Microbial Population Dynamics in the Phytoremediation of Petroleum Hydrocarbons in Hawaii

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Collaborative Greenhouse Project

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Francoise M. Robert – Microbial Aspect
PETROLEUM HYDROCARBON PHYTOREMEDIATION

DEGRADATION OF HYDROCARBONS BY MICROORGANISMS STIMULATED IN THE ROOT ZONE
RHIZOSPHERE:

Layer of soil influenced by root exudates and sloughed-off root cells

(< 2-3 mm)
Possible Plant-root Effects on Hydrocarbon Degraders

1) Population increase by root exudates & dead plant cells

2) Enzyme induction by analogs, e.g. phenolics, waxes etc.

3) Enhanced bioavailability of PHC by biosurfactants
POSSIBLE PLANT-ROOT EFFECTS ON HYDROCARBON DEGRADATION

• Cometabolism

Microorganism #1

Root exudates \rightarrow CO2 + H2O
(for growth)

Cyclohexane \rightarrow Cyclohexanol
(Not for growth) (Not for growth)
Main Screening Experiment
Microbial Aspect of Main Screening Experiment in the Greenhouse

Objectives

• Evaluate plant and contaminant influence on populations of hydrocarbon degraders

• Determine the effect of microbial population size on plant hydrocarbon reduction
Main Screening Experiment
Microbial Enumerations

- Rhizosphere of 6 plants and unplanted soil
- Contaminant (0 & 10,000 mg diesel/kg soil)
Various Types of Diesel Constituents

Various Diesel-degrading Microbes
Microbial Enumerations

- Total bacteria
  - R2A plate counts
- Phenanthrene degraders
  - Phenanthrene-overlay (mineral medium)
- Diesel degraders
  - Most-probable-number
- Pristane degraders
  - Most-probable-number
RESULTS
DIESEL CONTAMINANT DEPLETION
AT 98 DAYS (10,000 MG /KG SOIL SPIKE)

Day 0

Day 98

<table>
<thead>
<tr>
<th>Plant Type</th>
<th>Day 0 TPH-D (mg/kg)</th>
<th>Day 98 TPH-D (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No plant, d 0</td>
<td>10000</td>
<td>9000</td>
</tr>
<tr>
<td>Nerium oleander</td>
<td>8000</td>
<td>7000</td>
</tr>
<tr>
<td>Beach naupaka</td>
<td>6000</td>
<td>5000</td>
</tr>
<tr>
<td>False sandalwood</td>
<td>4000</td>
<td>3000</td>
</tr>
<tr>
<td>Common ironwood</td>
<td>2000</td>
<td>1000</td>
</tr>
<tr>
<td>No plant, d 98</td>
<td>10000</td>
<td>9000</td>
</tr>
<tr>
<td>Kou</td>
<td>8000</td>
<td>7000</td>
</tr>
<tr>
<td>Milo</td>
<td>6000</td>
<td>5000</td>
</tr>
<tr>
<td>Kiawe</td>
<td>4000</td>
<td>3000</td>
</tr>
<tr>
<td>B</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Major Questions

• Which source of carbon (diesel or plant) results in the highest microbial populations?

• Is there a correlation between rhizosphere population size and hydrocarbon depletion?
Populations of Total Bacteria

![Bar graph showing the populations of total bacteria over time for different conditions. The x-axis represents different treatments labeled S, K, KO, M, N, B, and F, and the y-axis represents the log of the number of microorganisms per gram of soil. Arrows indicate Day 0 and Day 98.](image-url)
Phenanthrene-Degrading Bacteria

![Graph showing the log of the number of microorganisms (g of soil) over time for different treatments. The x-axis represents different treatments labeled S, K, KO, M, N, B, and F. The y-axis represents the log of the number of microorganisms per gram of soil. The graph includes data from Day 0 and Day 98. The legend indicates the presence of a control and a die condition.](image-url)
Pristane Degraders

Day 0

Day 98

Log of No. of microorganisms / g of soil
Diesel Fuel Degraders

Control
Die

Day 0
Day 98
HYDROCARBON-DEGRADING POPULATION SIZE IS NOT A GOOD INDICATOR OF PLANT PROMOTION OF DIESEL DEGRADATION
Microbial Populations

