

Oil spills bioremediation in marine environment-biofilm characterization around oil droplets

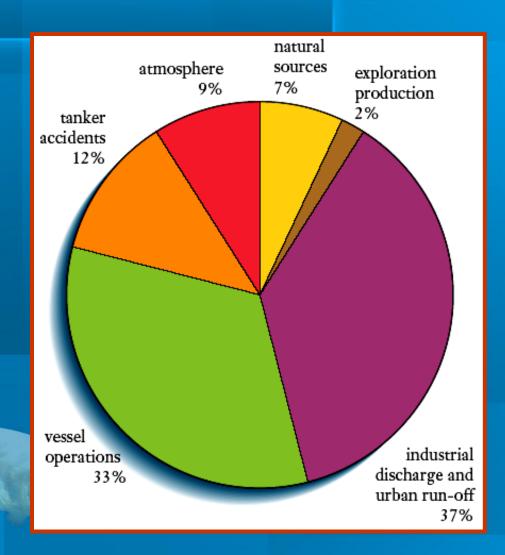
PhD Thesis

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Definition of the Problem

- Accidental crude oil spills are mainly caused by operations of extraction, transportation, storage, refining and distribution.
- Between 1.7 and 8.8 million metric tons of oil are released into the world's water every year.
- About 12% of the oil released into the marine environment is from tanker accidents



The need for action

- Marine shorelines are important public and ecological resources
- Oil spills have posed great threats and caused extensive damage to the marine coastal environments







Loss of species richness, downgraded sediment quality, a negative impact on offshore fish, crustacean fisheries with many long term adverse environmental effects and also on the touristic sector with many socioeconomical side effects.

BIOREMEDIATION OF OIL SPILLS

(after first response)

ENHANCED BIOREMEDIATION

NATURAL ATTENUATION

ADDITION of Nutrients, Surfactants and Microbes

NO-ACTION OPTION (only monitoring)

ENHANCED BIOREMEDIATION

BIOAUGMENTATION

 Large amounts of oildegrading bacteria are added to supplement the indigenous microbial population.

BIOSTIMULATION

 Addition of nutrients or other growth-limiting cosubstrates to stimulate the growth of indigenous oil degraders.

Biostimulation vs Bioaugmentation

Bioaugmentation

Research studies have failed to prove conclusively that seeding is effective (with bioengineered organisms or organisms enriched from different environments and grown in the laboratory to high numbers).

Bioaugmentation appears less effective than Biostimulation since

- 1. The influx of oil causes a relatively fast response in the hydrocarbon-degrading bacteria; however, in the absence of essential nutrients their growth is limited.
- 2. Seeded microorganisms usually show a short term benefit; however, over a longer period of time the indigenous populations catch up and the main limiting factor becomes the lack of N & P.

Autochthonous Bioaugmentation (ABA)

Uses exclusively microorganisms indigenous to the sites to be decontaminated

Key idea is to conduct the enrichment of contaminantdegrading bacteria under the same or very similar conditions to those where bioaugmentation will be performed

Isolated single strains or enriched cultures, which are obtained "before" or "after" the contamination of the target sites, are administered to the sites once contamination occurs

Oil Spill Problem

- Open water (e.g., Oil Spill off the Galicia coast of Spain- Prestige 2002)
 - Surface spills
 - Deep sea releases

> Shoreline (e.g., Oil Spill at Alaska- Exxon valdez 1989)

Major Challenges in Open-waters

Control of the release rates so that optimal nutrient concentrations can be maintained in the sea water over long time periods

Oleophilic Nutrients:

- To overcome the wash out problem, it is recommended to utilize oleophlilic organic nutrients (e.g., Uric acid ,Lecithin)
- The rationale for this approach is that oil biodegradation occurs primarily at the oil-water interface. Since oleophilic fertilizers are able to adhere to oil and provide nutrients at the oil-water interface, enhanced biodegradation should result without the need to increase nutrient concentrations in the bulk sea water

Major Challenges in Open-waters

Biosurfactants:

- Biosurfactants are amphiphilic compounds of microbial origin with considerable potential in commercial applications within various industries.
- The ability of biosurfactants to emulsify hydrocarbon- water mixtures enhances bioavailability of the hydrocarbons to the microbial population thus leads to degradation of hydrocarbons in the environment.

Shoreline cleanup (Sandy Beach)

Landfarming → High Efficiency and Low Cost

Design Parameters

- Aeration
- Moisture
- pH
- Other constrains

(Nutrients, microorganisms, etc.)



Main Objective

To examine the effectiveness of novel <u>combined</u> autochthonous bioaugmentation &/or biostimulation strategies in an oil contaminated environment

EPA's Bioremediation Agent Effectiveness test:

Designed to determine a product's ability to biodegrade oil by quantifying changes in the oil composition and microbial activity resulting from biodegradation.

Chemical analyses

→ alkanes and PAHs by GC-MS

Microbiological analyses

→ to determine hydrocarbon degraders by MPN procedure.

Molecular analyses

→ to determine microbial diversity of hydrocarbon degraders

Experimental approaches

- 1. Autochthonous bioaugmentation and/or biostimulation of seawater microcosm
- 2. Autochthonous bioaugmentation & biostimulation with isolated hydrocarbon degraders consortium of seawater microcosm
- 3. Landfarming of oil polluted beach sand through biostimulation
- 4. Landfarming of oil polluted beach sand through autochthonous bioaugmentation & biostimulation
- 5. Biofilm investigation on oil droplets & C20 with Confocal Microscopy (UFZ-Magdeburg)

Experimental approaches

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Microcosm Experimental Set Up

40-mL glass vials were charged with:

- 1. 20 mL of seawater containing the indigenous microorganisms
- 2. 0.5 % w/v of weathered (ASTM method D 86) blend Urals-Arabian light crude oil
- 3. The products were added to the solutions into such amount in order to achieve the optimized nutrient amendment C:N:P (100:10:1)
- 4. The prepared vials were shaken at 150 rpm at 20 °C until such time that they would be removed for sampling.
- 5. The entire vial is sacrificed for analysis (a 1-mL aliquot is removed from each vial for the microbiological analysis and the remainder of each flask is used for the chemical analysis).



Microcosm Experimental Design

Treatment		Weathered crude oil 0.5% w/v	Nutrients (KNO ₃ , KH ₂ PO ₄)	Nutrients (uric acid, lecithin)	Rhamnolipid biosurfactant	Preadapted indigenous population
Control		+				
NPK		+	+			
ULR		+		+	+	
NPKM		+	+			+
NPKMR		+	+		+	+
ULRM		+		+	+	+

Microcosm Experimental Set Up

100-mL glass flasks were charged with:

- 1. 50 mL of seawater containing the isolated consortium
- 2. The inoculum was diluted to have approximately 10⁵ cfu/ml.
- 3. 0.5 % w/v of weathered (ASTM method D 86) blend Urals-Arabian light crude oil
- 4. The products were added to the solutions into such amount in order to achieve the optimized nutrient amendment C:N:P (100:10:1)
- 5. The prepared flasks were shaken at 150 rpm at 20 °C until such time that they would be removed for sampling.
- 6. The entire flask is sacrificed for analysis (a 1-mL aliquot is removed from each flask for the microbiological analysis and the remainder of each flask is used for the chemical analysis).



Microcosm Experimental Design

Treatment	Weathered crude oil 0.5% w/v	Nutrients (KNO ₃ , KH ₂ PO ₄)	Rhamnolipid biosurfactant	Isolated Hydrocarbon Degraders Consortium (Eb8)
Control	+			+
NPKM	+	+		+
NPKMR	+	+	+	+

Soil Microcosm Landfarming Experimental Set Up

Stainless Steal trays were charged with:

- 1. 2 kg of sieved (<2mm) beach sand containing the indigenous microorganisms
- 2. 0.5 % w/w of weathered (ASTM method D 86) blend Uralsk light crude oil to reach oil contamination 5000 ppm.
- The products were added to the solutions into such amount in order to achieve the optimized nutrient amendment

 C:N:P (100:10:1)
- 4. water content was adjusted to 60% of the field-holding capacity, i.e. 20%
- 5. The prepared trays were mixed twice a week and incubated at RT until such time that they would be removed for sampling.
- 6. Samples were taken from each microcosm by sampling at various points, combining and mixing. 15 gr of sand were removed for the chemical analysis and 5 gr for the microbiological analysis.
- 7. Experiments were run for 45 days.

Soil Microcosm Experimental Design

Treatment	Weathered crude oil 0.5% w/w	Nutrients (KNO ₃ , KH ₂ PO ₄)	Nutrients (uric acid, lecithin)	Rhamnolipid biosurfactant	Isolated Hydrocarbon degraders
Control	+				
NPK	+	+			
ULR	+		+	+	
Control M	+				+
NPKM	+	+			+
ULRM	+		+	+	+

Soil Microcosm Landfarming Experimental Set Up

Glass trays (Pyrex) were charged with:

- 1. 1 kg of sieved (<2mm) beach sand containing the isolated hydrocarbon degraders
- 2. 0.5 % w/w of weathered (ASTM method D 86) Iranian light crude oil to reach oil contamination 5000 ppm.
- 3. The products were added to the solutions into such amount in order to achieve the optimized nutrient amendment C:N:P (100:10:1)
- 4. water content was adjusted to 60% of the field-holding capacity, i.e. 20%
- 5. The prepared trays were mixed twice a week and incubated at 20 °C until such time that they would be removed for sampling.
- 6. Samples were taken from each microcosm by sampling at various points, combining and mixing. 15 gr of sand were removed for the chemical analysis and 5 gr for the microbiological analysis.
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Soil Microcosm Experimental Design

Treatment	Weathered crude oil 0.5% w/w	Nutrients (KNO ₃ , KH ₂ PO ₄)	Nutrients (uric acid, lecithin)	Rhamnolipid biosurfactant	Isolated Hydrocarbon degraders
Control	+				
NPK	+	+			
ULR	+		+	+	
Control M	+				+
NPKM	+	+			+
ULRM	+		+	+	+

Biofilm Microcosm Experimental Set Up

250-mL glass flasks were charged with:

- 200 III glass Hasks Were offargea With.
- 1. 100 mL of ONR7 medium containing the isolated consortium Eb8 or E4
- 2. 0.5 % w/v of weathered (ASTM method D 86) blend Iranian light crude oil
- 3. The products were added to the solutions into such amount in order to achieve the optimized nutrient amendment C:N:P (100:10:1)
- 4. The recommended dose dispersant: oil (1:10) by the manufacturer
- 5. The prepared flasks were shaken at 150 rpm at 20 °C (Eb8) or at 14 °C (E4) until such time that they would be removed for sampling.
- 6. A few μLs aliquot is removed from each flask were placed to a specifically designed 1,02mm Deep Chamber, stained with Syto9 (bacteria) and immediately examined by CLSM.



Biofilm Experimental Design

Treatment With Eb8(20°C)/E4(14°C)	Weathered crude oil 0.5% w/v	Nutrients (KNO ₃ , KH ₂ PO ₄)	Rhamnolipid biosurfactant	Corexit	marichem	S200
Eb8R/ E4R	+		+			
Eb8RNP/ E4RNP	+	+	+			
Eb8C/ E4C	+			+		
Eb8CNP/ E4CNP	+	+		+		
Eb8MNP/ E4MNP	+	+			+	
Eb8SNP/ E4SNP	+	+				+

Biofilm Microcosm Experimental Set Up

petri dishes were charged with:

- 1. 100 mL of ONR7 medium containing the isolated consortium Eb8 or E4
- 2. Droplets of C20 (500000 ppm, 0.4 μ l) were placed on a sterile plastic slide
- The products were added to the solutions into such amount in order to achieve the optimized nutrient amendment ——>C:N:P (100:10:1)
- 4. The prepared petri dishes were shaken at 100 rpm at 20 °C (Eb 8) or at 14 °C (E4) until such time that they would be removed for sampling.
- 5. After 5, 7, 11, 14 and 18 days, pieces with 2 C20 droplets per slide were cut off, stained with Syto9 (bacteria) and immediately examined by CLSM.

C20 Biofilm Experimental Design

Treatment with C20	Nutrients (KNO ₃ , KH ₂ PO ₄)	Rhamnolipid biosurfactant	Consortium Eb8	Consortium E4
Eb8 (20°C)	+		+	
E4 (14°C)	+			+
Eb8R (20°C)	+	+	+	+
E4R (14°C)	+	+		+

Chemical Analyses (1)

Quantification of the hydrocarbon target analytes was performed by GC-MS according to a modified EPA method (40 CFR Ch. I, Pt 300, App. C)

LIQUID EXTRACTION

- 1. Flasks contents were sacrificed and extracted by adding approximately 50 mL of DCM spiked with a surrogate recovery standard (4 ng/ μ L of each d₁₀-anthracene, 5 $_{\alpha}$ -androstane in solvent of the final extract)
- 2. After mixing for several minutes, the flask was set aside to allow the dichloromethane and water layers to partition.
- 3. The dichloromethane layer was passed through a funnel packed with anhydrous sodium sulfate.
- 4. The dichloromethane was evaporated in rotavapor concentrator.

