Enzymatic Bioremediation of Petroleum Hydrocarbons

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Characteristics of Enzymatic Biotreatment of Oil Spills

- Biodegrades crude oil rapidly into smaller components and finally into CO2 and H2O
- Non-toxicity - demonstrated on fw and marine organisms – fish, plankton, and bacteria
- Non-toxic to humans
- Stimulates population growth in naturally occurring bacteria
- Works to completion within a period of 4-6 weeks – mechanism of action protects shorelines, beaches, marine life and human health within hours.
Four Types of Remediation for Oil Spills

NCP Product Types

- Dispersants
- Nutrient Additives
- Microbiological Cultures
- Enzyme Activities
  - Example – OSE-II®
Mechanism of Enzyme Bioremediation Action

- Contains
  - Bio-surfactants
  - Enzymes
  - Nutrients
Testing

- Tests performed by numerous intl. govt. agencies and independent labs

- Non-Toxic

  - Safe for marine and aquatic organisms at effective applications levels
  - No specific precautions need be taken
  - No vapors emitted; thus, no inhalation problems
  - Safe for humans (OSHA)
  - No special handling or protective equipment or clothing required
Efficacy
Degradation of 96 Analytes through Time
Using a Bioenzymatic treatment (OSE-II)
vs. Nutrient Controls

- **Non-Nutrient Control**
- **Nutrient Treatment**
- **Bio-enzymatic Treatment (OSE-II)**

Concentration of Analytes (ppm)

Time (Days)
TPH BIODEGRADATION TESTS OF OSE II
PERFORMED BY INDEPENDENT LABORATORIES

TPH Biodegradation Tests
Using Enzymatic
Treatment of Crude Oil
Performed by
Independent Labs
(OSE-II)
Data: Oil decompose rate of OSEII bio stimulation
Analysis By University of Yamanashi (JAPAN)

Proceedings oil decompose

About ppm (parts per million):
ppm is rate of contamination level of each soil.
We multiply amount of soil by ppm.
For Example: If the rate is 12300ppm from amount of
3000kg soil which means contain oil about 37kg (1.23%).
*Usually most of pollution rate is 1000-7000ppm.

Proceedings Benzene level

Before test start
1 week after
2 week after
3 week after

Less than 0.001ppm

Benzene level (ppm)

TPH = Total Petroleum Hydrocarbon
TPH include hexane, benzene, toluene, xylenes, naphthalene, and fluorene,
other constituents of gasoline, jet fuels, of mineral oils, and of other
petroleum products.
Enzymatic Bioremediation (OSE-II)
Dissipation of Spilled Dielectric Insulating Oil (by GC/MS)
28 Days

(U.S. Dept. Interior, BOEM; and Dept. Envtl. Sci., LA State Univ.)
Toxicity
Pimephales promelas
(Fathead Minnow)

http://sepond.com/fish-stocking/fathead-minnows
Mortality of *Pimephales promelas*

\[ Y = 233.0 - 172.0X + 31.1X^2 \]

OSE-II Concentration (mg/L, log transform)

Percent Mortality (Angular Transform)
Enzymatic Bioremediation (OSE-II)  
Toxicity  
*Pimephales promelas* (Fathead Minnow)

LC50 = 11,800 mg/L
Enzymatic Bioremediation (OSE-II)
Toxicity - Mortality
*Pimephales promelas* (Fathead Minnow)

Concentration (OSE-II) (mg/L, Log_{10} + 1 transform)

Mortality (no. inds., n = 20)

LC50 = 5,856.34 mg/L
Ceriodaphnia dubia
(Freshwater water flea – planktonic)

Enzymatic Bioremediation
Toxicity - *Ceriodaphnia dubia*
(planktonic crustacean)
(Using OSE-II)

\[ Y = \frac{37.3}{1+e^{-(x-428.1)/16.8}} \]

**Graph:**
- **X-axis:** Concentration (mg/L) (Sqrt Transform)
- **Y-axis:** Percent Mortality
- **LC-50** indicated on the graph.
Enzymatic Biotreatment (OSE-II)
Toxicity - Mortality
*Ceriodaphnia dubia* (f.w. water flea)

Concentration (mg/L, Log$_{10}$ + 1 transform)

Mortality (no. ind.s, n = 20)

