Guidelines for the Bioremediation of Freshwater Wetlands

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Topics to be discussed

• Wetland environment
• Summary of St. Lawrence River Study
• Bioremediation on water
• Guidance for implementation of bioremediation
  ▪ Decision tree
    ◆ Pretreatment assessment
    ◆ Bioremediation planning
    ◆ Implementation, assessment, termination
  ▪ Conclusions
Wetland Environment

- Freshwater oil spills most likely to affect marshes and wetlands
- Only research data available is ORD-funded study in Quebec on St. Lawrence River

- Multiple plots studying effect of ammonium and nitrate addition with and without plants
St. Lawrence River Study

• Oil penetration very low due to wet, clayey soil (typical of all wetlands)
  ▪ Oil raked into top 3 cm to assure penetration
• Oxygen became limiting a few mm below ground surface
• Very quiescent, very little wave action
• Tidal effects
Treatments Studied

- Natural attenuation (no amendments)
- Ammonia addition with plants cut back to suppress growth
- Ammonia addition with plants intact
- Nitrate addition with plants intact
Change in Total Alkanes Normalized to Hopane

mg alkanes/mg hopane

time, weeks

- NA
- NH₃ Cut
- NH₃ Intact
- NO₃ Intact
Change in Total PAHs Normalized to Hopane

mg PAHs/mg hopane

time, weeks

- NA
- NH₃ Cut
- NH₃ Intac
- NO₃ Intac
Summary St. Lawrence Findings

- No treatment differences noted for biodegradation of total alkanes and PAHs except for plots with plants cut
  - Highly suggestive that oxygen was limiting
  - Presence of healthy plant roots may be important for biodegradation to take place
  - More physical loss of oil from plots with plants cut back
Conclusions from St. Lawrence Study

• Biostimulation may not be appropriate for rapidly degrading oil in a contaminated freshwater wetland *if significant oil penetration has taken place*

• Lack of oxygen is the most likely cause for the retarded biodegradation in a wetland where oil has penetrated to any significant depth

• If restoration is the primary goal, fertilizer addition might be appropriate
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
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
Step 1: Pretreatment Assessment

- **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 to 100s of mg/kg): less likely to be limited by N and P; thus, natural attenuation may be appropriate

  ▪ **Intermediate** (~1-80 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**
  - Determine background concentration of N, P
  - Determine historical range of N, P at the spill site
    - If low, biostimulation likely to be effective
    - If high, consider natural attenuation
Step 1: Pretreatment Assessment

- **Types of shorelines**
  - High energy not amenable: washout too rapid and 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
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

- Treatability studies and considerations
  - Tiered screening protocol for testing products and listing on the NCP Product Schedule
  - Microcosm tests: batch and semi-continuous or continuous flow
  - Nitrate- vs. Ammonium-based fertilizers
  - Human and ecotoxicity impacts
  - Environmental factors
    - Water soluble fertilizers
    - Slow-release fertilizers
    - Oleophilic fertilizers
Step 2: Bioremediation Planning

- **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
Step 2: Bioremediation Planning

• **Optimal nutrient concentration**
  - **Field studies**
    - Prince William Sound: rates accelerated by 1.5 mg/L 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
Step 2: Bioremediation Planning

• 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
  ▪ 4 types of fertilizers:
    ♦ Slow-release briquettes (problematic)
    ♦ Dry, granular (easy and flexible)
    ♦ Liquid oleophilic (easy but expensive)
    ♦ Water-soluble inorganic solutions (complicated equipment)
Step 2: Bioremediation Planning

- **Sampling and Monitoring Plan**
  - **Important variables**
    - Interstitial nutrients *(very important)*
    - Dissolved oxygen
    - Concentration of oil and its constituents *(GC/MS)*
    - Microbial activity *(MPNs)*
    - Environmental effects *(ecotoxicity)*
    - Others *(temperature, pH)*
  - **Samples should cover entire depth of oil penetration**
  - **Statistical considerations**
Step 3: Assessment/Termination

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

• How To Measure Biodegradation
  ▪ Must be able to distinguish between physical vs. biodegradative loss
  ▪ Normalize to a conservative internal marker
  ▪ Monitor changes in concentrations of individual oil constituents
Step 3: Assessment/Termination

• Physical vs. Biodegradative Loss

- Distinguished by measuring biomarkers
- Biomarkers (molecular fossils) found in oil are complex organic compounds:
  - Composed mostly of carbon and hydrogen
  - Show little or no change in structure from parent compound in living cells
  - Highly resistant to biodegradation
Step 3: Assessment/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**
  - Acyclic Diterpanes (pristane and phytane)
  - Cyclic Triterpanes (hopanones, steranes)
Structure of $C_{30}^{\text{17\textae}(H)}, 21\textendash(H)$-Hopane ($C_{30}H_{52}$)
Step 3: Assessment/Termination

- **Normalize Data to Biomarker**
  - Measure concentrations of individual oil components, including hopane
  - Divide the concentrations of each component by the concentration of hopane
  - Losses will be adjusted for physical loss
Step 3: Assessment/Termination

- What If Oil Has No Biomarker?
  - Normalize to a less readily biodegradable constituent, such as C₂-, C₃-, or C₄-chrysene

- Observe the relative rate of decline of alkanes
  - The higher the molecular weight, the slower the biodegradative loss

- Observe rate of decline of parent PAHs to alkylated homologs
  - Alkylated homologs will biodegrade slower
Step 3: Assessment/Termination

- 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
- Not considered a primary response technology
- Relatively slow process (weeks to months)
- Toxic hydrocarbons destroyed, not just moved to another environment
- Biggest challenge: maintaining nutrients in pore water
  - For wetlands, achieving aerobic conditions
- If background nutrients are high, may not need to use bioremediation for cleanup
  - Could still be considered for ecosystem recovery
CONCLUSIONS

• Bioaugmentation not likely to enhance biodegradation
• If impact area is high energy shoreline, bioremediation less likely to be effective
• Apply nutrients as dry granules at intermittent intervals
• Measure effectiveness by GC/MS, normalize oil components to hopane
• Conduct cadre of ecotoxicological assays to assess endpoints other than hydrocarbon concentrations