Biodegradation and Bioremediation in Oiled Marine Environments



Considerations in understanding Biodegradation and developing, testing and applying bioremediation products

Nature

Bioremediation vs Biodegradation

Bio<u>degradation</u> is the process whereby natural organisms degrade oil compounds.

Bio<u>remediation</u> is a human activity targeted at *enhancing the rates* at which oil compounds are naturally degraded by organisms

What's New With <u>Biodegradation</u>?

- Hydrocarbon degrading bacteria are <u>present in all</u> seas at low concentrations
- The science of <u>genomics</u> is identifying many previously unknown hydrocarbon-degrading marine microbes
- Natural <u>biodegradation rates</u> of specific hydrocarbons are being measured
 - they take days to weeks in water
 - Months to years if oil buried in beach sediment
- There are now <u>dozens of papers</u> in the peer reviewed literature

What's New With <u>Biodegradation</u>?

- <u>It takes a Village</u>. No single microorganism can degrade all compounds in oil. When oil spills in the water, dozens of species "bloom", each choosing their preferred hydrocarbon compounds
- <u>The Rate at which Oil Degrades Increases with Surface Area of oil</u>. Dispersed oil particles (natural or otherwise) offer thousands of times more surface area than slicks, tar mats or tar balls.
- <u>Degradation Rates decrease with Temperature</u>. Compounds in oil undergo natural biodegradation in Arctic conditions, but at slower rates than in warm environments
- Oxygen and nutrients are consumed (old news)

Nature

Biodegrading Microbes in the Ocean

Figure S4. Bacterial richness detected in oil plume. A total of 951 subfamilies were detected in 62 bacterial phyla using Phylogenetic microarray (PhyloChip) analysis (see supplemental methods). Only 16 subfamilies in one subphylum (γ-proteobacteria) were significantly enriched in the plume relative to outside the plume.

The "Village"

Genomics identifies functional species* of hydrocarbondegrading bacteria



Firmicutes	E MILENA
	I NKBTU
Basteroidetes	OP11
Actinobacteria	Sufebadili
Planotomycetes	Synergistetes Aminanaer
Chloroflexi	Thermi
Acidobacteria	wse wse
Verru comior obia	Aquificae
Cyanobacteria	CD12
Gemmatimona detes	EM19
Marine_group_A	Entoth eonella
Spirochaetes	F\$274-7B-03
Unclass Fied	E Fusebacteria
Nitrospirae	Kazan-3B-22
9N02	Moorella
Fibrobacteres	NC10
Lentisphaerae	01aA90
OP3	OP1
NS3	OP10
Desuffovibrionales	OP9_JS1
Elusimicrobia T01	P9X263A04
TM7 -	SC3
Chlamydiae	SC4
Chlorobi	SHA-95
AC1	Thermosulfido bacterium
Caldithrix KSB1	Thermoterrab acterium
Haloanaeroblales	Thermotogae
VHS-85-50	TMB
NPS-2	WCH B1-27
ABY1_OD1	W51
BRC1	283

* Some Scientists call them Operational Taxonomic Units



What's New With <u>Bioremediation</u>?

How can we help Nature speed the Process?

National Contingency Paln (NCP) Subpart J (including the National Product Schedule NPS) 49 Products Listed Effectiveness is compared to Nutrient Control USEPA proposed revisions of SUBPART J (Now available out for public comment)

National Response Team (NRT)

Bioremediation Fact Sheet being revised

Scientific (Peer Reviewed) Literature

Dozens of papers testing new products, formulations and methods

Vendor Advertisements and Brochures

We need to confirm the claims using science

The World of Oil Spill Biodegradation Science

- I conducted a Literature Search of journal papers on marine oil spill bioremediation
- 130 peer reviewed papers since 2010
- <u>Operationally</u>, most products have not been field-tested: little peer-reviewed science published at this scale
- There are peer reviewed papers on many products that are <u>not</u> on the EPA Product schedule

Technologies Being Tested as reported in the Scientific Literature

- Nutrients and Oxidants
- Mechanical
- Microbial consortia
- Oleophilic fertilizer
- Urea
- Dispersants
- Clay Minerals
- Polymers
- Molasses

- Nano Materials
- Magnetites
- Chitosan (from fish wastes)
- Sawdust
- Mushroom spent compost
- Phyto-remediants
- Rhizo-remediants
- Temperature Increase
- Modified fishmeal
- TiO₂

Examples

Manzano-Agugliaro, F.; Hernandez Escobedo, Q.C.Zapata Sierra, A.J. (2010) **Use of bovine manure for ex situ bioremediation of diesel contaminated soils in Mexico**. *Itea-Informacion Tecnica Economica Agraria*, **106**, 197-207.

Usman, M.; Faure, P.; Lorgeoux, C.; Ruby, C.Hanna, K. (2013) **Treatment** of hydrocarbon contamination under flow through conditions by using magnetite catalyzed chemical oxidation. *Environmental Science and Pollution Research*, **20**, 22-30.