Chemical Analyses (1)

SOIL EXTRACTION

- Sand samples were dried with anhydrous sodium sulfate, spiked with a surrogate recovery standard (200 ppm of each d10anthracene, 5α-androstane) and finally extracted with soxhlet apparatus using 300 mL of DCM
- 2. The dichloromethane was evaporated in rotavapor concentrator.
- 3. The volume was blown down to dryness (nitrogen).



Chemical Analyses (2)

- 6. A known weight of 5-10 mg of the dried oil was dissolved in was transferred onto the Bond Elut TPH SPE cartridge and eluted, under a positive pressure, with 4ml of *n*-C6 (FI- aliphatics) and 4ml of DCM (FII- aromatics).
- 7. The two fractions were blown down to dryness (nitrogen)
- 8. The weight of FI- aliphatics and FII- aromatics was recorded and redesolved in 1 mL *n*-C6 and 1 mL DCM respectively
- 9. The final concentration of the internal standards added in each fraction right before the injection is 1 ppm. This solution contained 4 deuterated compounds: d_8 -naphthalene, d_{10} -phenanthrene, d_{12} -chrysene and d_{12} -perylene.

Depletion of target analytes

%Depletion =
$$\frac{[(Ao/H_o) - (A_s/H_s)]}{(A_o/H_o)} \times 100$$

- A_s= Concentration of target analyte in the sample
- A_o= Concentration of target analyte in the initial sample
- H_s= Concentration of 17a(H), 21b(H) C₃₀-hopane in the sample
- H_o = Concentration of 17a(H), 21b(H) C_{30} -hopane analyte in the initial sample

Kinetic Analysis (Batch)

Cell Growth

$$r_X = \frac{dX}{dt} = \mu X$$

- μ specific growth rate (1/h)
- Substrate Utilization

$$r_{S} = \frac{dS}{dt} = -q_{S}X$$

• q_s specific degradation rate (µg/cells h)

Estimation of q, by the integral method

Integration of Substrate equation yields:

$$S_t - S_0 = -\overline{q}_s \int_0^t X dt$$

Average specific degradation rate is obtained by LS estimation

Microbiological Analyses (1)

Microbial enumerations of hydrocarbon degraders are performed at each sampling event using a microtiter MPN determination

- 1. The growth medium was a B-H minimal salts mediun supplemented with crude oil as the hydrocarbon substrate.
- 2. The MPN plates were 96-well microtiter tissue culture plates
- 3. Each well contains 180 μ L BHS, 5 μ L crude oil and 20 μ L of the appropriate dilution of sample
- 4. The samples were diluted in a 9 mL B-H solution (pH 7).

Microbiological Analyses (2)

- 5. Tenfold serial dilutions were performed, and the plates were inoculated by adding 20 μL of each dilution to I of the 12 columns of eight wells.
- 6. The inoculated plates were incubated at 20 °C for 2 weeks.
- 7. At the end of the incubation period, 50 μ L of p-iodonitrotetrazolium violet dye (3.5 g/L) was added to each well of the tissue culture plates.
- 8. The dye turns from colorless to red (is reduced) in the presence of actively respiring microorganisms.

	Mol	ecular Analyses	
Target	Primer	Sequence (5'-3')	Product

GTGCCAGCMGCCGCGGTAA

GGACTACHVGGGTWTCTAAT

CGCCGTGTGAATGACAAGGG

CGACGCTTGGCGTAAGCATG

GACGTCGCCACACCTGCC

GGGCCATACAGAGCAA

CGTTGTGCGCATAAAGGTGCGG

CTTGCCCTTTCATACCCCGCC

References

Kuczynski et

al., 2012

MacKew et

al., 2007

MacKew et

al., 2007

MacKew et

al., 2007

(bp)

291

132

217

145

	Mol	ecul	ar A	Inal	yses
Townsk					

515F

806R

ALCalkB2F867

ALCalkB2R999

THALalkBF125

THALalkBR342

CYCphnAF243

CYCphn-AR388

genes

alkB2

alkB

phnA

16SrDNA

RT-PCR Conditions

Thermal cycling was as follows:

- Initial denaturation for 5 min at 95°C (holding stage)
- 40 cycles of 95°C for 15 s
- 60°C for 1 min.

Reaction volume/well (20 µl) contained:

- 2 μl of sample DNA
- 10 μl of 2× SYBR Green PCR Master Mix
- 100 nM of each primer

Molecular Analyses

Target genes	Primer	Sequence (5'-3')
16SrDNA	27F	AGAGTTTGATCMTGGCTCAG
bacteria	1492R	GGTTACCTTGTTACGACTT

PCR Conditions

Thermal cycling was as follows:

- Initial denaturation for 5 min at 94°C (holding stage)
- 30 cycles of 94°C for 1 min
- 55°C for 1 min
- 72°C for 2 min
- Final extension at 72°C C for 10 min

Reaction volume/well (50 μl) contained:

