MICROBIAL BIOREMEDIATION OF POLYCYCLIC AROMATIC HYDROCARBONS (PAHS) IN OILY SLUDGE WASTES

M I C K Y V I N C E N T
**I N T R O D U C T I O N**

- Petroleum-hydrocarbon compositions vary greatly in its complex mixture of hydrocarbons and other organic and inorganic compounds.
  1. The saturates
  2. The aromatics
  3. The resins
  4. The asphaltenes

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**Location of refinery**

- Ontario
- Quebec
- Western Canada
- Eastern USA
- Latin America
- South East Asia
- Middle East

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- The aromatics are often referred to as Polycyclic Aromatic Hydrocarbons (PAHs).
• PAHs are fused-ring compounds that are structurally complex.
• They are highly recalcitrant under normal conditions because of their strong molecular bonds.
• PAHs are mainly found in the areas surrounding petroleum-refining plants, accidental oil spills and pipe leakages, and rainwater runoff from roadways.
• Many of the constituents of PAHs are not only carcinogenic and mutagenic, but they are also potent immunotoxicants.
• PAHs impact are reported on critical habitats such as the benthic ecosystems, which may ultimately get into the marine food chain.
• Generally, PAHs and other hydrocarbons compounds are readily biodegraded and eliminated from the environmental by indigenous microorganisms, such as bacteria and fungi.

• It was only after high profile incidences like the Exxon Valdez oil spill (1989) that EPA was finally forced to establish all out researches to determine the viability of microbial PAHs degradation for bioremediation.

• According to Phillips (2000), “Biodegradation can be an effective and inexpensive approach to remediating soils which contain PAHs and other hydrocarbon compounds”

<table>
<thead>
<tr>
<th>Oily waste</th>
<th>Initial oilconc. (ppm)</th>
<th>Oil degradation (%)</th>
<th>Time (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drilling oil</td>
<td>50,000</td>
<td>99.0</td>
<td>7</td>
</tr>
<tr>
<td>Drilling mud</td>
<td>50,000</td>
<td>90.0</td>
<td>14</td>
</tr>
<tr>
<td>Lubricant oily sludge</td>
<td>50,000</td>
<td>85.0</td>
<td>10</td>
</tr>
<tr>
<td>Wastewater oily biosolids</td>
<td>26,000</td>
<td>92.3</td>
<td>10</td>
</tr>
<tr>
<td>Oily clay fines</td>
<td>52,000</td>
<td>91.8</td>
<td>14</td>
</tr>
<tr>
<td>Coker catcher fines</td>
<td>63,000</td>
<td>89.5</td>
<td>21</td>
</tr>
</tbody>
</table>
• Microbial bioremediation of PAHs from oily sludge wastes are dependent on these three factors:
  1. Physical characteristics of the PAH constituents.
  2. The choice of microbial consortium.
  3. Factors affecting the biodegradation mechanism.
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2. The choice of microbial consortium.
3. Factors affecting the biodegradation mechanism.

- The PAHs molecular sizes affects bioavailability greatly.
- Prolonged exposure to soil particles reduces biodegradation.
- Total PAHs concentrations is also an important determinant. Documented recommended concentration is around 5% (>10% can be toxic).
- As a rule of thumb: If a hydrocarbon sludge contains more than 10% oil, oil recovery procedure is recommended prior to the bio remediation.
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Indigenous microbes vs. introduction of foreign consortia?
Wild strains vs. engineered bugs (GMOs)?
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BIOREMEDIATION STRATEGIES

- Biosurfactants.
- pH.
- Nutrients.
- Salinity.
- Oxygen.
- Temperature.
- Water activities/moisture contents.
The chosen strategy for microbial bioremediation of PAHs is to integrate biostimulation, bioaugmentation of biopiles.

1. Effective, low cost and causes minimal environment impact.
2. A confinement made of concrete can prevent excessive run-off or absorption into surrounding soil.
3. Capacities to handle as much as 10,000 m³ oily sludge per year.
4. Elimination of Volatile Organic Compounds (VOC)
   - *Rhodococcus spp.* and *Pseudomonas spp.* Had the ability to degrade the volatile fraction 45% and 55% in 2 days and 4 days, respectively.
5. Denitrification of nitrogenous compounds.
   - Crude oil contains up to 2.1% nitrogen and nitrogenous hydrocarbons that are both toxic and mutagenic.
   - Bacterial species such as *Azoarcus, Bacillus, Brevibacterium,* and *Corynebacterium.*
QUANTIFYING BIOREMEDIATION

- A battery of chemical analysis, for target contaminant levels, and bioassays for measuring soil toxicity to be done to ensure efficiency of bioremediation strategies:

1. Bioassays.
   - Soil toxicity test by performing the response of Sheep Red Blood Cells (SRBC), lettuce seed germination and earthworm survival assays were performed by several researchers.
   - Effluent toxicity test can be assessed by using *Daphnia similis*.
   - Resting-cells assay by using the cells of *Pseudomonas stutzeri* P-16 and *P. saccharophila* P-15.

2. Chemical analysis.
   - Normally performed by using Gas Chromatography Mass Spectrometer (GC-MS) and Flame Ionization Detector (FID).
Changes in Crude Oil Composition during Bio-

Conc., wt.% in soil

<table>
<thead>
<tr>
<th></th>
<th>Asphal</th>
<th>NSOs</th>
<th>Aros</th>
<th>Sats</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>2.0</td>
<td>1.0</td>
<td>1.0</td>
<td>3.0</td>
</tr>
<tr>
<td>21</td>
<td>2.0</td>
<td>1.0</td>
<td>1.0</td>
<td>3.0</td>
</tr>
<tr>
<td>64</td>
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<td>1.0</td>
<td>1.0</td>
<td>3.0</td>
</tr>
<tr>
<td>120</td>
<td>2.0</td>
<td>1.0</td>
<td>1.0</td>
<td>3.0</td>
</tr>
</tbody>
</table>

Changes in PAHs

<table>
<thead>
<tr>
<th>4% Crude in Soil: Concentration of ppPAH (mg/kg soil) over Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day</td>
</tr>
<tr>
<td>2-ring total</td>
</tr>
<tr>
<td>3-ring total</td>
</tr>
<tr>
<td>4-ring total</td>
</tr>
<tr>
<td>5-ring total</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>4% Diesel in Soil: Concentration of ppPAH (mg/kg soil) over Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day</td>
</tr>
<tr>
<td>2-ring total</td>
</tr>
<tr>
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BIOREMEDIATION STRATEGIES

- A case study: ESPI (Malaysia-Brunei).
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ESPI solution is to provide an engineered biopile and the utilisation of a microbe developed by ESPI for this specific purpose.

With the facility, which has a capacity of 500m3, waste residues are reduced from a TPH of 30% to a disposal standard of < 1% in a 45 day period.

The biopile is a cost effective solution and has the added advantage that the material produced for disposal is extremely fertile and can be used in SCOT for beautification.
Indeed there is great future for the application of microbial biodegradation for oily sludge wastes contaminated with PAHs.

Simply put, this method is cheaper, requires low start-up capital, and needs few expensive high-tech machinery and non-labor intensive.

Furthermore, candidate microbes or bugs are either easily isolated from the natural environment or may even be purchase from commercial supplier.
THANK YOU
MICKY VINCENT