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Evaluation of Fungal Enzyme Extracts to Catalyze Remediation of Heavily Weather Crude Oil Contaminated Soil

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Background

- Crude oil spills – straight and branched chain alkanes, aromatics and cycloalkanes
- Lighter easier to degrade by microorganisms
- Heavier fractions remain
- Typical removal include – thermal, landfill, chemical oxidation

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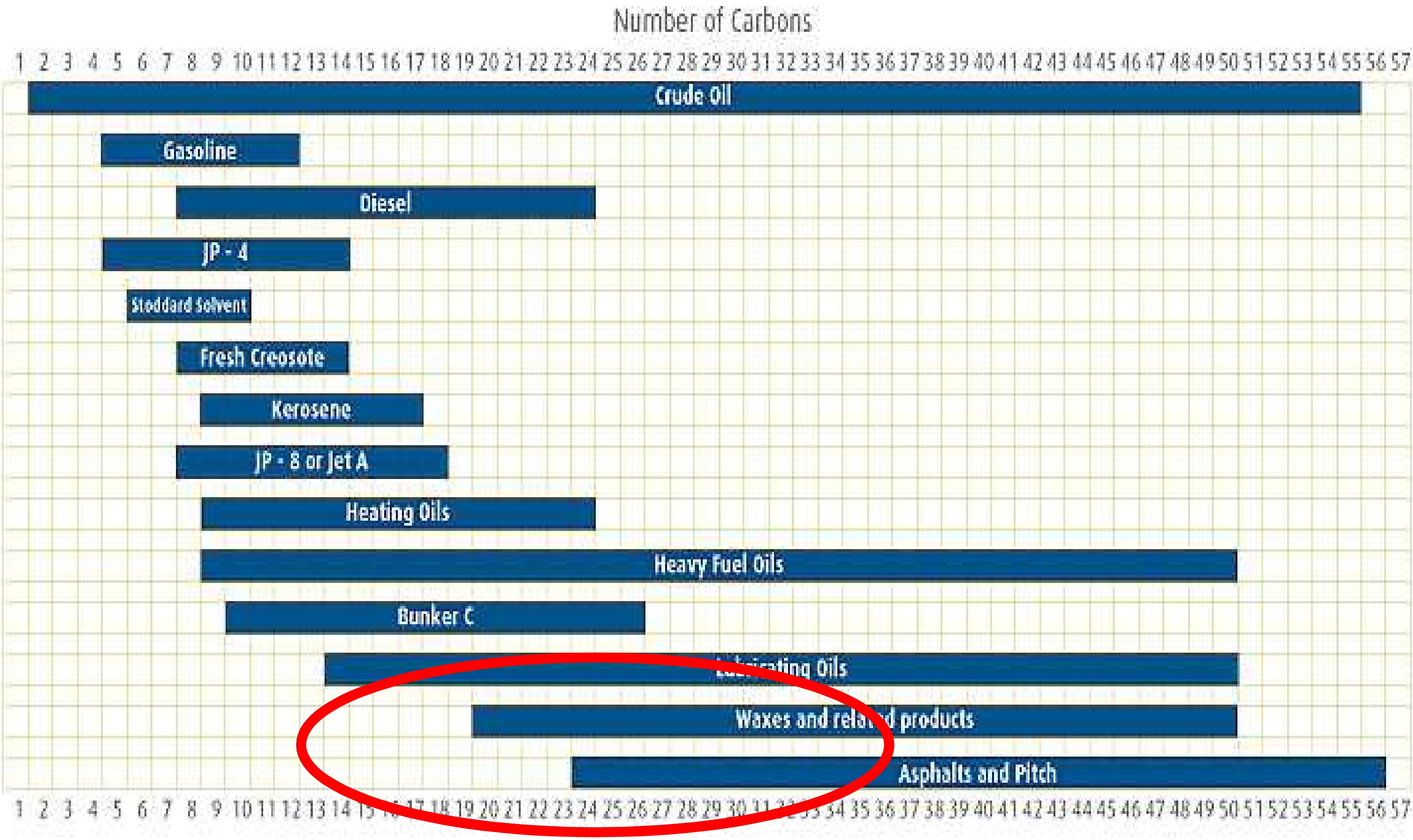
Objective

To develop a method to treat heavily weathered crude contaminated soil using *encapsulated fungal enzymes*

Hypothesis

Fungal enzymes can non-selectively break down long-chain hydrocarbons possibly into shorter chain hydrocarbons.