2 μl of sample DNA

5 μl of 1× PCR Buffer solution

5 μl of 0.5 μM of each primer

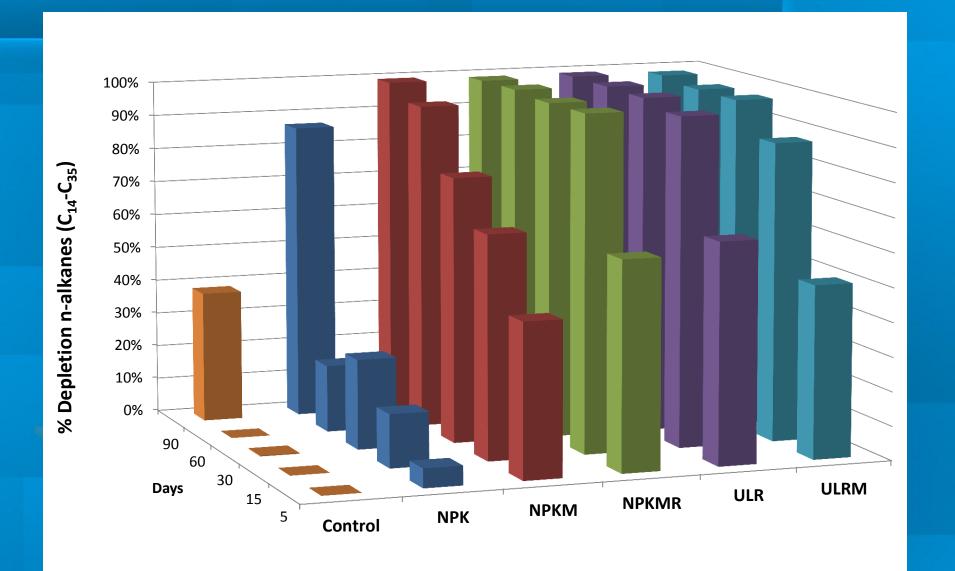
 $0.05 \mu l$ of $0.01 \, mM$ of dNTPs

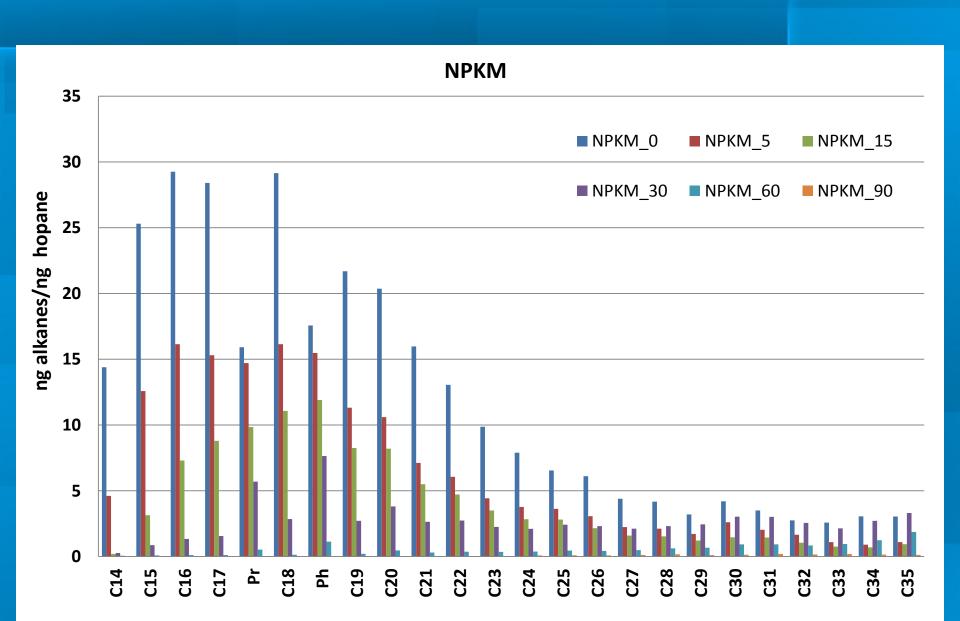
0.3 μl of 0.03U/ μl Taq Polymerase

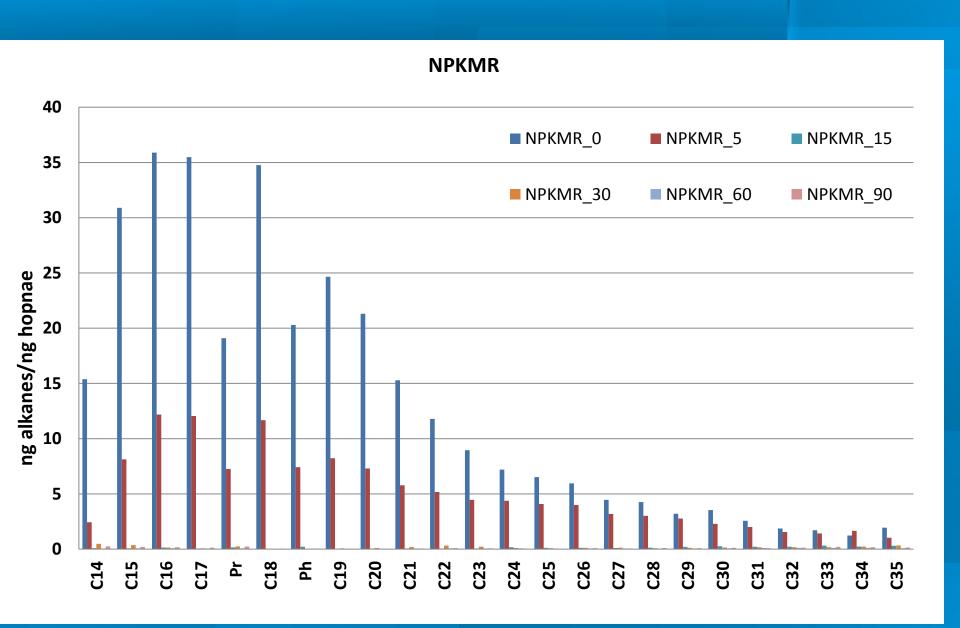
Experimental Results

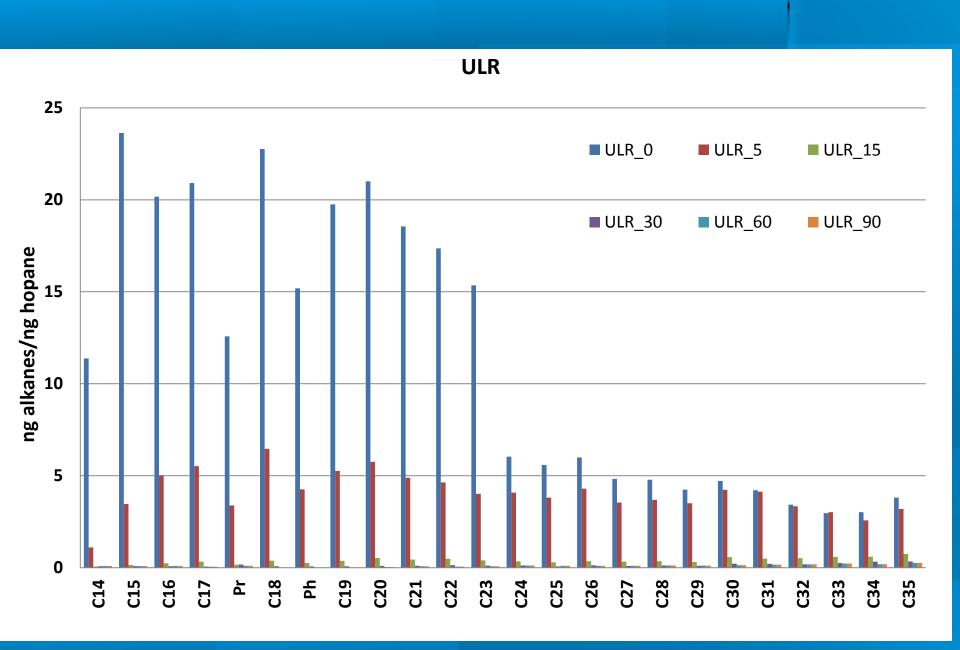
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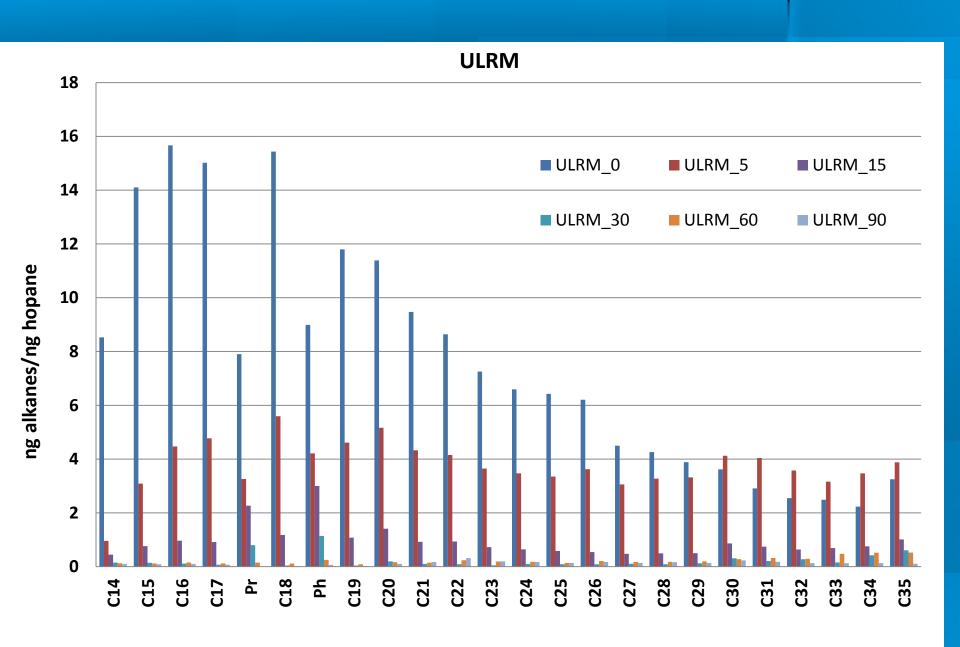
n-alkanes overall degradation profile



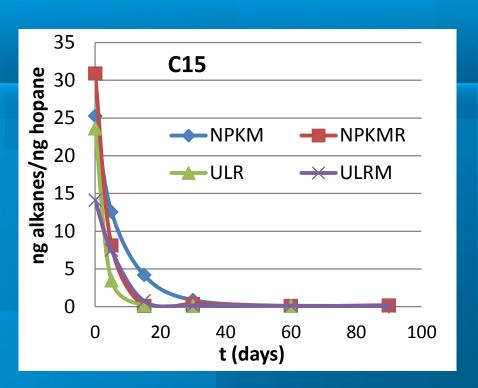


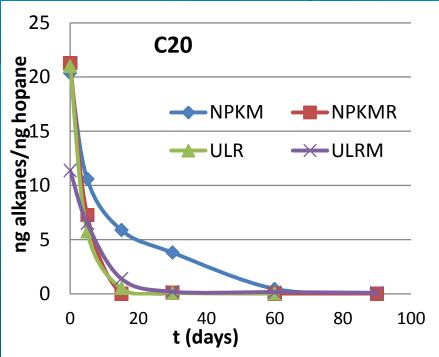






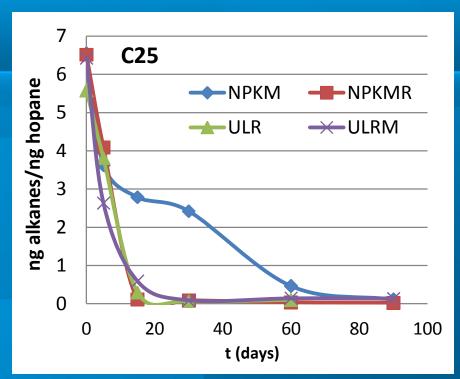
Degradation Kinetics of C15 & C20

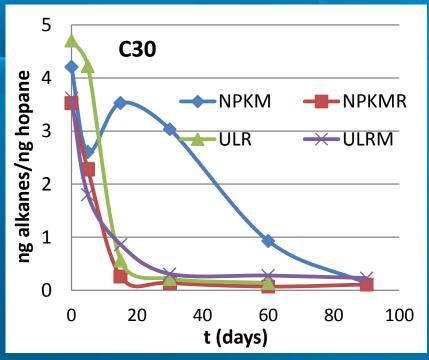




		C15		C	20
	μ	q_{s}	q_s/q_{s0}	q_{s}	q_s/q_{s0}
NPKM	0.004	65	1	51.3	1
NPKMR	0.023	1239.3	19	854.8	17
ULR	0.024	898.4	14	783.2	15
ULRM	0.010	105	1.6	84.1	1.6

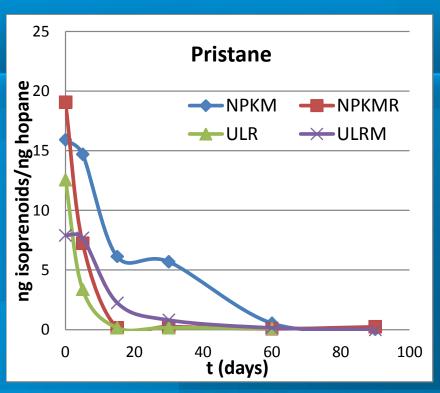
Degradation Kinetics of C25 & C30

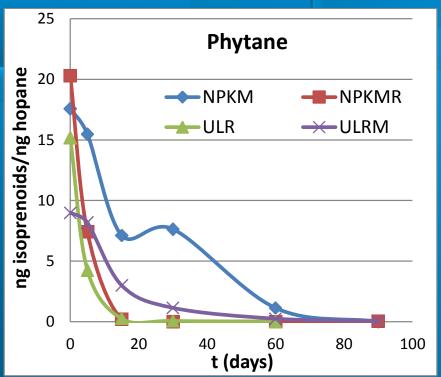




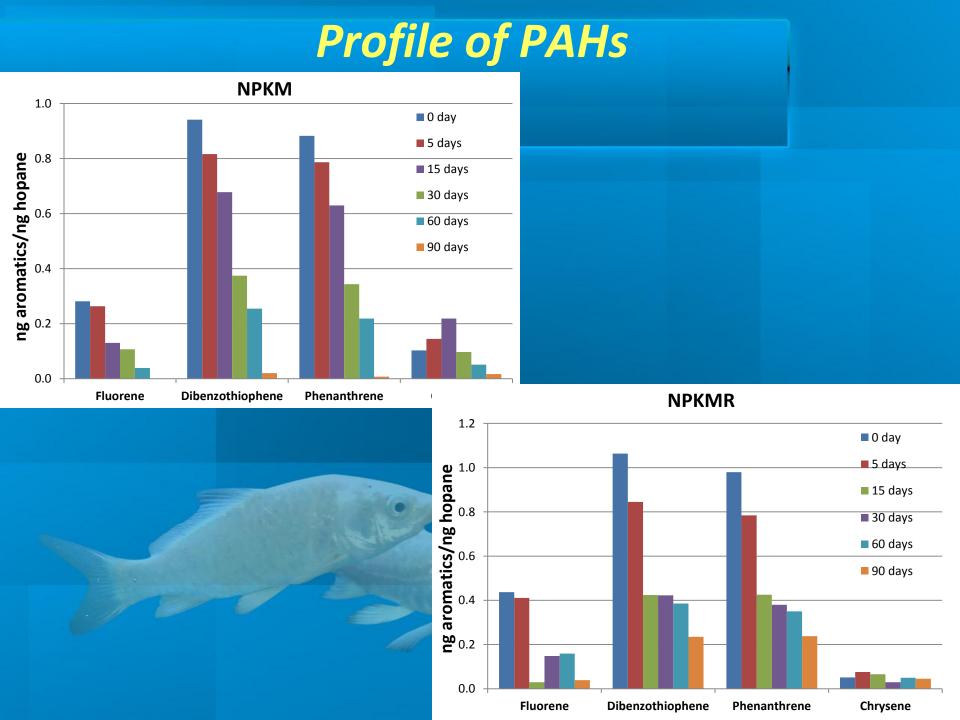
	C25		C30	
	q_s q_s/q_{s0}		q_{s}	q_s/q_{s0}
NPKM	15.7	1	8.4	1
NPKMR	257.3	16	131.3	16
ULR	202.3	12	158.3	19
ULRM	47.7	2	24.9	3

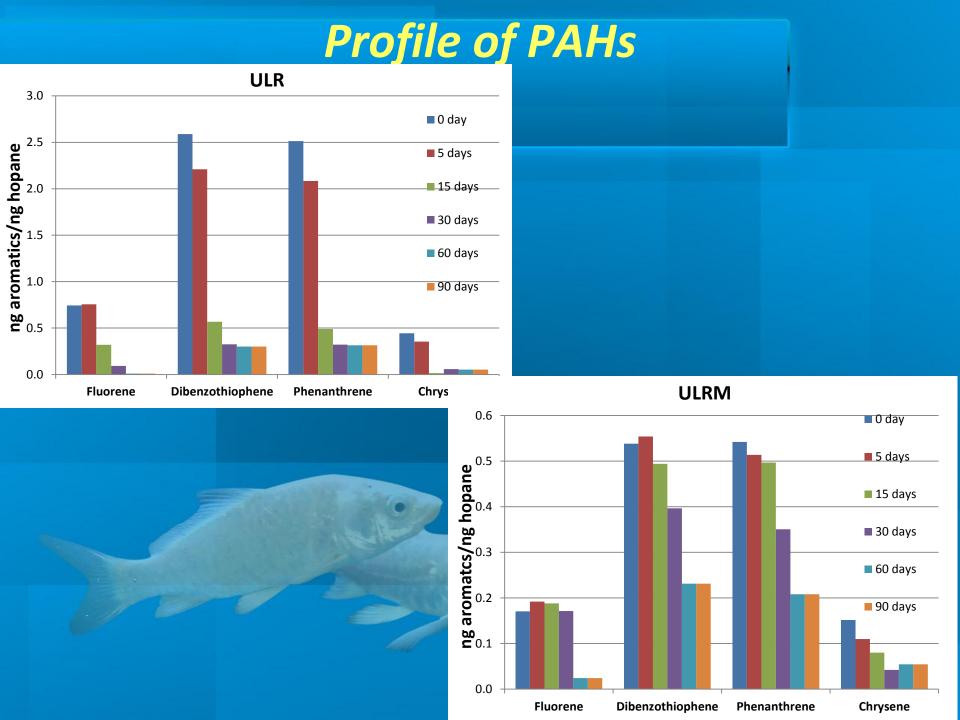
Degradation Kinetics of Pristane & Phytane



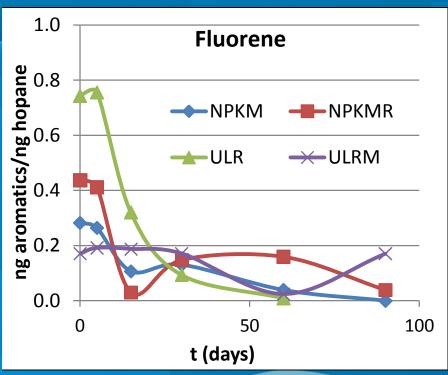


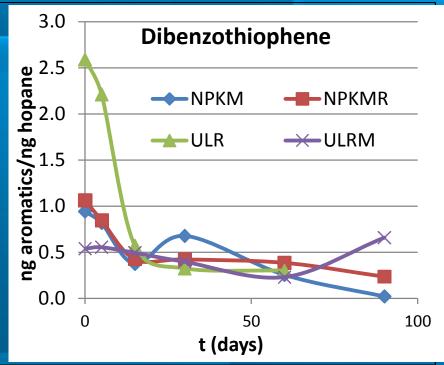
	Pristane		Phytane	
	q_s q_s/q_{s0}		q_{s}	q_s/q_{s0}
NPKM	39.6	1	42.4	1
NPKMR	760.6	19	807.3	19
ULR	474.7	12	571	13
ULRM	53.4	1.3	59	1.3





