Biodegradation of Emulsified MC252 Oil in Coastal Salt Marshes
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Outline

• Emulsified MC252 oil
• What we knew about biodegradation in salt marshes
• Initial salt marsh core studies using emulsified MC252
Emulsion or “mousse”- primary form of oil at coast
Stability of oil:water emulsions

Stable emulsions form due to surface-active components: asphaltenes and resins

From McLean and Kilpatrick, 1997
Outline

• Emulsified MC252 oil
• What we knew about biodegradation in salt marshes
• Initial salt marsh core studies using emulsified MC252
1. Both aerobic and anaerobic microbial processes involved in crude oil biodegradation in salt marshes
2. Mixed results for fertilization impacts on acceleration of crude oil components in marshes

<table>
<thead>
<tr>
<th>Sum of Alkanes</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0054 ± 0.0027</td>
</tr>
<tr>
<td>0.0059 ± 0.0025</td>
</tr>
<tr>
<td>0.0058 ± 0.0009</td>
</tr>
</tbody>
</table>
3. Oil limits oxygen flux by creating a physical barrier but also increases $O_2$ demand from 3-5x through increased aerobic and sulfate reduction (sulfide oxidation)
Outline

• Emulsified MC252 oil
• What we knew about biodegradation in salt marshes
• Initial salt marsh core studies using emulsified MC252
Study Details

- Salt marsh cores (Lafourche Parish, LA) 6” dia × 36” long and incubated inside a greenhouse.
- Emulsified oil (100 g) added to core surface
- Replicate cores used to evaluate three treatment conditions; (i) natural attenuation with existing nutrient concentrations, (ii) nitrogen amendment and (iii) nitrogen + sulfate amendment.
Study Details

• **Short-term biodegradation** (0-3 months). For each treatment condition, cores were sacrificed for oil analysis every ~30 days. The cores were cut into 2 cm sections from the top and oil was extracted and analyzed from the soil sections 0-2 cm, 2-4 cm and 8-10 cm (GC-MS of PAHs, denaturing gradient gel electrophoresis, nutrients)

• ** Longer-term** (320 days)-replicate cores sacrificed after ~10 months
# MC252 PAH Compounds

(mg PAH/kg soil after 100 mL of emulsion)

<table>
<thead>
<tr>
<th>Compound</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Naphthalene</td>
<td>(&lt;0.1)</td>
</tr>
<tr>
<td>- C1-NAP</td>
<td>0.51</td>
</tr>
<tr>
<td>- C2-NAP</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>- C3-NAP</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>- C4-NAP</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>Phenanthrene</td>
<td>(3.1)</td>
</tr>
<tr>
<td>- C1-PHEN</td>
<td>15.2</td>
</tr>
<tr>
<td>- C2-PHEN</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>- C3-PHEN</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>- C4-PHEN</td>
<td>0.53</td>
</tr>
<tr>
<td>Dibenzothiophene</td>
<td>(&lt;0.1)</td>
</tr>
<tr>
<td>- C1-DBZ</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>- C2-DBZ</td>
<td>8.0</td>
</tr>
<tr>
<td>- C3-DBZ</td>
<td>5.6</td>
</tr>
<tr>
<td>Chrysene</td>
<td>(2.5)</td>
</tr>
<tr>
<td>- C1-CHRYS</td>
<td>1.3</td>
</tr>
<tr>
<td>- C2-CHRYS</td>
<td>1.2</td>
</tr>
<tr>
<td>- C3-CHRYS</td>
<td>1.0</td>
</tr>
</tbody>
</table>

\[ \Sigma \text{PAHs} = 50-70 \text{ mg/kg in 0-2 cm} \]
Phenanthrene and C1-phenanthrene

CTRL

N added

N+sulfate
SHORT-TERM

C2-dibenzothiophene  C3-dibenzothiophene

CTRL  N added  N+sulfate
Distribution of ΣPAH’s with depth

SHORT-TERM

0-2 cm

2-4 cm

8-10 cm

ug's

0 5000 10000 15000 20000 25000 30000 35000
Phenanthrene and C1-phenanthrene

![Bar chart showing compound/C30 hopane levels for CTRL, N added, and N+sulfate after 326 days.

- CTRL: 1.6
- N added: *
- N+sulfate: lower levels

After 326 days, the chart indicates a significant increase in the compound/C30 hopane ratio for the CTRL group, while the other groups show lower values. The asterisk (*) indicates a statistically significant difference compared to the CTRL group.]

LONGER-TERM
LONGER-TERM

C2-dibenzothiophene  C3-dibenzothiophene

after 326 days
Distribution of $\Sigma$PAH’s with depth

LONGER-TERM

[Graph showing the distribution of PAHs with depth, comparing 0-2 cm and 2-4 cm layers between the N added and CTRL conditions.]
DGGE-SRB on 0-2 cm soil samples

All sequenced bands were identified as *Anaerolinea spp* (Chloroflexi) with similarity indices ranging from 0.71-0.75.
<table>
<thead>
<tr>
<th>CTRL 62d</th>
<th>N add 62d</th>
<th>N/S 62d</th>
<th>CTRL 91d</th>
<th>N add 91d</th>
<th>N/S 91d</th>
<th>Replicate</th>
<th>CTRL</th>
<th>cores</th>
<th>326 days</th>
</tr>
</thead>
</table>

Eubacterial-DGGE on 0-2 cm soil samples

Diverse populations in all treatments

*Bacteroidetes spp*
Future directions

Resolve differences in PAH degradation studies in marshes

FIGURE 1. (A) Total (C15–C44) alkane hopane ratio versus time in aerated microcosms. (B) Total PAH (phenanthrene, C1–C2; naphthalene, C1–C2) hopane ratio versus time.