Soil Requirements



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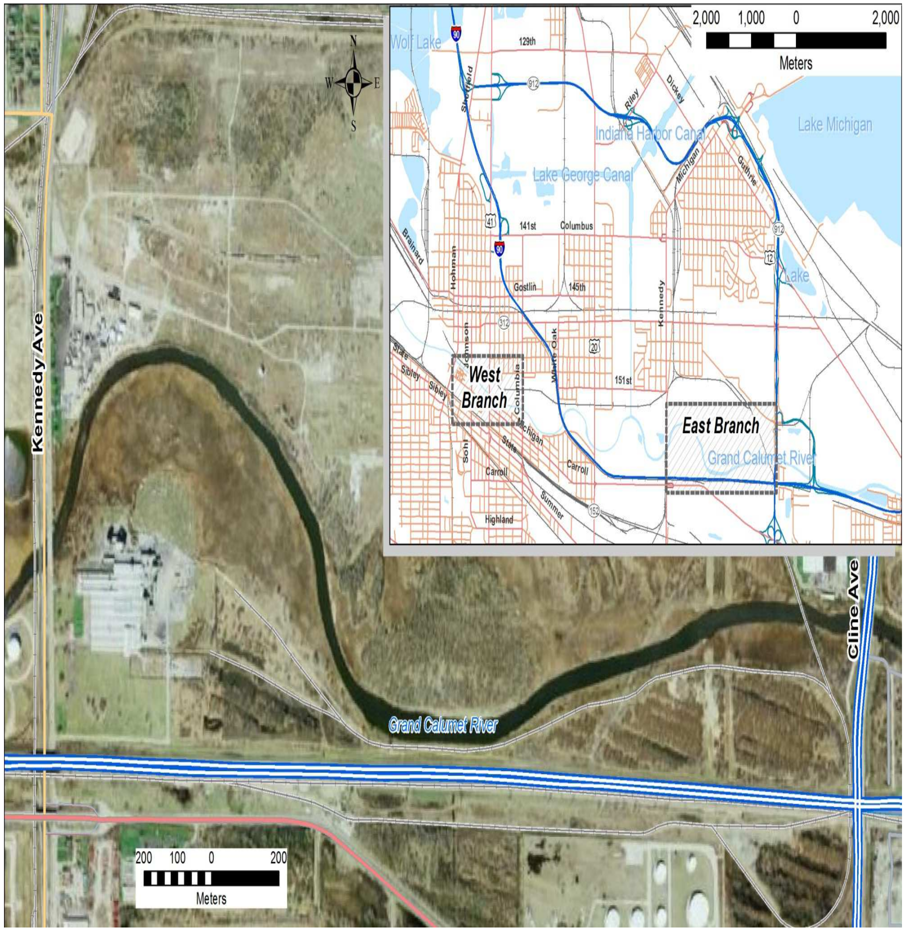
Contaminated Soil

- Grand Calumet River Sediments
- Contamination from multiple industries including oil refineries on the banks of the river
- Contamination in place since 1970's
- Contaminants include PCBs, heavy metals, crude oil, and PAHs



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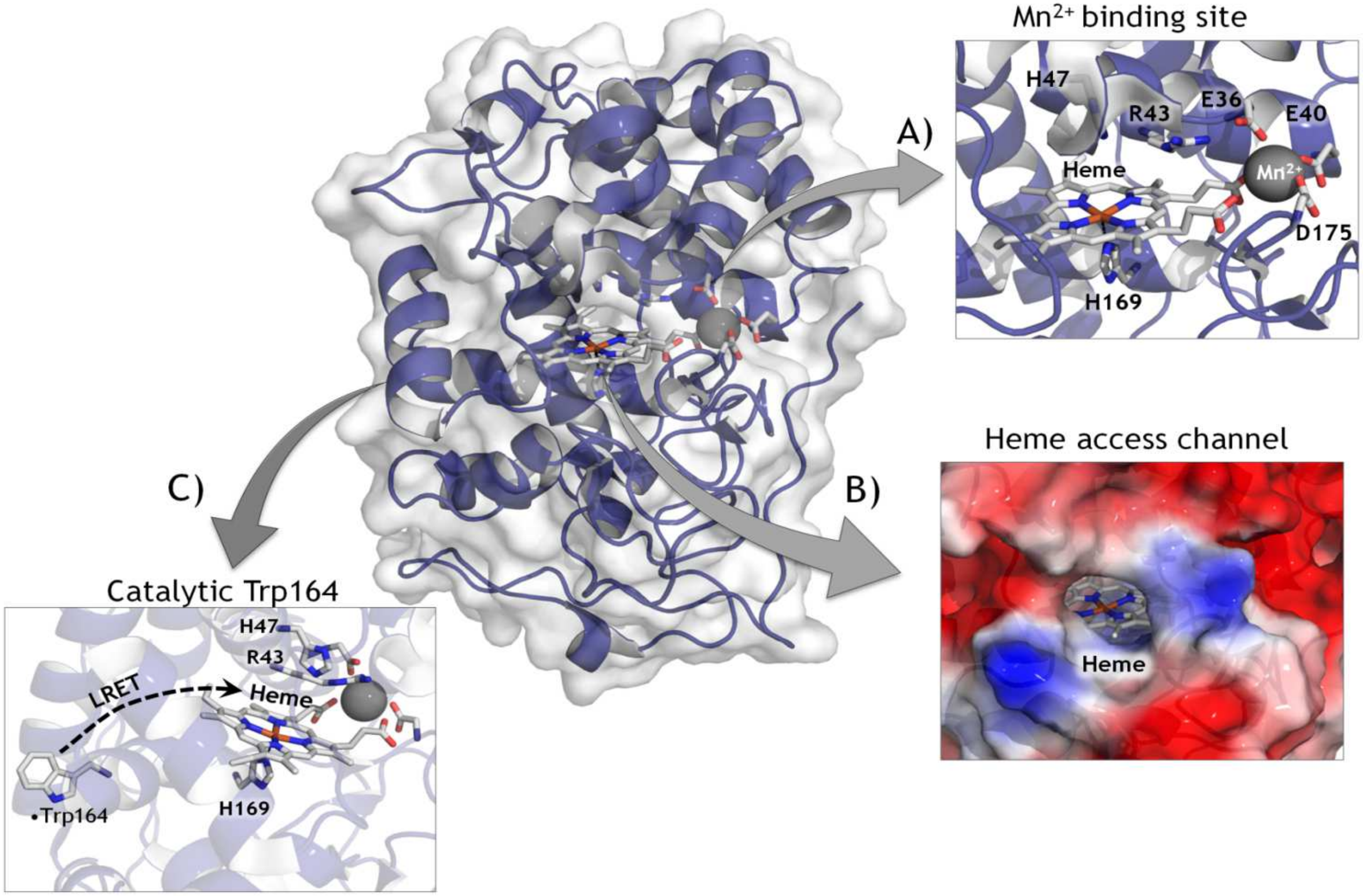
Soil Characteristics

TOTAL PAHs (mg/kg dry wt.)		Total PCB (Aroclor-1248)	Oil and Grease	DRO	RRO
(n=16)	(n=34)	(mg/kg dry wt)	mg/kg	mg/kg	mg/kg
164.0	463.6	0.31	17,500	6,400	10,000

DRO – C10 to C28
RRO – C25 to C36

Oxidoreductase Enzymes

- Manganese peroxidase, laccase and lignin peroxidase



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Encapsulation

To provide reactive ingredient (enzyme) in an easily applicable form without the risk of introducing non native fungal species.