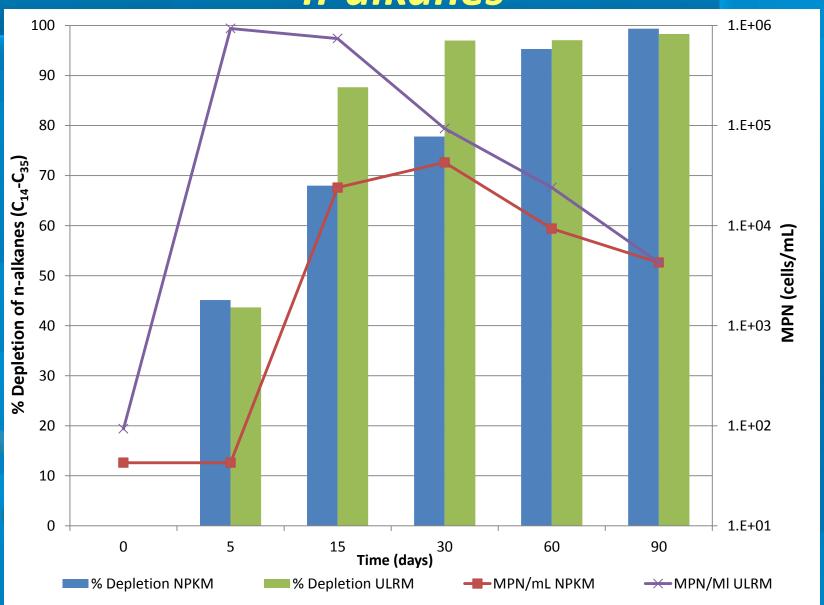
Degradation Kinetics of Fluorene & Dibenzothiophene



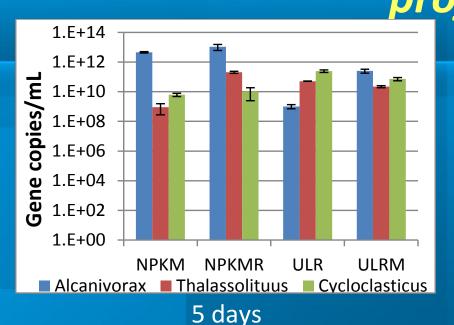


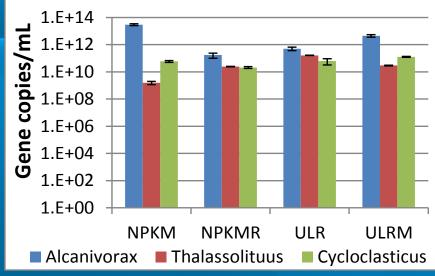
	Fluorene		Dibenzothiothene	
	q_s q_s/q_{s0}		q_{s}	q_s/q_{s0}
NPKM	0.28	1	0.79	1.2
NPKMR	0.46	1.6	0.96	1.5
ULR	1.72	6	6.76	11
ULRM	0.29	1	0.62	1

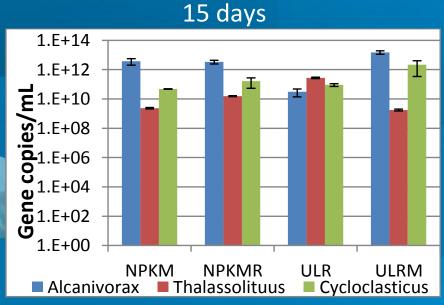
MPN profile & overall degradation of n-alkanes



alkane hydroxylase & PAH dioxygenase genes profile

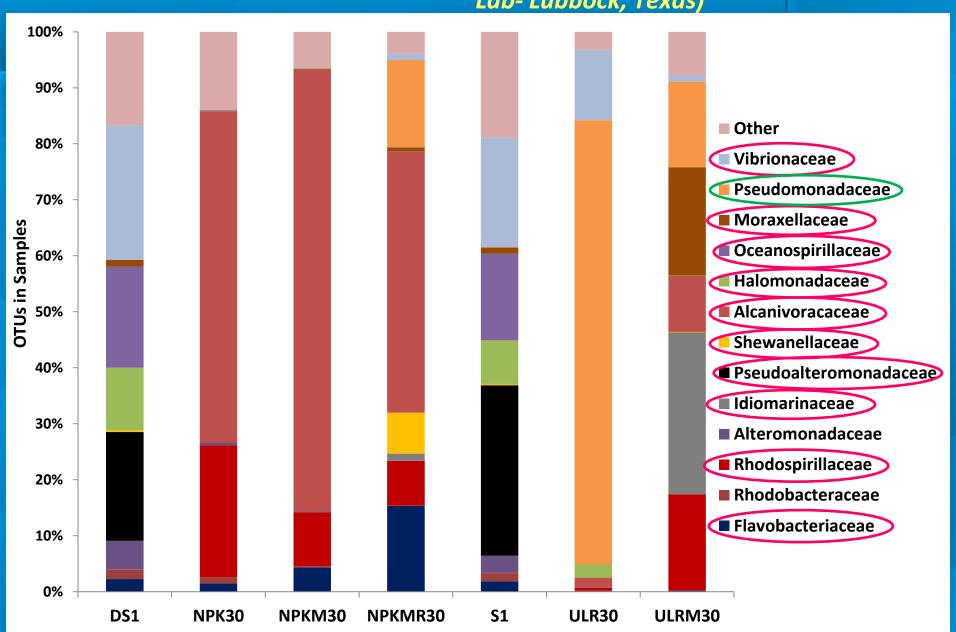






30 days 60 days

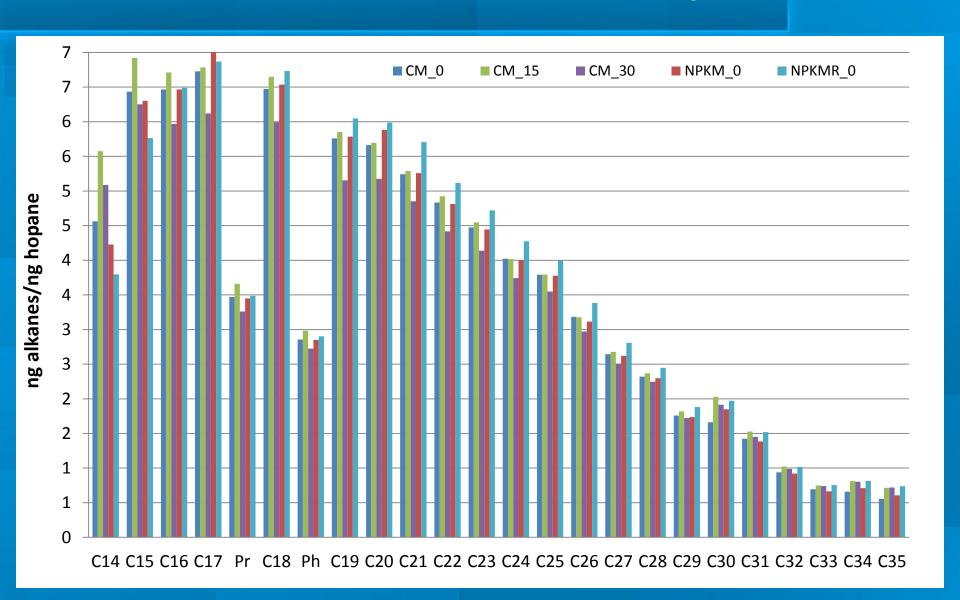
Families Response (Pyrotag-sequencing at Research and Testing Lab- Lubbock, Texas)

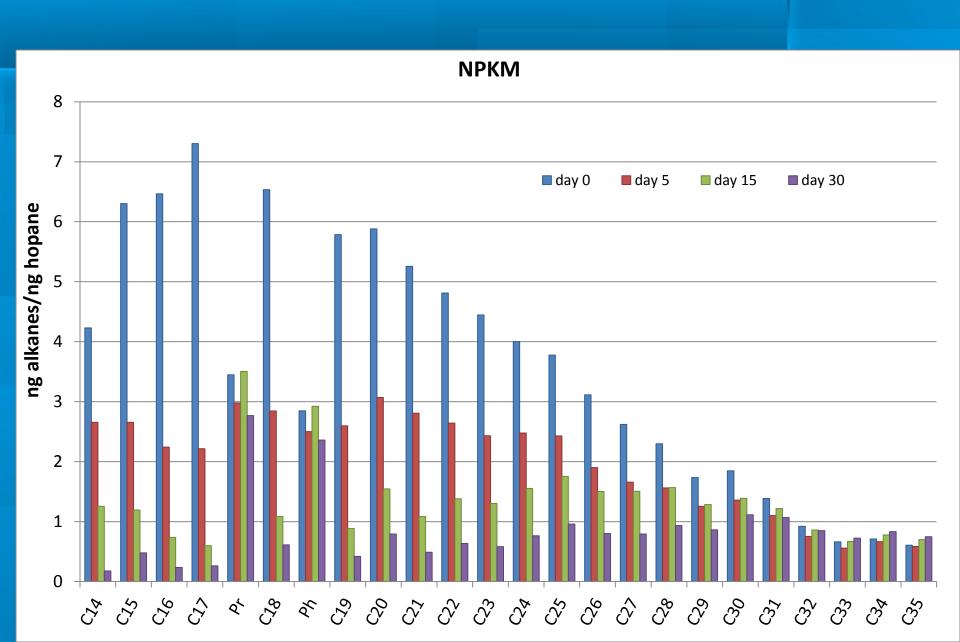


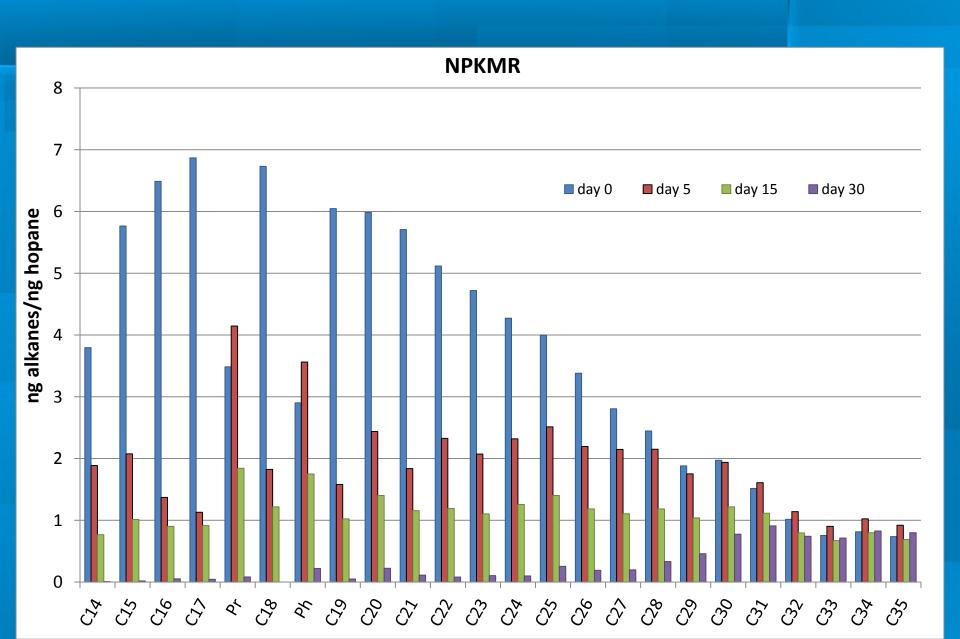
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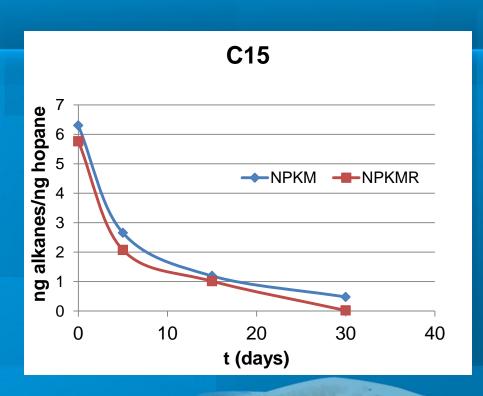
Concentration profile of alkanes at time 0 & Control at 0, 15, 30 days

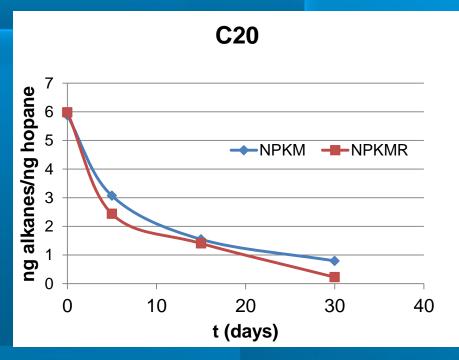






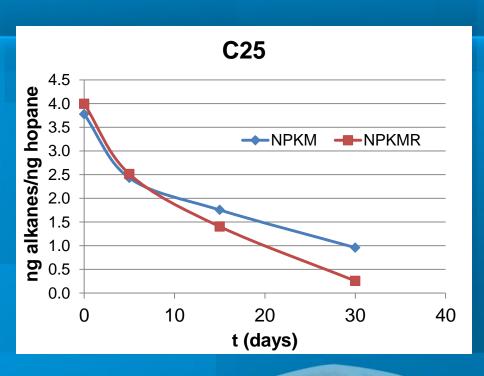
Degradation Kinetics of C15 & C20

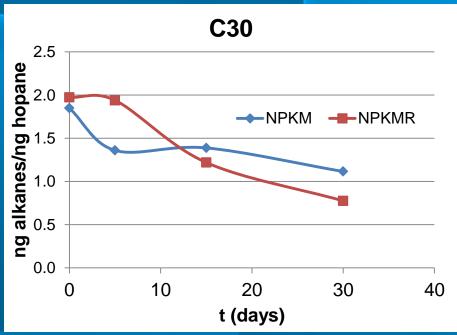




		C15		C20	
	μ	q_{s}	q_s/q_{s0}	q_s	q_s/q_{s0}
СМ	0.011	9	1	24	1
NPKM	0.031	250	28	218	21
NPKMR	0.083	196	23	197	20