Concentration of Application and Efficacy

LC$_{50} >$ 16,000 mg/L

(Huther & Assocs., 2008)
Mysidopsis bahia
(Estuarine/marine mysid shrimp)

http://www.dailykos.com/story/2010/5/30/871254/-
Enzymatic Bioremediation
Toxicity - *Mysis bahia* (mysid shrimp)

LC-50 = 2,100
Artemia salina
(Freshwater brine shrimp, “Sea Monkeys”)

http://geneticsandbeyond.blogspot.com/2013/06/the-puffins-lair-to-be-pink-or-not-to-be.html
Enzymatic Bioremediation
Toxicity - *Artemia salina* (planktonic crustacean) (Using OSE-II and Water)

Percent Mortality

Time (hrs)

25 mg/L

0 25 50 75
Enzyme Bioremediation (OSE-II) Toxicity

Mortality in *Artemia salina* (freshwater plankton)

**OSE-II Alone**

- LC$_{50}$ = > 100 mg/L
- Mortality (no. inds., n = 20)

**No. 2 Fuel Oil**

- LC$_{50}$ = 12.6 mg/L
- Mortality (no. inds.)

**OSE-II and No. 2 Fuel Oil (1:10)**

- LC$_{50}$ = 29.4 mg/L
- Mortality (no. inds., n = 20)
Additional Test Organisms

**Oncorhyncus mykiss**  
(Pacific salmon)

**Photobacterium phosphorum**  
(m-negative phosphorescent bacterium)

**Daphnia magna**  
(fw plankton)

**Gasterosteus aculeatus**  
(fw three-spined-stickleback)

**Menidia sp.**  
(Neotropical Silverside)

**Mysidopsis sp.**  
(estuarine/marine shrimp)
LC-50s for Two Corexit Products and OSE-II Enzymatic Bioremediation Product, on Five Test Organisms

<table>
<thead>
<tr>
<th>Test Species</th>
<th>LC-50 (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oncorhynchus mykiss (salmon)</td>
<td>3000</td>
</tr>
<tr>
<td>Photobacterium phosphoreum</td>
<td>6000</td>
</tr>
<tr>
<td>Gasterosteus aculeatus (three-spined stickleback - fish)</td>
<td>9000</td>
</tr>
<tr>
<td>Dahnia magna (planktonic f.w. crustacean)</td>
<td>12000</td>
</tr>
<tr>
<td>Menidia Sp. (Neotropical silverside - fish)</td>
<td>9000</td>
</tr>
<tr>
<td>Mysidopsis sp. (mysid shrimp)</td>
<td>9000</td>
</tr>
</tbody>
</table>

*Contains 2-BTE
Toxicity to Humans

http://www.thesleuthjournal.com/whats-left-gulf-mexico/
DEPARTMENT OF LABOR

OCCUPATIONAL SAFETY AND HEALTH
LABOR STANDARDS AND SAFETY DIVISION
(OSHA)

August 23 1989

North Country Investment
2522 Arctic Blvd.
Anchorage, Alaska 99503

Attn: Steve Kacz

Dear Mr. Kacz:

An inquiry was made to this office concerning Sky Blue Chems
"Oil Spill Eater." Specifically, we were asked to assess whether
or not the use of this product would pose any health concerns by
reason of the properties of the constituents.

Upon review of the material safety data sheet and other documents,
we see no special toxicological concern with the ingredients that
would pose a significant health concern with its application as
described.

We would appreciate knowing in advance of any field tests or uses
of this product.

Sincerely,

Dennis L. Smythe
Chief of Compliance

cc: Ron Biggers
Bacterial Enhancement
Enzymatic Bioremediation (OSE-II)
Bacterial Enhancement

(Bio-Aquatic Testing, NELAC Certified)
Enzymatic Biotreatment (OSE-II)
Bacterial Response

(Bacterial Count (MPN))

OSE-II
Nutrient Addit.
Control

(U.S. Dept. Interior, BOEM; and Dept. Envlt. Sci., LA State Univ.)
Respirocity Test
Indicator of Oxygen-Enhanced Bacteria