Battelle Encapsulation Technology Experience:

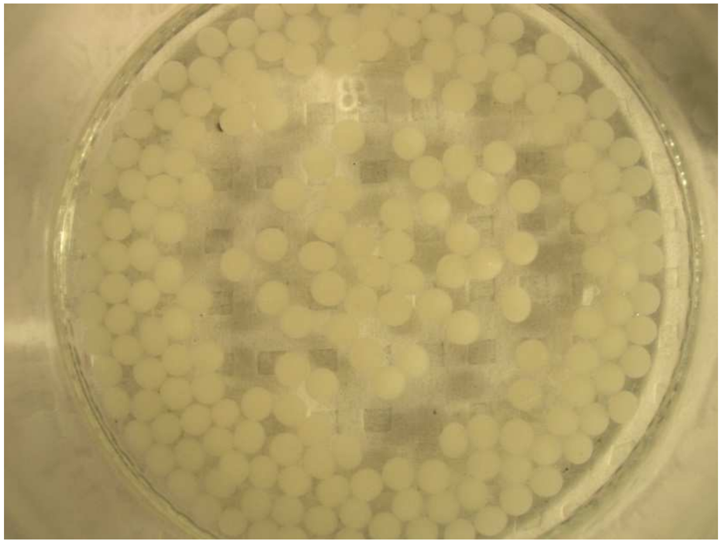
- Microencapsulation *via* spray drying with mixture of polymer and solvent (solid material)
- Encapsulation into hydrogel particles using non aqueous dispersion process (Battelle US Patent 8193142)
- Encapsulation using complex co-aservation
- Encapsulation via electrospray

Selection of Encapsulant

Matched suitable encapsulation route with critical process metrics to gain high probability of success.

Encapsulant benefits:

- Room temperature process
- Bio-based & biodegradable matrix
- Absorbs water
- Fast process
- VOC free / No solvents
- Variable particle size
- Stable pH 4 to 6 range



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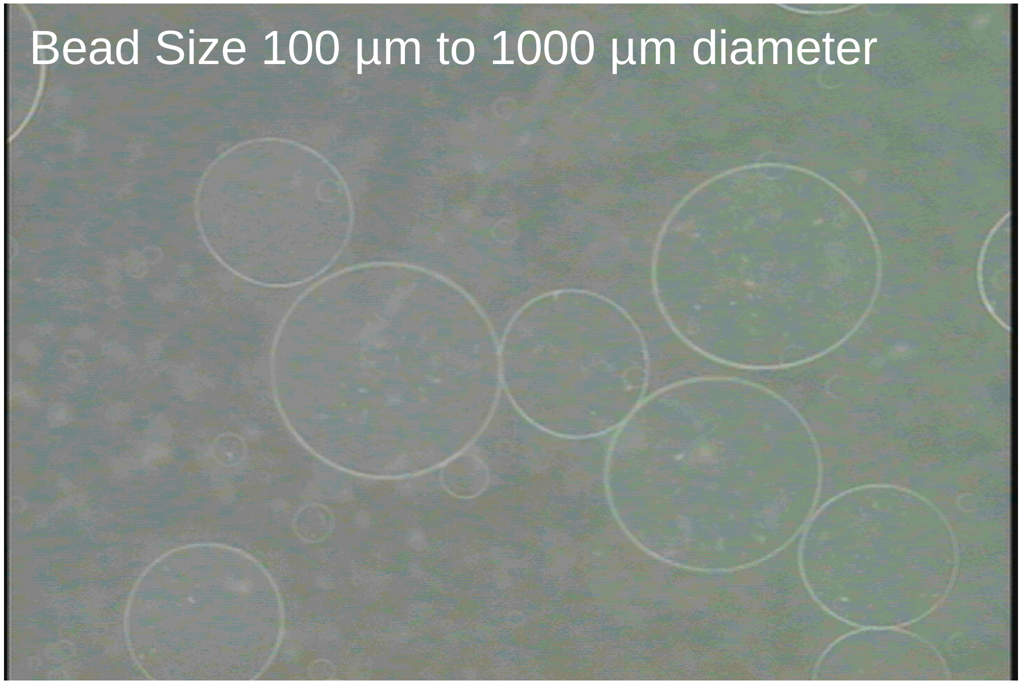
Encapsulated Enzyme



No Loss of activity seen after encapsulation

Activity before encapsulation = 2.2 U/mL and 1.76 U/mL
Activity after encapsulation = 2.015 U/mL and 1.861U/mL

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Treatments

Treatment	Soil	Enzyme	Peroxide
Purified Enzyme			
1	☺		
2	☺	☺	
3	☺		☺
4	☺	☺	☺
Encapsulated Enzymes			
1	☺	☺	
2	☺	☺	☺