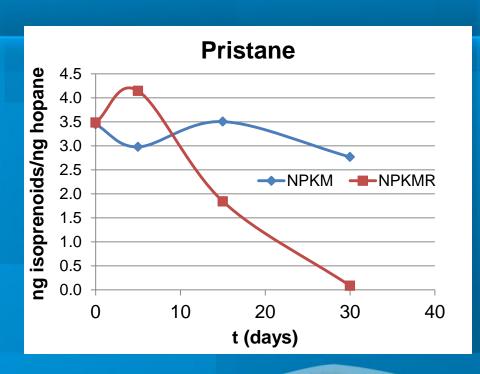
Degradation Kinetics of C25 & C30

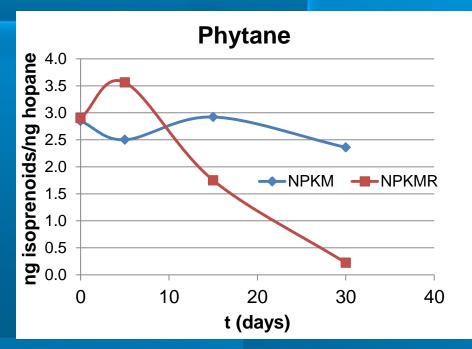




	C25		C	30
	q_s q_s/q_{s0}		q _s	q_s/q_{s0}
СМ	12	1	~0	-
NPKM	121	10	31	-
NPKMR	128	11	41	-

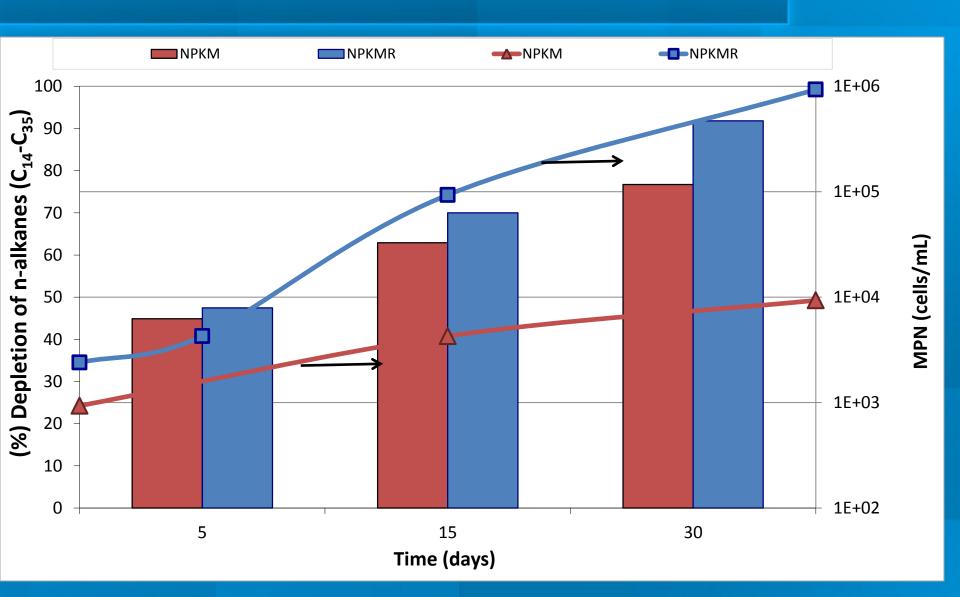
Degradation Kinetics of Pristane & Phytane

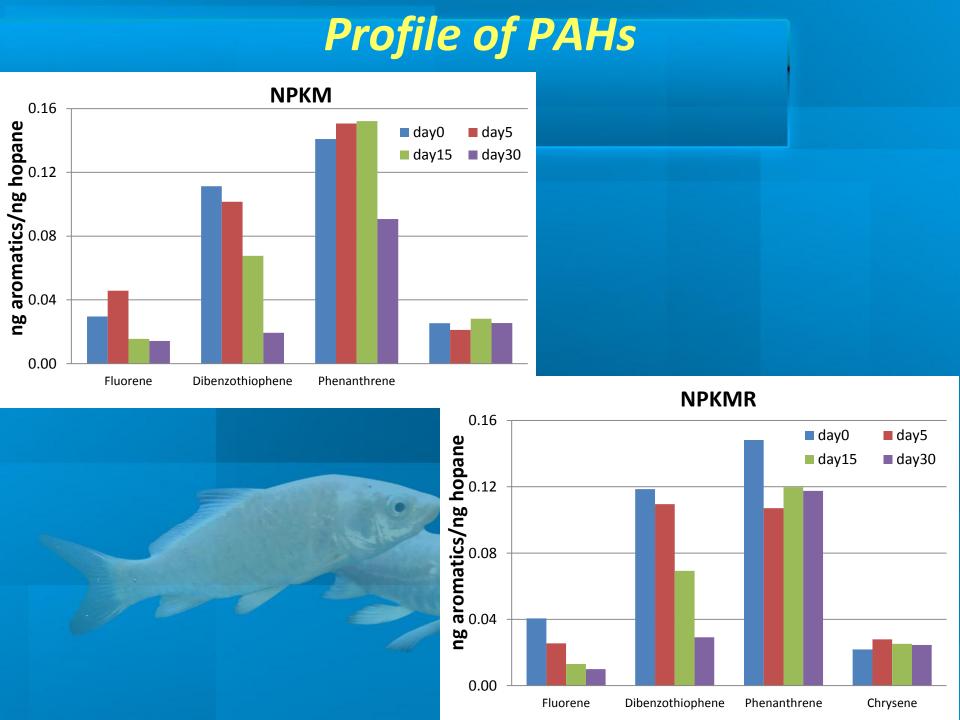




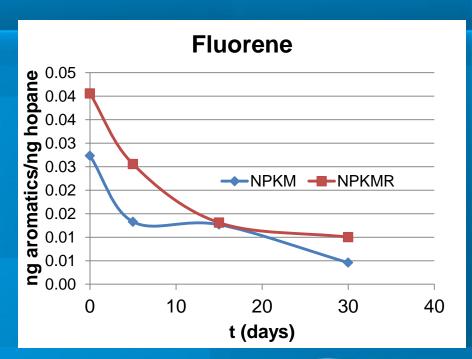
	Pristane		Phytane	
	q_s q_s/q_{s0}		q _s	q_s/q_{s0}
СМ	10	1	6	1
NPKM	29	3	21	3
NPKMR	116	12	92	12

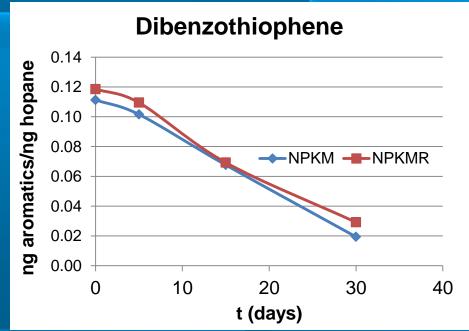
MPN profile & overall degradation of n-alkanes





Degradation Kinetics of Fluorene & Dibenzothiophene



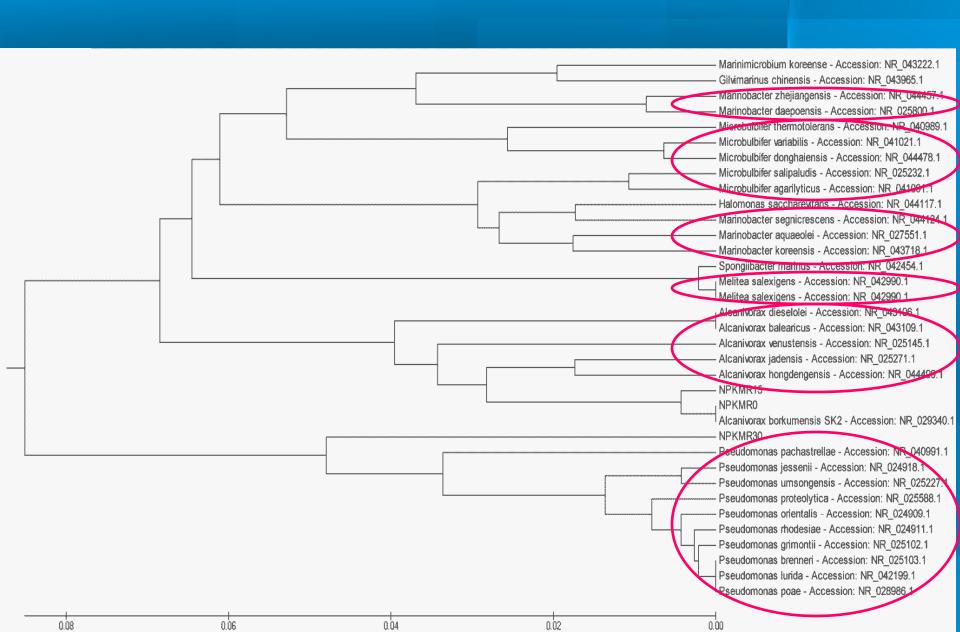


	Fluorene		Dibenzothiophene	
	q_s q_s/q_{s0}		q_{s}	q_s/q_{s0}
СМ	~0	-	~0	-
NPKM	0.97	-	3.94	-
NPKMR	1.04	-	3.06	-