Total Bacteria

Phenanthrene & Pristane Degraders

Diesel Degraders
Diverse Group of Diesel Degraders

Milo Exudates

Diesel

Sandalwood Exudates
Phytoremediation Benefit Model
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  – Mike Thomas
• C. Ray
  – Jennifer Morita
• C. Ray
  – Kei Kitakata
Major Conclusions

- Increased number of hydrocarbon-degraders by diesel alone and all uncontaminated plants.
- Large populations of hydrocarbon degraders in contaminated rhizospheres is not a reliable indicator of phytoremediation success.
- Three different types of carbon preferences were observed among the soil microorganisms.
- Qualitative differences in root exudates potentially account for differences in plant performance in hydrocarbon-level reduction.
Soil Sampling for Microbial Enumerations

• Rhizosphere
  – Roots with layer of soil < 1 mm
  – Roots shaken in mineral medium for 1 hour
  – Serial dilutions in mineral medium

• Bulk Soil
  – Decimal dilutions in mineral medium after 1-hr shaking
Rhizosphere Effect (R/S)

\[ R/S = \frac{\# \text{ microorganisms in rhizosphere}}{\# \text{ microorganisms in bulk soil}} \]

At the field site:

- Kiawe, \( R/S = 28 \) for total bacteria
- Buffelgrass, \( R/S = 60 \) for total bacteria
Greenhouse Simulation of Phytoremediation at the Field Site in Trisector-Planters

Sandy Loam
Silt
Sandy Loam
Microbial Populations at the Field Site

![Bar graph showing microbial populations at the field site.](image_url)
The Experiment

• Roots were grown in lower section with the contaminant for 200 days
• Contaminant was 6 individual diesel constituents
  – 3 n-alkanes (hexadecane, eicosane, docosane) (500 mg/kg each)
  – 2 PAHs (phenanthrene, pyrene) (200 mg/kg each)
  – 1 branched alkane (pristane) (200 mg/kg)
• Microbial populations enumerated for kou, false sandalwood, and unplanted soil
Hydrocarbon Reduction
Plant Influence in the Presence of Contaminant

![Graph showing log # microorganisms/g soil for Kou, FSW, and Soil samples with labels for total bacteria, phen degradation, and hexadecane degradation.]
Correlation Between Population Size and Hydrocarbon Degradation

- **Kou**
  - Reduced Phen (>69%) and Hex (27.5%)

- **FSW**
  - Reduced Phen (>69%); hex (0%)

- **Soil**
  - Reduced Phen (<31%); hex (0%)
### AERATED TRISECTOR-PLANTERS
*(ALL TREATMENTS ARE CONTAMINATED)*

<table>
<thead>
<tr>
<th></th>
<th>TOTAL BACTERIA</th>
<th>PHE – DEG. BACTERIA</th>
<th>HEXADECANE DEGRADERS</th>
</tr>
</thead>
<tbody>
<tr>
<td>FALSE SANDAL WOOD</td>
<td>5</td>
<td>23</td>
<td>955</td>
</tr>
<tr>
<td>KOU</td>
<td>7</td>
<td>38</td>
<td>7413</td>
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</table>
RHIZOSPHERE EFFECT (R/S) IN MAIN SCREENING EXPERIMENT (AVERAGE OF 6 PLANTS)

<table>
<thead>
<tr>
<th></th>
<th>TOTAL BACTERIA</th>
<th>PHE. - DEG. BACTERIA</th>
<th>DIESEL- DEG.</th>
<th>PRISTANE- DEG.</th>
</tr>
</thead>
<tbody>
<tr>
<td>WITHOUT DIESEL</td>
<td>34</td>
<td>46</td>
<td>257</td>
<td>19</td>
</tr>
<tr>
<td>WITH DIESEL</td>
<td>5</td>
<td>Ca. 1</td>
<td>Ca. 1</td>
<td>Ca. 1</td>
</tr>
</tbody>
</table>