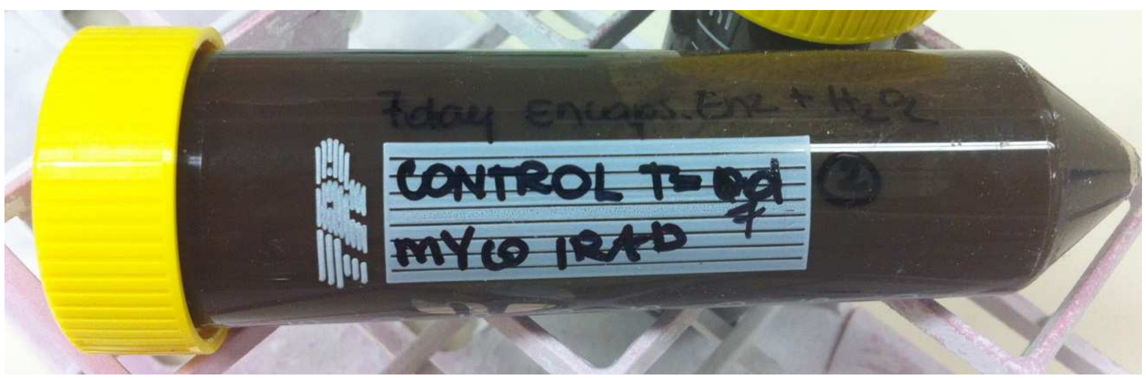
Treatments

- 20 g soil
- 2 mL purified enzyme at 2.2 U/mL
- 100 µL 10 mM hydrogen peroxide added every other day
- TPH measured after 7 days and 14 days
- All treatments prepared in duplicates