Nucleotide Sequence Analysis Results

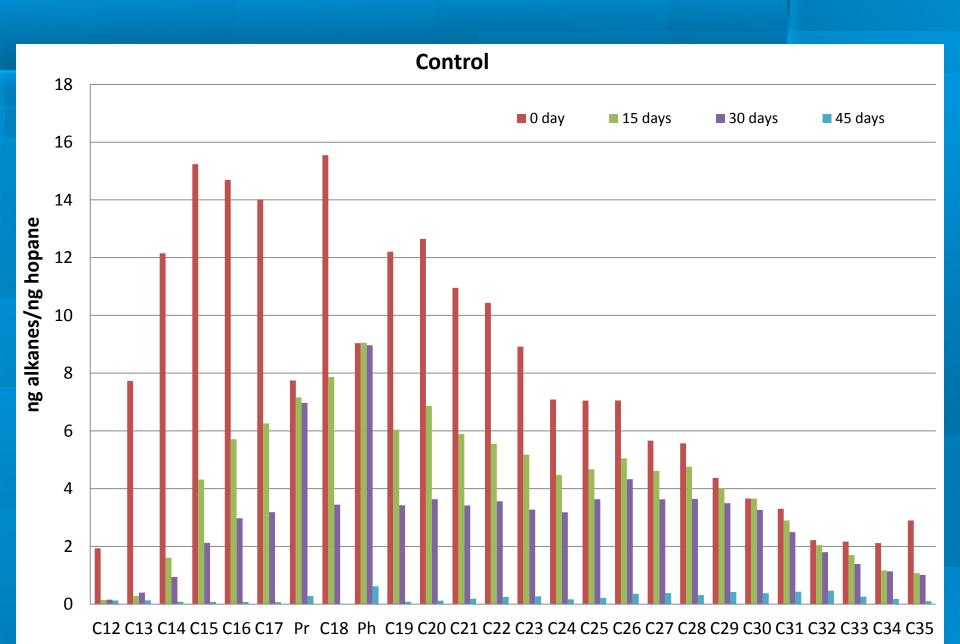


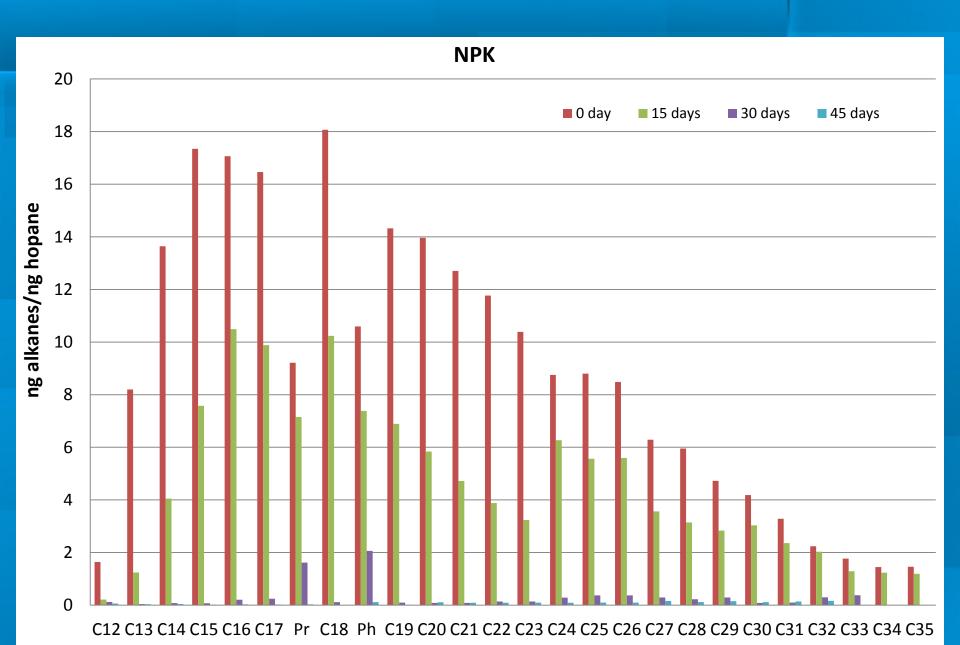
Nucleotide Sequence Analysis Results

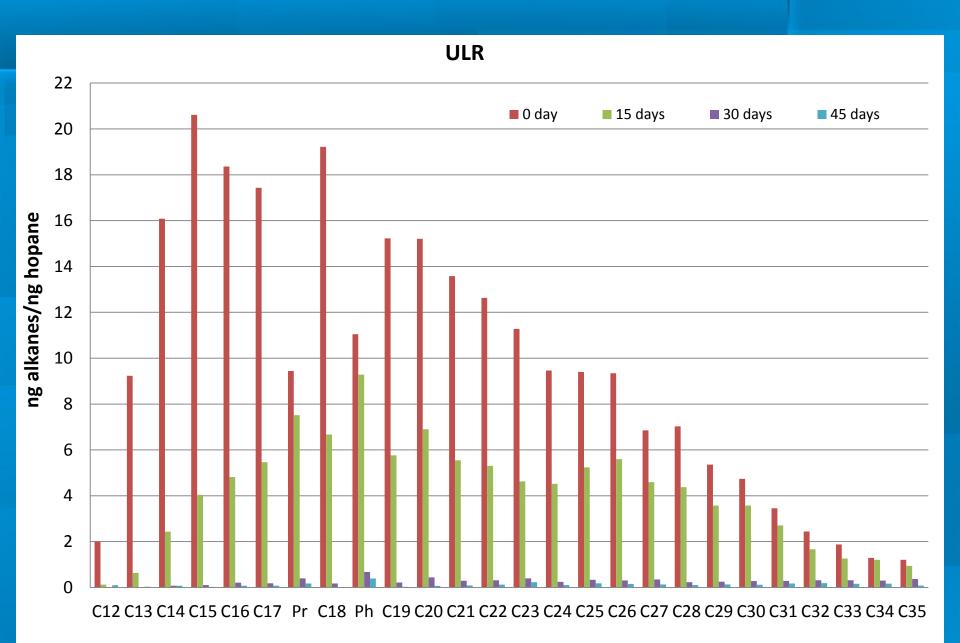


Experimental Results

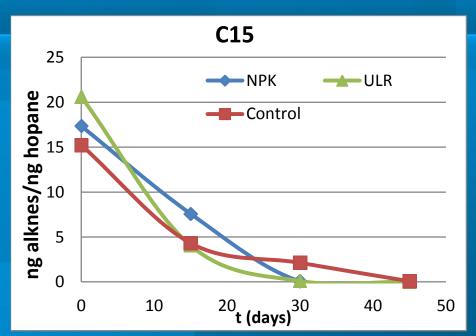
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- 5. Biofilm investigation on oil droplets&C20 with Confocal Microscopy (UFZ-Magdeburg)

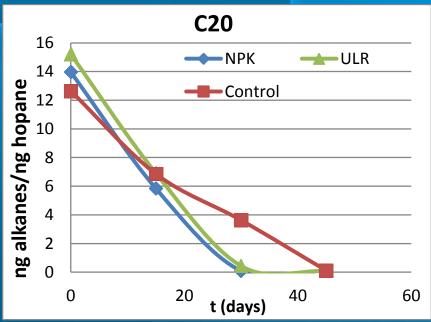






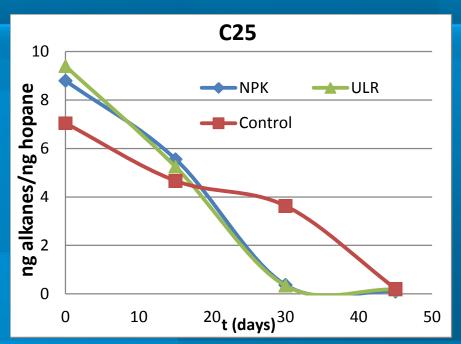
Degradation Kinetics of C15 & C20

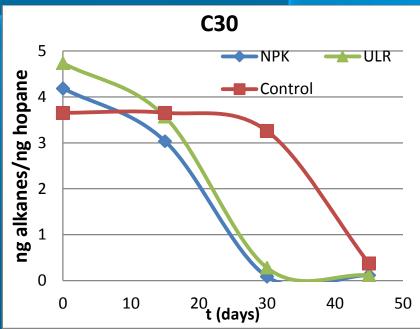




		C15		C	20
	μ	q_{s}	q_s/q_{s0}	q _s	q_s/q_{s0}
Control	0.005	61.3	1	50.6	1
NPK	0.014	201.1	3.3	161.6	3.2
ULR	0.017	202.7	3.3	145.9	2.9

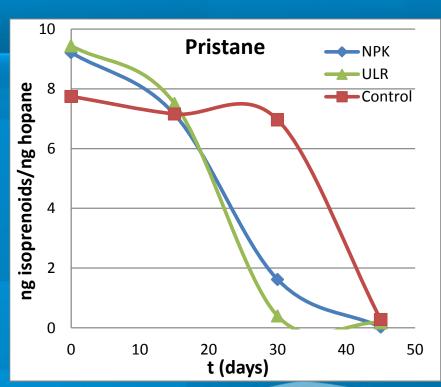
Degradation Kinetics of C25 & C30

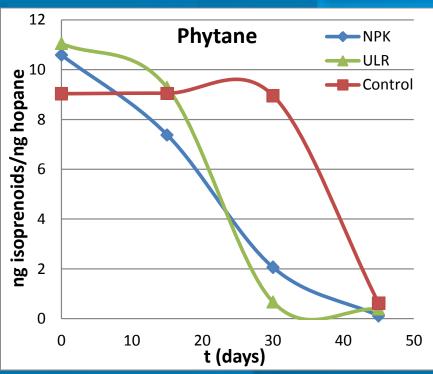




	C25		C30	
	q_s q_s/q_{s0}		q _s	q_s/q_{s0}
Control	27.6	1	13.3	1
NPK	98.2	3.5	47.7	3.6
ULR	89.5	3.2	44	3.3

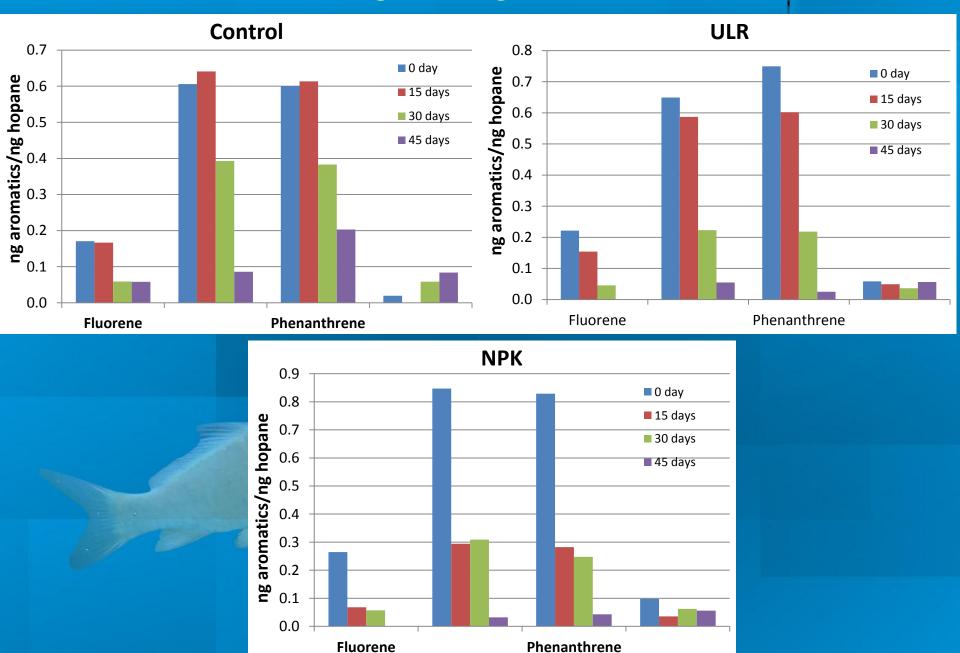
Degradation Kinetics of Pristane & Phytane



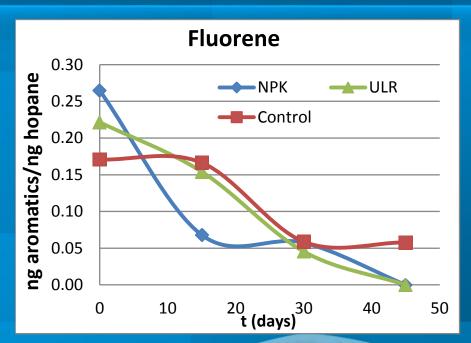


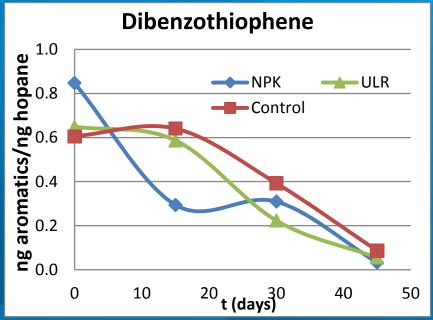
	Pristane		Phytane	
	q_s q_s/q_{s0}		q_{s}	q_s/q_{s0}
Control	30.2	1	34	1
NPK	88.4	3	99.3	3
ULR	89.4	3	102.5	3

Profile of PAHs



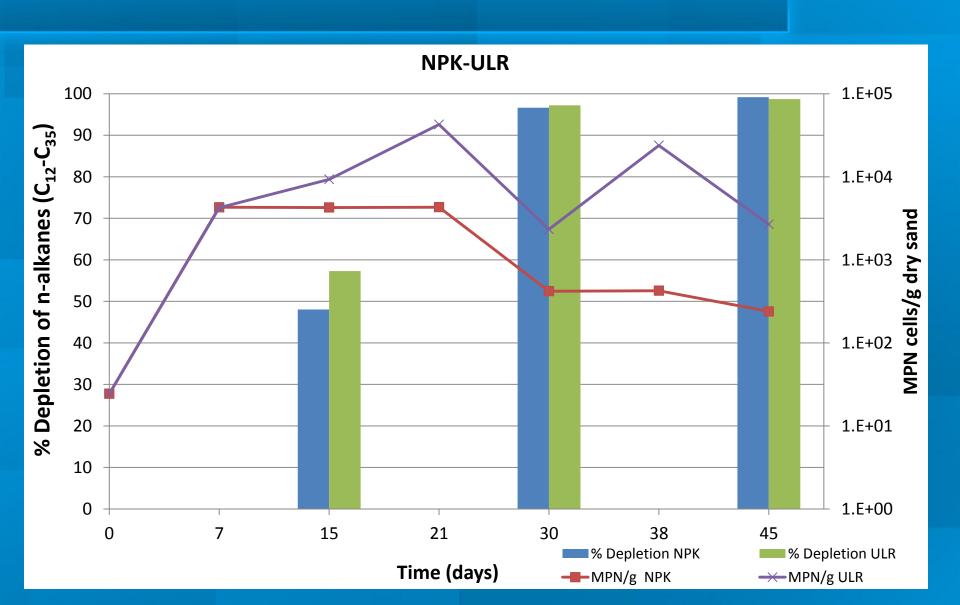
Degradation Kinetics of Fluorene & Dibenzothiophene





	Fluorene		Dibenzothiophene	
	q_{s}	q_s/q_{s0}	q _s	q_s/q_{s0}
Control	0.46	1	2.10	1
NPK	2.29	5	6.44	3
ULR	1.74	4	4.21	2