Comparative Products

<table>
<thead>
<tr>
<th>Product</th>
<th>Oxygen Uptake (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Product 1 10</td>
<td>250</td>
</tr>
<tr>
<td>Product 1 30</td>
<td>450</td>
</tr>
<tr>
<td>Product 2 10</td>
<td>300</td>
</tr>
<tr>
<td>Product 2 30</td>
<td>500</td>
</tr>
<tr>
<td>Bioenzymatic Treatment (OSE-II) 10</td>
<td>750</td>
</tr>
<tr>
<td>Bioenzymatic Treatment (OSE-II) 30</td>
<td>850</td>
</tr>
</tbody>
</table>
Enhancement of Bacterial Population Growth on Crude Oil after application of Enzymatic Bioremediation treatment (OSE-II)
Cost Comparisons
BP/Deepwater Horizon Spill

Est. cost of treatment – in situ burning, mechanical recovery, chemical dispersants, damage claims, fines.
- Est. spillage – 200M gallons
- Total est. cost of remediation - $42B
- Est. cost of clean-up = $210/gal oil

Cost of alternate approach – Enzymatic Bioremediation
- Total est. cost of remediation - $800M
- Est. cost of clean-up = $4/gal oil (Savings – 98.1%)
Countries using Enzymatic Bioremediation on Oil Spills with Governmental Approval

- Australia
- Bahrain
- Colombia
- Greece
- Iraq
- Iran
- Kenya
- Kuwait
- Mexico
- New Zealand

- Nigeria
- Oman
- Philippines
- Qatar
- Saudi Arabia
- South Korea
- Trinidad and Tobago
- United Arab Emirates
- United Kingdom
- United States (Military - Army, Navy, Coast Guard, Air Force, Marines)
Acknowledgements

The Lawrence Anthony Earth Organization (LAEO)
- Ms. Diane Wagenbrenner
- Ms. Elizabeth Montgomery
- Ms. Barbara Wiseman

Mr. Steven Pedigo, The OSE-II Corporation

Louisiana Universities Marine Consortium (LUMCON)

The 2016 Clean Gulf Conference – Ms. Cassie Davie
<table>
<thead>
<tr>
<th>Clean-up</th>
<th>Dispersants</th>
<th>Mechanical</th>
<th>Enzymatic Bioremediation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Toxicity</td>
<td>0%</td>
<td>2-8%</td>
<td>~ 100%</td>
</tr>
<tr>
<td>(To marine spp. &amp; humans, particularly w. 2-butoxy-ethanol - 2-BTE)</td>
<td>Toxic (derived from oil)</td>
<td>Non-toxic (detoxifies oil quickly)</td>
<td></td>
</tr>
</tbody>
</table>
60 YEAR OILED SOIL
Tacoma, Washington
Sept-Nov 1990

Start 1,800 TPH
End 10 TPH

SOUND ANALYTICAL SERVICES
TACOMA, WASHINGTON

TPH

2,000
1,800
1,600
1,400
1,200
1,000
800
600
400
200
0

DAYS

20 30 40 50 60 70 80
Tier II Efficacy Data
Enymatic Bioremediation Treatment
(OSE-II)

Percent Reduction after 21 Days

Percent Reduction (%)

Chrysene
Total Aromatics
Phenanthrenes
Fluorene
Pristane
C-30
Phytane
Total n-Paraffins
C-18

Analytes
<table>
<thead>
<tr>
<th>Species</th>
<th>Duration (hrs)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>48</td>
</tr>
<tr>
<td><em>Menidia beryllina</em></td>
<td>6698</td>
</tr>
<tr>
<td><strong>Menidia bahia</strong></td>
<td>8839</td>
</tr>
</tbody>
</table>

*Static

**Static renewal
Enzymatic Biotreatment
(OSE-II)
Juvenile Mysid Shrimp
Toxicity - Survivorship

Time (hrs)
0
24
48
72
96

Mortality
(no. inds.)
0
2
4
6
8
10

10,000 mg/L
100 mg/L
10 mg/L
1 mg/L

0 mg/L (Control)

LC50 - 2,100 mg/L
1,000 mg/L

(EPA/NETAC)
Mechanism of Action (cont.)

- Neutralizes toxicity to soil and aquatic or marine organisms
- Enzymes separate oil molecules and enhance their metabolic breakdown
Additional Advantages of Enzymatic Bioremediation

- No specific precautions need be taken
- No vapors emitted; thus, no inhalation problems
- A one-time application is sufficient to achieve effectiveness