Laboratory Experimental Approach

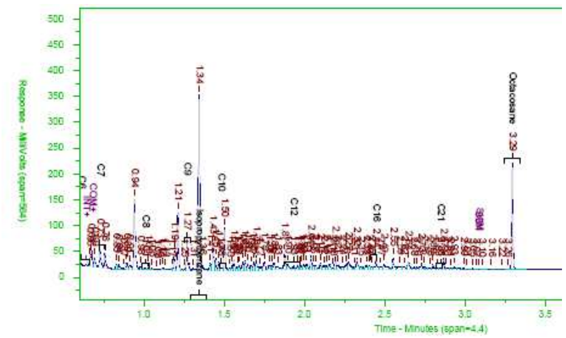
Obtain purified enzyme

Apply enzyme to contaminated soil, add H_2O_2 as substrate

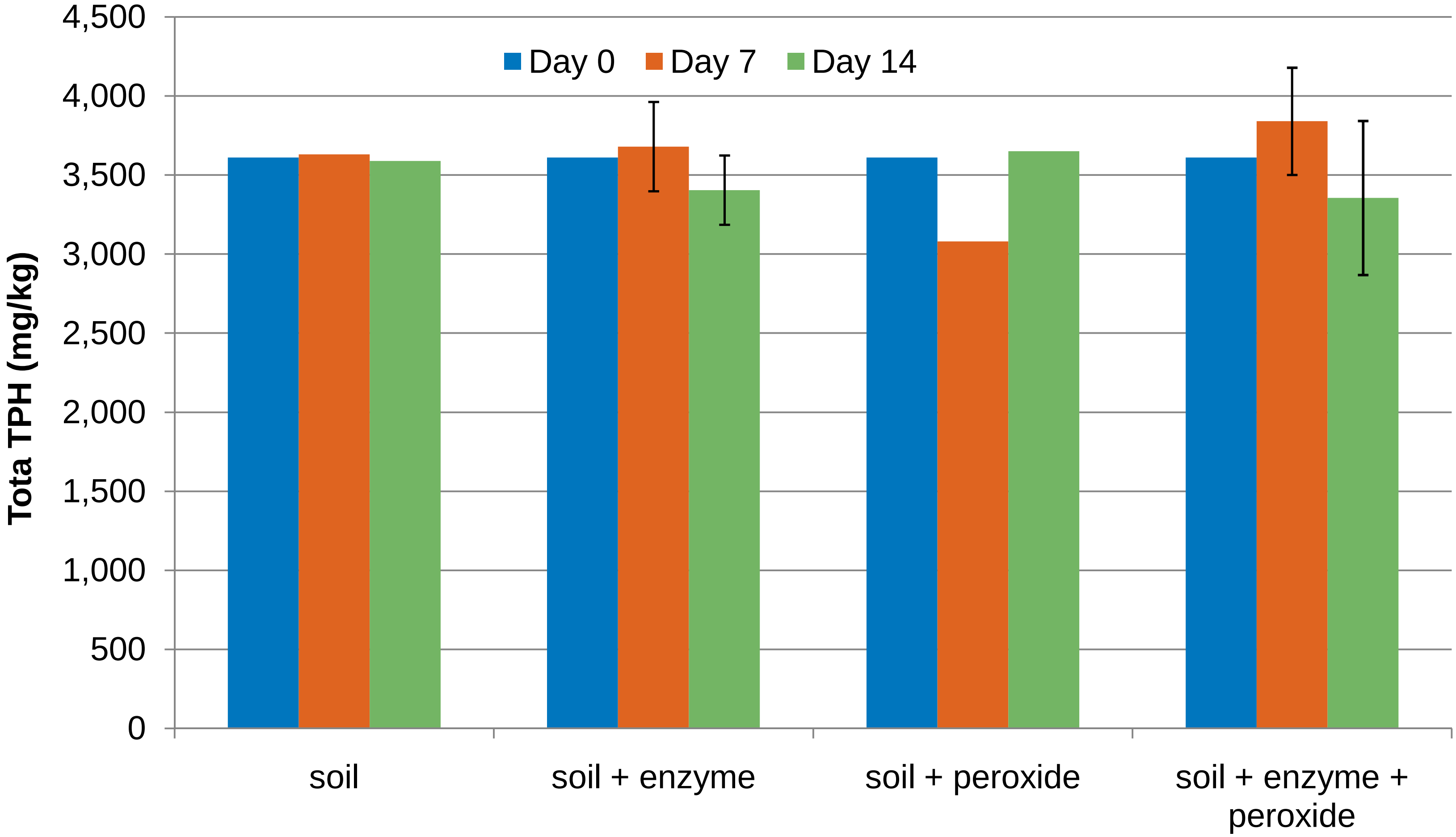


Incubate at Temp 25^oC

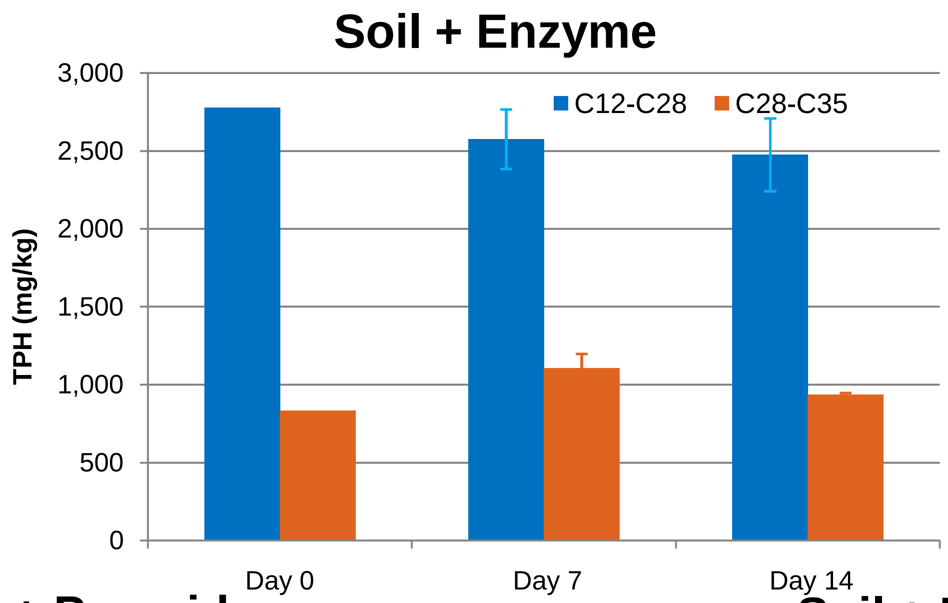
Analyze Soil Method TX1005 - TPH

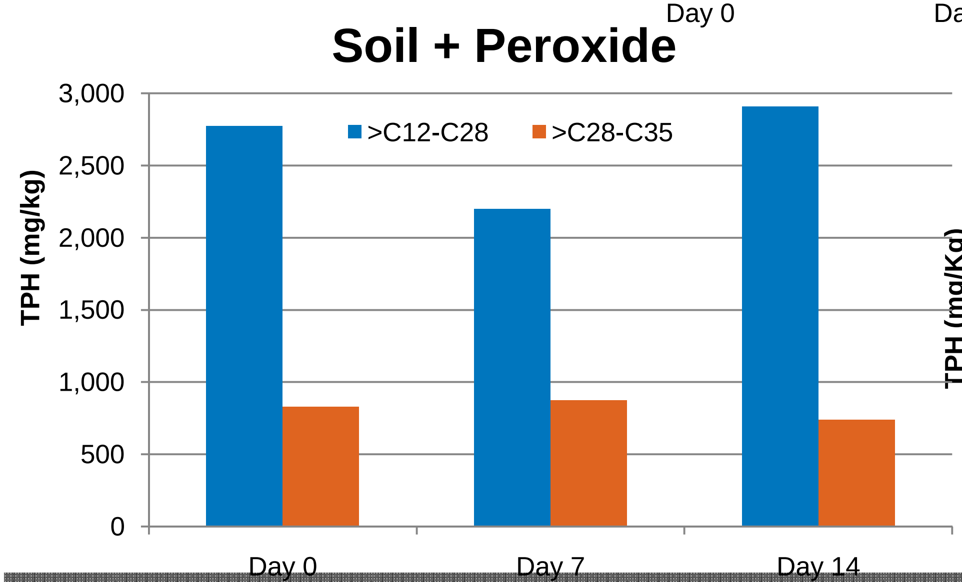


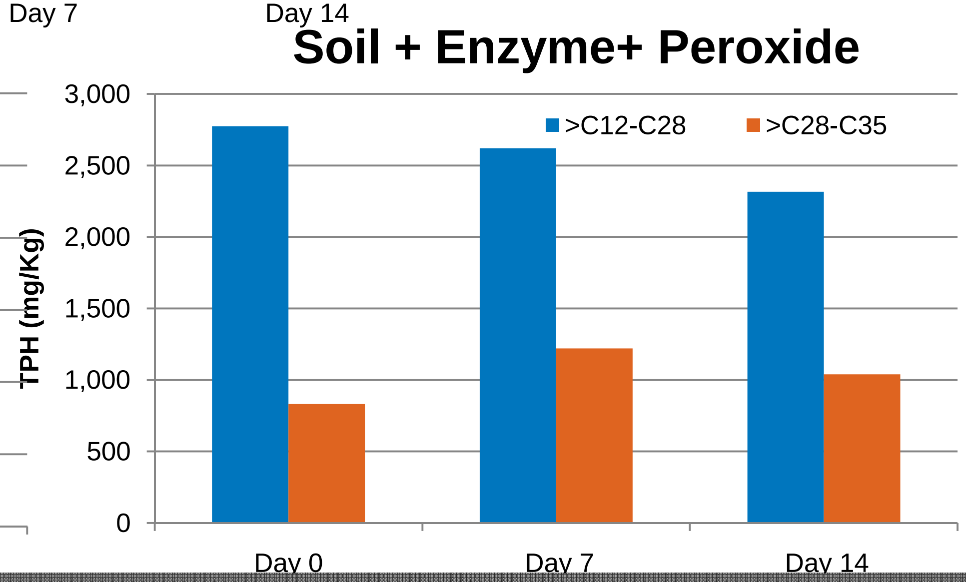
Total TPH Results – Pure Enzyme



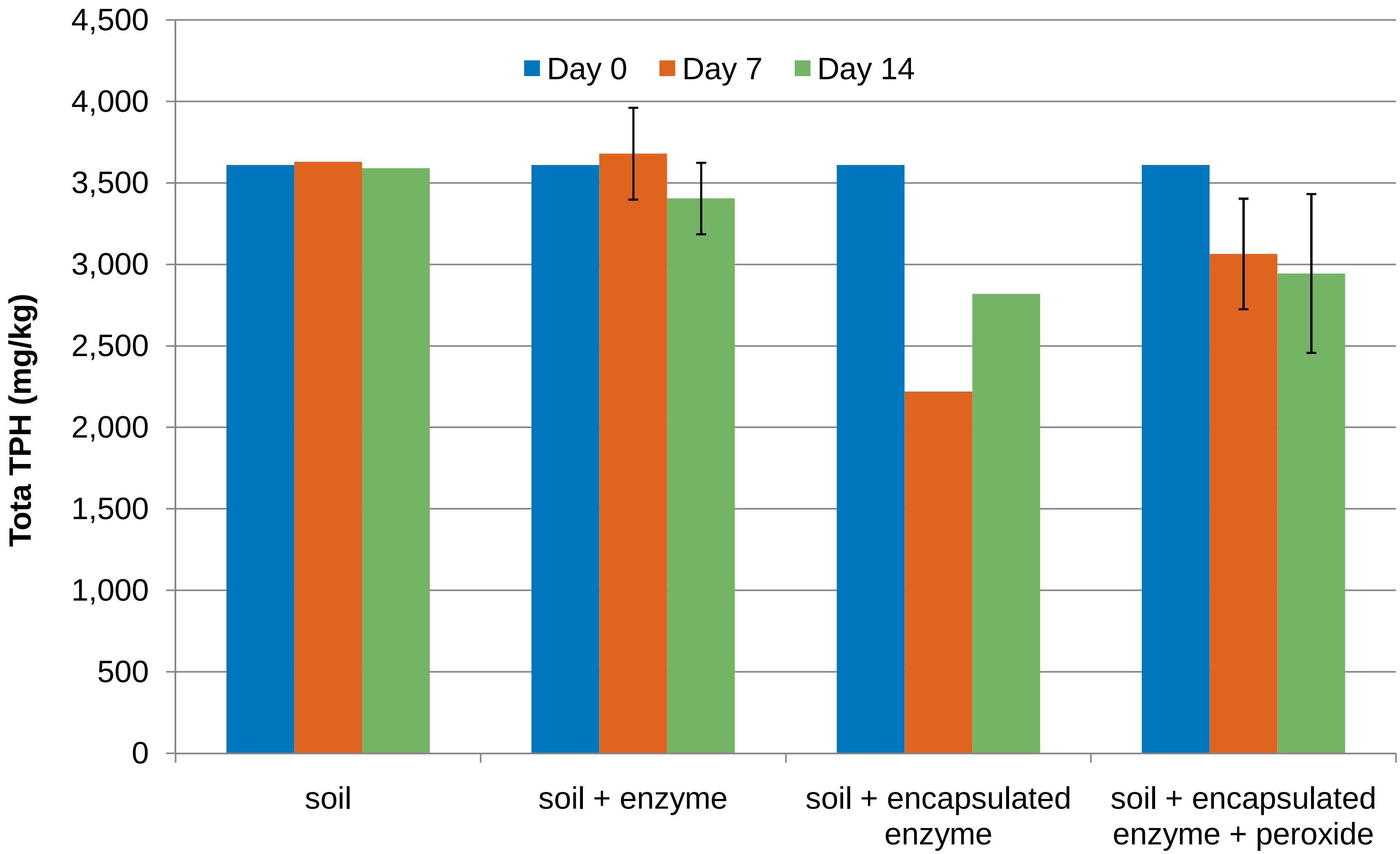
TPH Fractions – Pure Enzyme



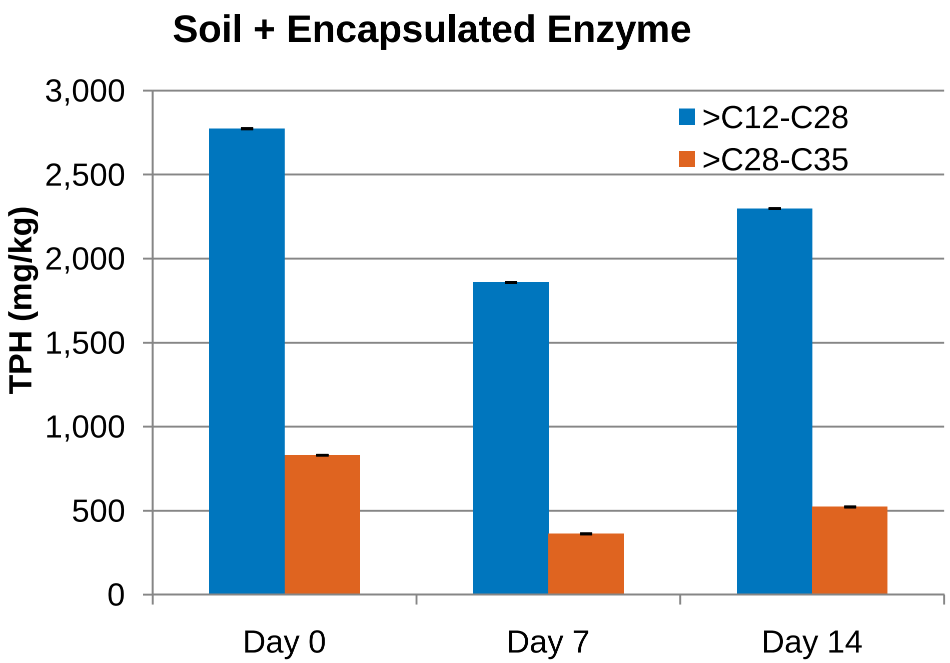


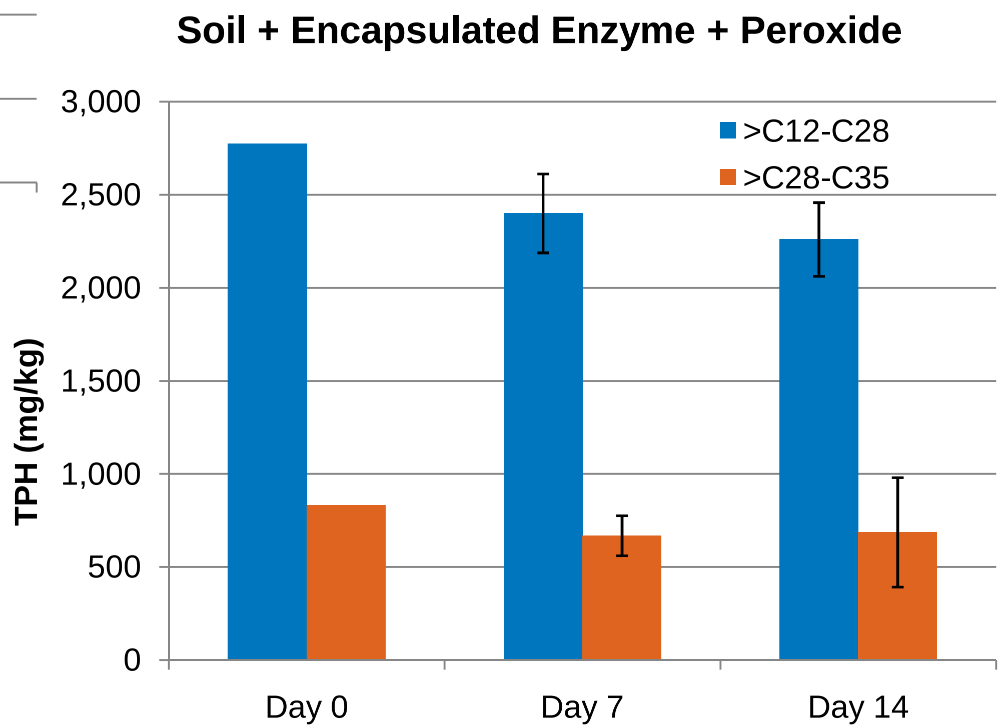


Total TPH Results – Encapsulated Enzyme



