid phase)
- Algal solid phase bioassay
- *Daphnia* survival
- Amphipod survival
- Gastropod (mollusc) survival
- Fish bioassays
CONCLUSIONS

- Bioremediation a proven technology
- Primarily a polishing step
- Relatively slow process (weeks to months)
- Toxic hydrocarbons destroyed, not just moved to another environment
- Biggest challenge: maintaining effective nutrient concentration
- If background nutrients are high, may not need to use bioremediation
CONCLUSIONS

- Bioaugmentation not likely to enhance biodegradation
- If impact area is high energy shoreline, bioremediation less likely to be effective
- Wetland environments may be amenable to natural attenuation
- Apply nutrients as dry granules
- Measure effectiveness by GC/MS, normalize oil components to hopane
- Conduct cadre of ecotoxicological assays to assess endpoints