Yateem, A. (2013) **Rhizoremediation** of oil-contaminated sites: a perspective on the Gulf War environmental catastrophe on the State of Kuwait. *Environmental Science and Pollution Research*, **20**, 100-107. Warr, L.N.; Perdrial, J.N.; Lett, M.-C.; Heinrich-Salmeron, A.Khodja, M. (2009) **Clay mineral-enhanced bioremediation of marine oil pollution**. *Applied Clay Science*, **46**, 337-345.

Ummadisingu, A.Gupta, S. (2012) Characteristics and kinetic study of chitosan prepared from seafood industry waste for oil spills cleanup. Desalination and Water Treatment, 44, 44-51.

Simarro, R.; Gonzalez, N.; Fernando Bautista, L.Carmen Molina, M. (2013) **Biodegradation of high-molecular**weight polycyclic aromatic hydrocarbons by a wood-degrading consortium at low temperatures. *Fems Microbiology Ecology*, **83**, 438-449.

Formulations

- Liquids
 - Water soluble
 - Oleophilic (Inipol)
 - Subsurface Injection products PES
- Pellets and Granules (Customben)
- Product Applications combined with Tilling
- Nutrients

"Substrates" Tested

- Water, sediment, slicks, mostly in the laboratory, some in the field
- Field Studies: mainly oiled beaches, marsh and mangrove sediments (but not in US since 1994)
- A half dozen papers have addressed bioremediation in water with variety of treatments
- Using various oils and fuels, but no "standard" oil

Other Bioremediation Methods

- <u>Phyto-remediation</u> repopulate damaged marsh with new plantings.
- <u>Oxygenating</u> sub-surface sediments in gravel intertidal beaches
- <u>Disperse</u> oil back into water so natural biodegradation can proceed (products with surfactants)

Gaps

 Practically no literature on effectiveness of NPS products in scaled-up (operationallyrelevant) studies

 Hardly any literature on the ability of treatments to reduce toxicity of treated oiled sediments or water

The Anecdotal "Science" of Bioremediation

- Advertisements have very few citations to effectiveness of treatments at <u>operational scales</u>
- Videos showing oil disappearing are not supported by hard data on biodegradation; some appear to disperse
- Many anecdotes:
 - We did a bucket test and were impressed
 - Our products have been sold and used worldwide
- Literature suggests researchers have <u>abandoned tests with</u> <u>microbial formulations</u> in favor of testing "biostimulants"

Improving the Information for Decision-Makers

Four Scales of Testing Effectiveness and Effects of Bioremediation Treatments



1 - Bench-scale Laboratory, LSU



2- Marsh Mesocosm, NOAA



3- Intentional Beach Plot Oiling, Delaware



4- Actual Oil Spill, Galveston Bay, 1990

What Needs To Be Monitored?

- Total Petroleum Hydrocarbons
- Polycyclic Aromatic Hydrocarbons and Alkanes
- Hopane and Analyte-to-Hopane Ratios
- Nutrients (N, P)
- Microbial Biomass
- Microbial Composition (actual degraders)
- CO2 Production
- Toxicity Reduction of Treated Substrate
- Visuals

Elements of Monitoring (Design)

- <u>Time Series</u> for rate trends (>3 sampling events)
- **Controls** (Sterile and Nutrient Treatment)
- <u>Replication</u> (N>3, preferably 5)
- <u>Randomization</u> of Treatments
- <u>Publish</u> Results in Peer-reviewed Journals

Define What Marine/Aquatic Situations are targets of the Technology Testing

Open Water? Above high tide (Supra-tidal)

Intertidal Shorelines Sand and Gravel Beaches Mudflats Marshes Mangroves Coral Reefs

Define What Oil is the Testing Target

Fresh Crude Oil?

- Light API 40 Medium - API 25
- Heavy API 15

Weathered Crude Oils

Oils are NOT Alike!

<u>Fuel Oils (fresh or weathered)</u> Diesel Intermediate Fuel Oils Bunker Oil

Emerging Oils

Bakken Oil Sands Biofuels

Logistical Considerations

- <u>Application Rate</u>: One-time or continuous?
- <u>Wash Out</u>: Product removal by tidal flushing
- <u>Scale</u>: amount of product really needed?
- <u>Access</u>: Disturbance, mechanical injury
- Product/Oil Ratio
- <u>Monitoring</u>:
 - Visual? Chemical? Toxicity Reduction?
 - Replication?

Goal of Bioremediation:

What are you trying to accomplish?

- Remove visible oil?
- Reduce concentrations of toxic oil components?
- Reduce toxicity of oiled substrate?
- Enhance recovery & recolonization of marine life?
- Reduce damage to historic and cultural resources?

Field Testing Needed

- Delaware (1994) was the last full scale field testing in the US with robust, scientifically satisfying, published results.
- Unfortunately the study did not test any <u>commercial</u> product. But the design is clearly applicable.
- Intentional shoreline oiling experiments are being done in other countries.

Thank You

And

Please Review USEPA's proposal for revisions to the National Contingency Plan, Subpart J

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