Total TPH Results – Encapsulated Enzyme





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% Loss Summary

Treatment	Days	Total TPH	C12 – C28	C28 – C35
Soil + Enzyme	Day 7 Day14	-2% 6%	7% 11%	-33% -13%
Soil + Peroxide	Day 7 Day 14	15% -1%	21% -5%	-5% 11%
Soil + Enzyme + Peroxide	Day 7 Day 14	-6% 7%	6% 17%	-47% -25%
Soil + Encapsulated Enzyme	Day 7 Day 14	38% 22%	33% 17%	56% 37%
Soil + Encapsulated Enzyme + Peroxide	Day 7 Day 14	15% 18%	14% 19%	20% 17%

Summary

- Difficult to interpret due to limited data
- Increase in TPH may be due to reduction in fractions >C35 not measured by current method.
- Noticeable decreases seen in encapsulated
- Some decreases seen in enzyme treatment

Next Steps

- Continue experiment over a longer period of incubation
- Utilize higher units of enzyme
- Optimize encapsulation conditions – hydrogel formulation
- Conduct experiment in soil without drying and measure changes in microbial activity

Metagenomics and Metaproteomics

- To understand the shift in microbial population as a result of application of fungal enzymes and degradation of TPH
- To detect suite of microbial proteins directly involved in TPH degradation
- Use data to optimize treatment