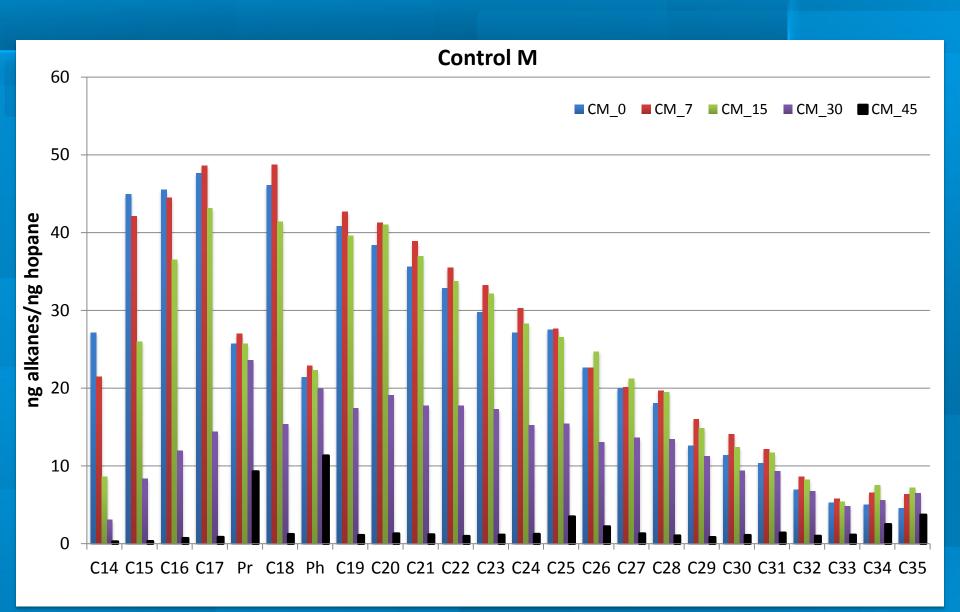
MPN profile & overall degradation of n-alkanes



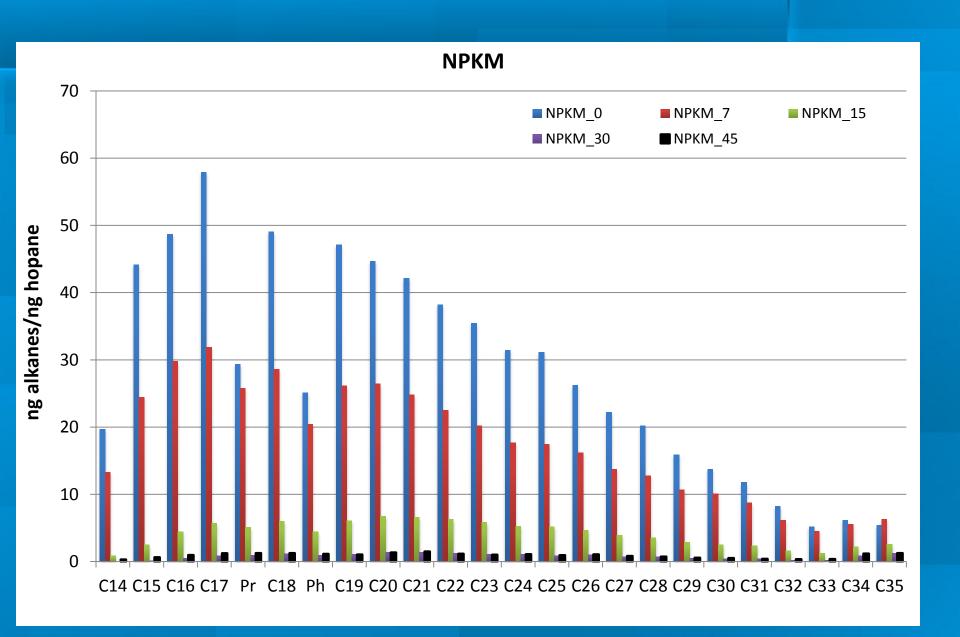
Experimental Results

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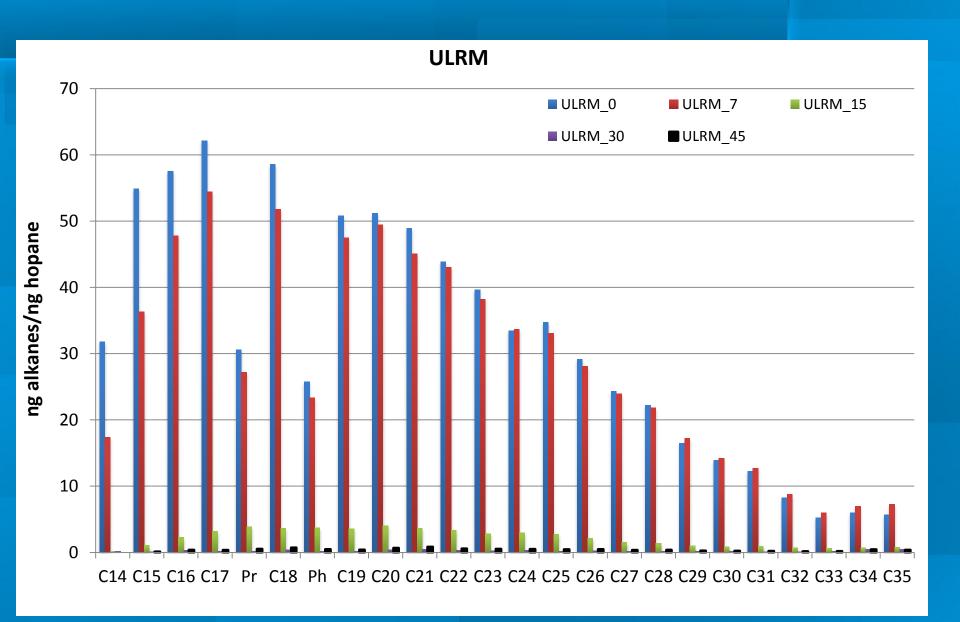
Profile of n-alkanes



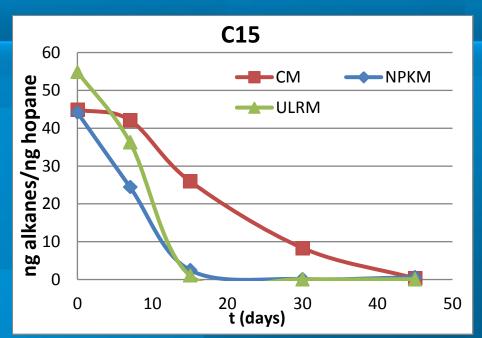
Profile of n-alkanes

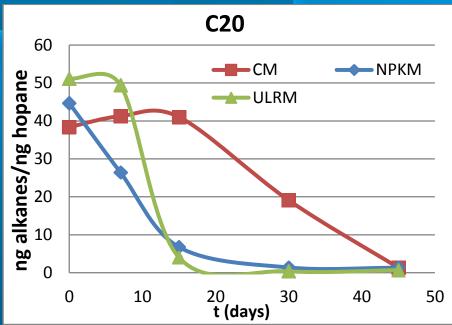


Profile of n-alkanes



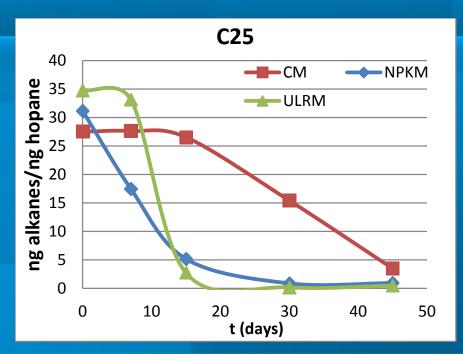
Degradation Kinetics of C15 & C20

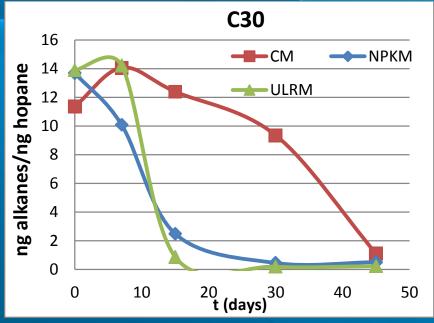




	C15		C20	
	q_{s}	q_s/q_{s0}	q¸	q_s/q_{s0}
Control M	160.7	1	133.7	1
NPKM	1203.8	7.5	1096.1	8.2
ULRM	1252.1	7.8	1097.2	8.2

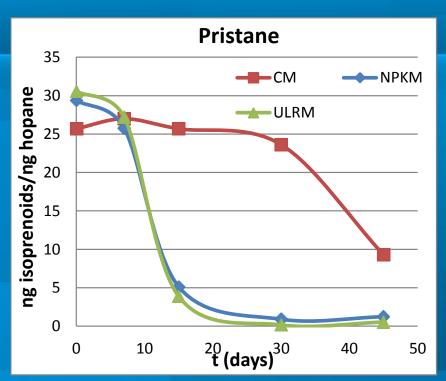
Degradation Kinetics of C25 & C30

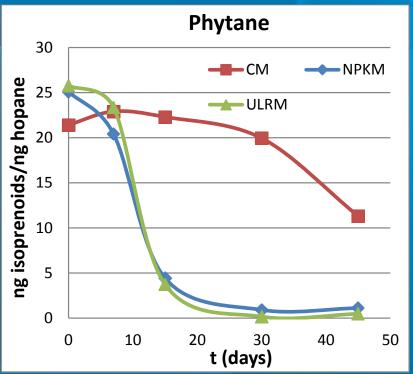




	C25		C30	
	q_{s}	q_s/q_{s0}	q _s	q_s/q_{s0}
Control M	86.6	1	36.9	1
NPKM	751.9	8.7	324.3	8.8
ULRM	745.5	8.6	302.7	8.2

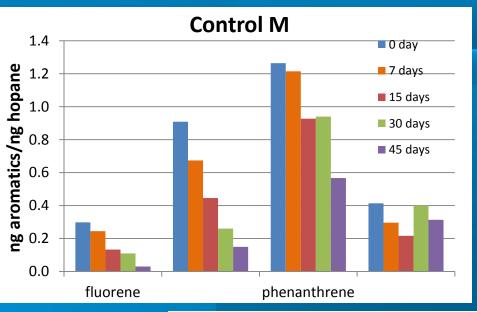
Degradation Kinetics of Pristane & Phytane

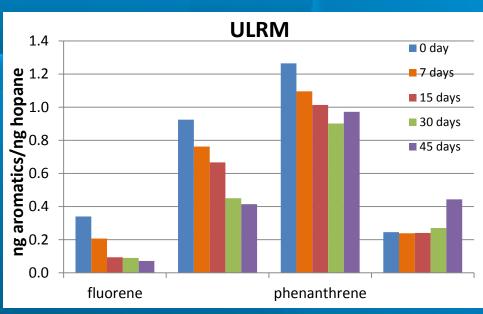


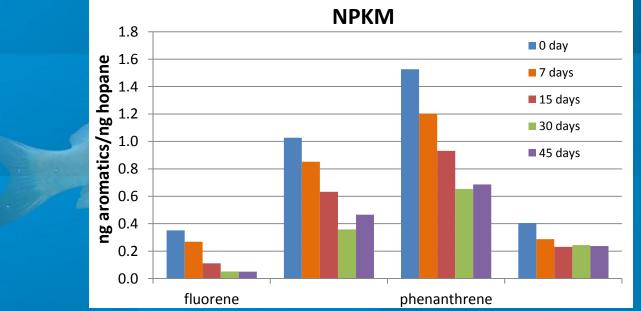


	Pristane		Phytane	
	q_{s}	q_s/q_{s0}	q _s	q_s/q_{s0}
Control M	59.2	1	36.4	1
NPKM	700.9	12	598.3	17
ULRM	621	10.5	512.6	14