Application of Omic Technologies

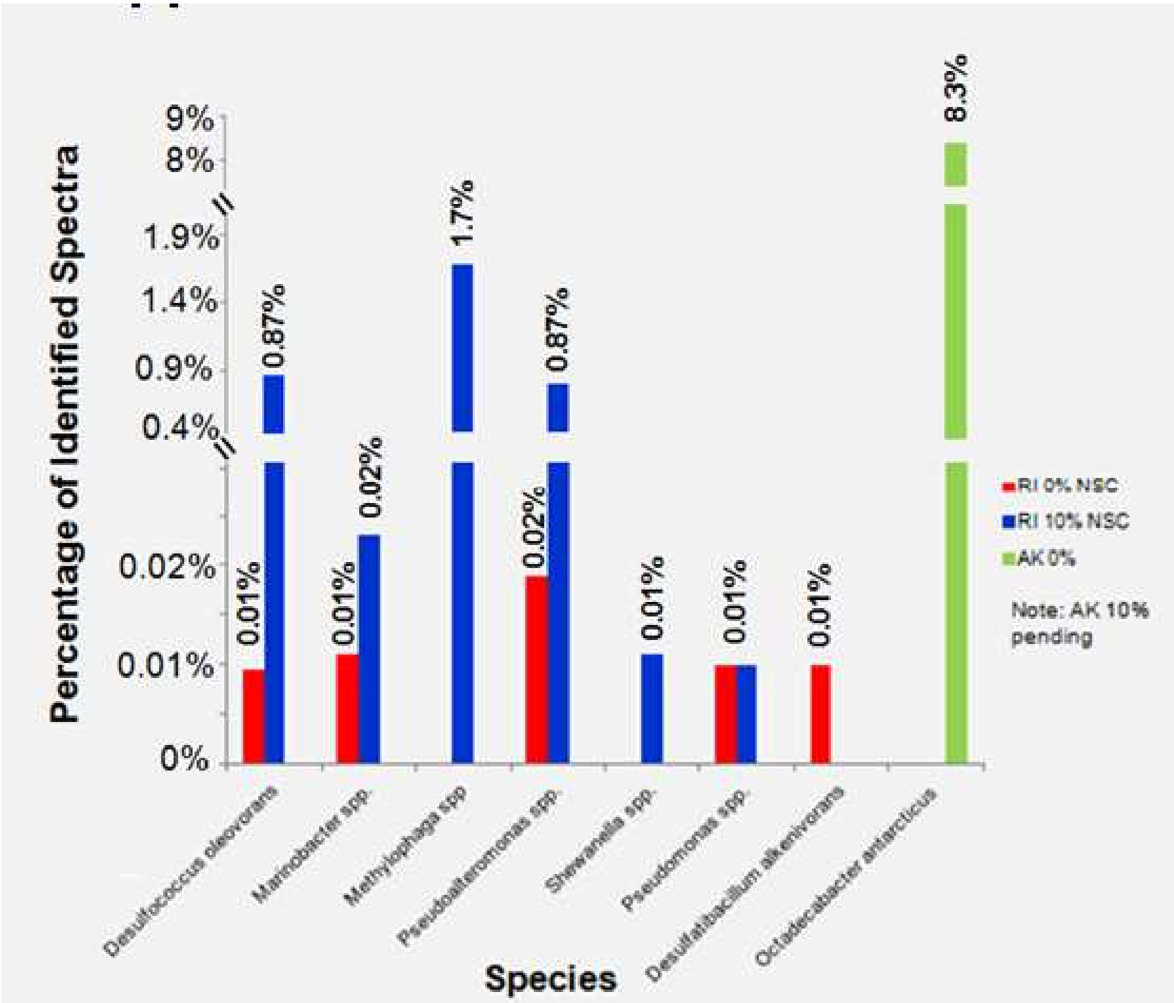
Biodegradation: Baseline and time/dose response characterization

- Community structure (microorganisms)
- Functional potential (genes)
- Functional biomarkers (proteins)

Omic Technologies - Metagenomics

Organism	Relation to Oil Degradation	% of Bacterial Sequences			
		RI		AK	
		0% NSC	10% NSC	0% NSC	10% NSC
Methylophaga thiooxydans	Aerobic Gammaproteobacteria; can grow on low carbon # and methylated sulfur compounds[4]	0.03%	19%	0.6%	4%
Pseudoalteromonas spp.	Aerobic Gammaproteobacteria; several species considered oil degraders[5]	0.1%	13%	1%	9%
Marinobacter hydrocarbonoclasticus	Aerobic Gammaproteobacteria; implicated in PAH and other pollutant degradation[5]	0.1%	19%	1%	4%
Alcanivorax spp.	Aerobic Gammaproteobacteria; principal carbon source is linear-chain alkanes[5]	0.4%	2%	0.07%	0.2%
Pseudomonas spp.	Aerobic Gammaproteobacteria; many species are known oil degraders[5]	6%	1%	1%	30%
Vibrio spp.	Gammaproteobacteria; identified in Mexico beach sands from Gulf of Mexico oil spill[6]	0.4%	8%	0.1%	0.2%
Denitrovibrio acetiphilus	Aerobic bacteria isolated from off-shore oil recovery simulated conditions[7]	0%	5%	0.04%	0%
Shewanella spp.	Gammaproteobacteria; one of dominant bacteria in Arctic sea ice treated with crude oil[8]	0.3%	3%	0.6%	0.4%
Desulfatibacillum alkenivorans	Anaerobic Deltaproteobacteria sulfate reducer; degrades medium-chain alkanes and alkenes[9]	0.2%	0.4%	0.4%	0.02%
Desulfococcus oleovorans	Anaerobic Deltaproteobacteria; sulfate-reducer; shown to degrade long-chain alkanes[10]	0.1%	0.3%	0.4%	0.1%
Octadecabacter antarcticus	Alphaproteobacteria; not associated with oil degradation	0.04%	0.08%	19%	8%
Escherichia coli	Facultative anaerobic gammaproteobacteria; not associated with oil degradation	4%	0.2%	0.6%	0.8%

Omic Technologies -Proteomics



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Questions

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Application to Field Treatment

- Formulation of encapsulated enzyme with hydrogen peroxide embedded
- Apply encapsulated enzyme into vadose zone soils using backhoe
- Encapsulant is resistant to mechanical stress due to size
- Measure TPH concentration to determine when to reapply enzyme
- Monitor microbial community

Field Approach Schematic

Heavy crude contaminated soil

Collect and analyze soil samples

Reapply encapsulated enzyme

Apply encapsulated enzyme to soil

Mix amended soil

Comparison to Other In Situ Technologies

Technology	Applicability	Cost
Soil vapor extraction	limited	$
Chemical Oxidation	+	$$$$
Bioventing	limited	$
Encapsulated Fungal Enzymes	+	$$