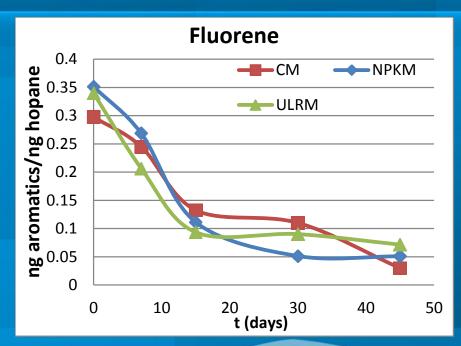
Profile of PAHs

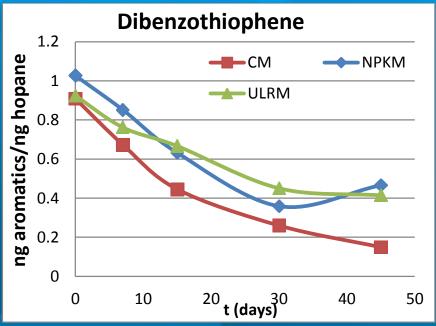






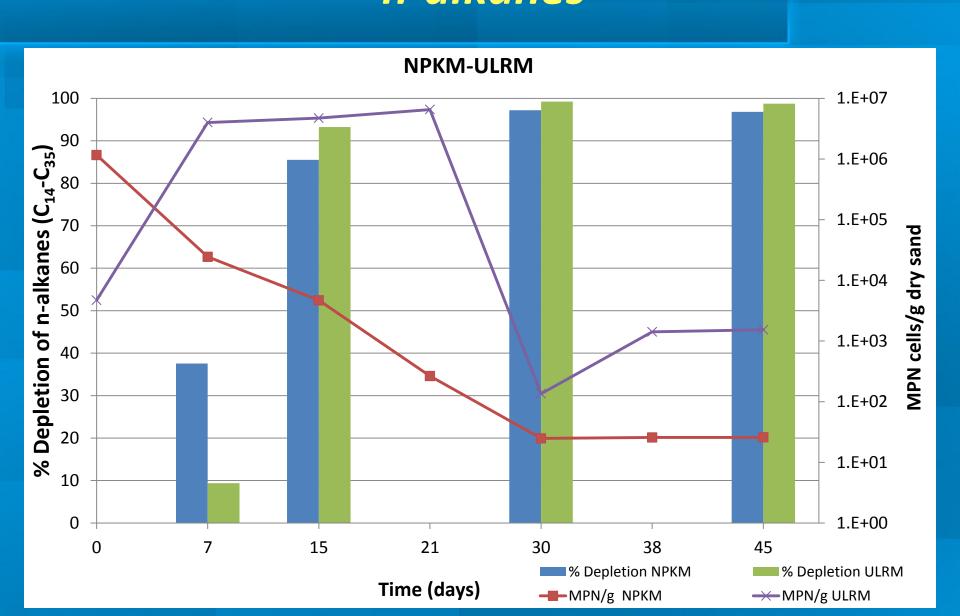
Degradation Kinetics of Fluorene & Dibenzothiophene



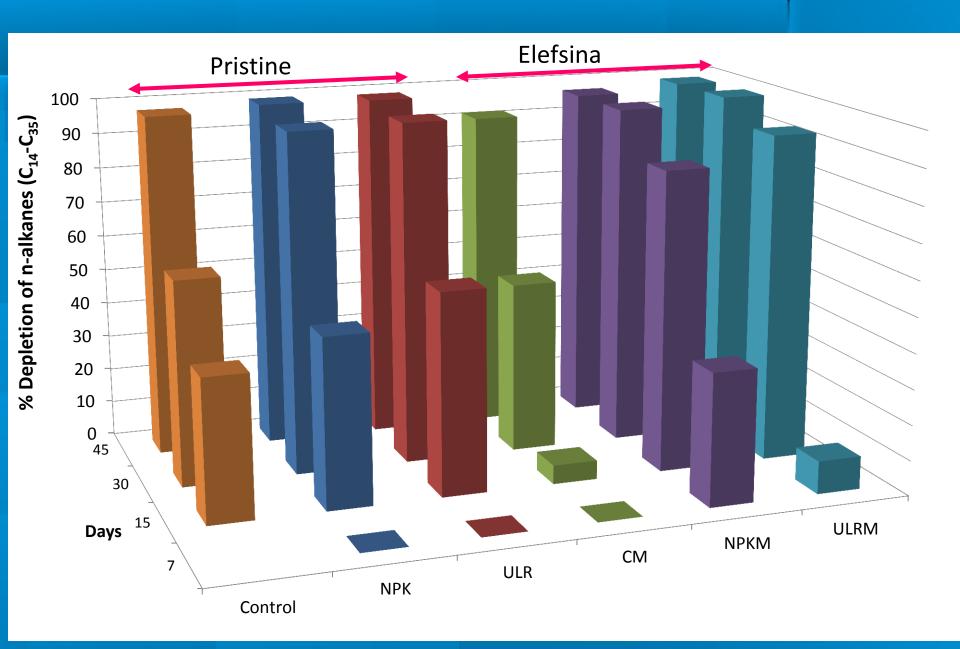


	Fluorene		Dibenzothiophene	
	q_{s}	q_s/q_{s0}	q _s	q_s/q_{s0}
Control M	0.96	1	2.74	1
NPKM	2.86	3	6.38	2.3
ULRM	1.52	1.6	2.89	1

MPN profile & overall degradation of n-alkanes



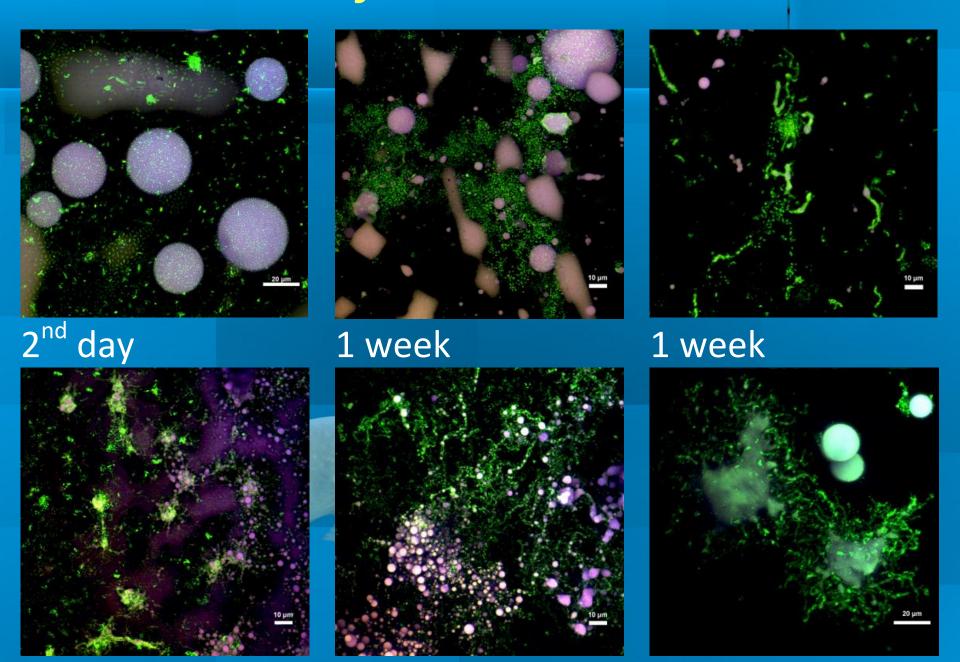
n-alkanes overall degradation profile



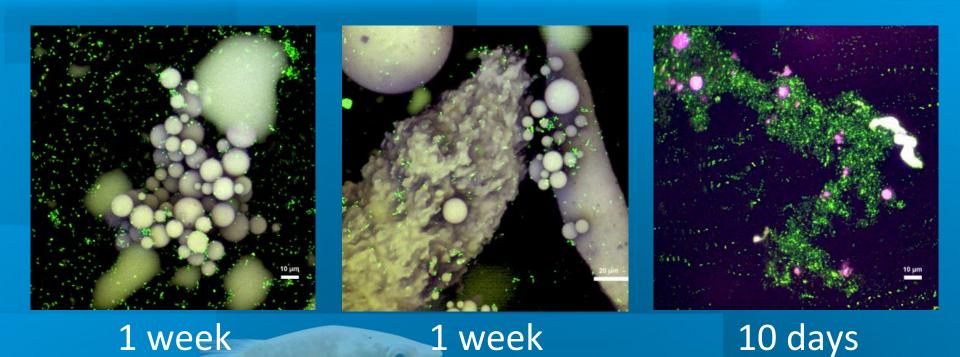
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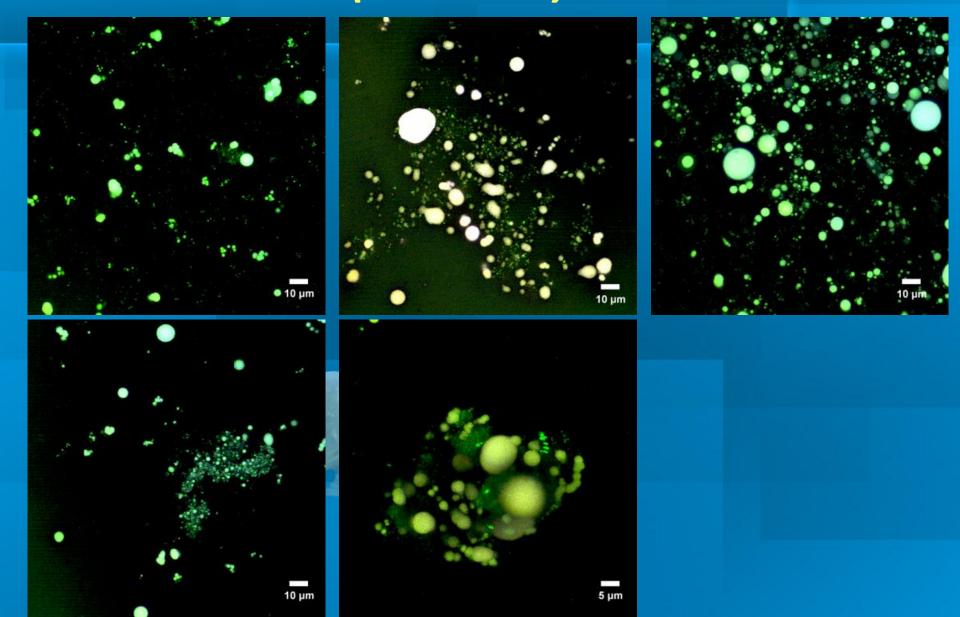
Crude oil from ONR7 Culture



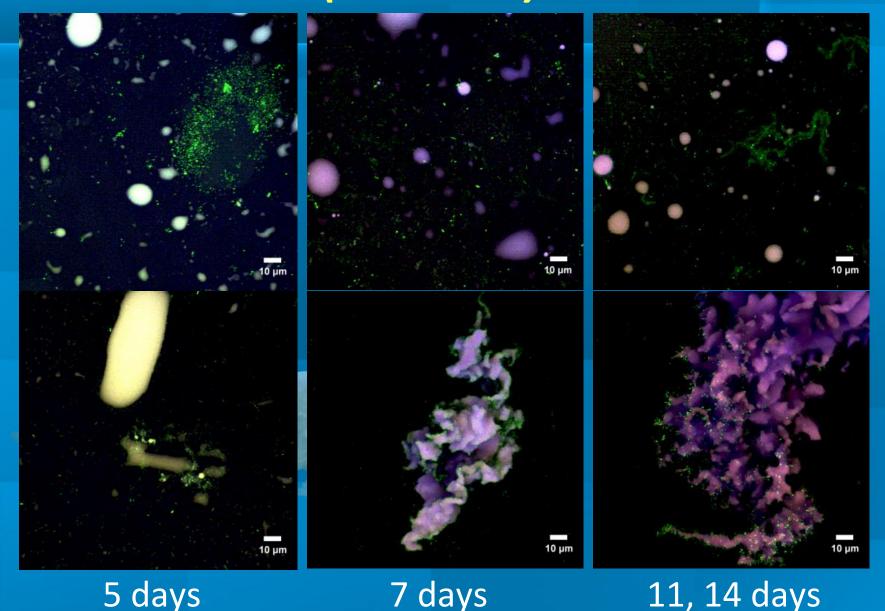
Crude oil from ONR7 Culture



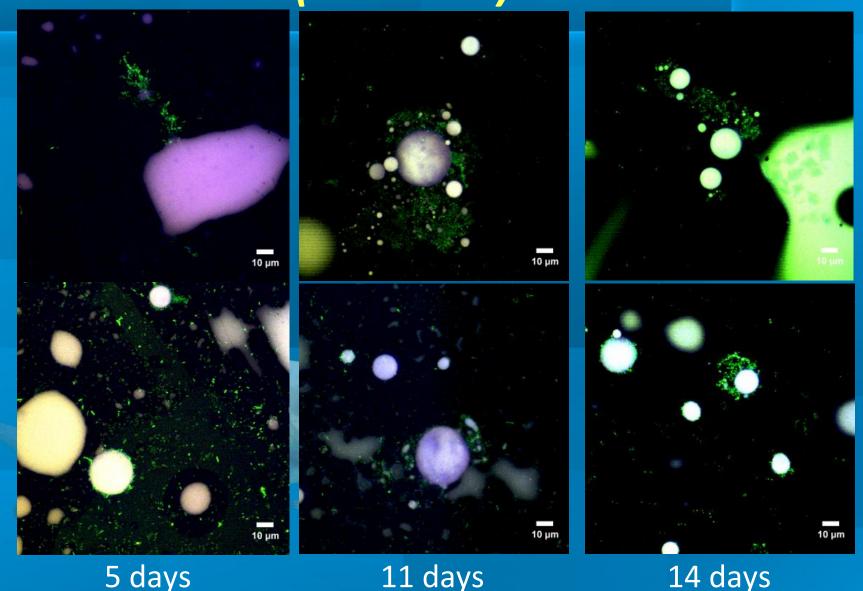
Crude oil from ONR7 Culture with Rha (Eb8 & E4)



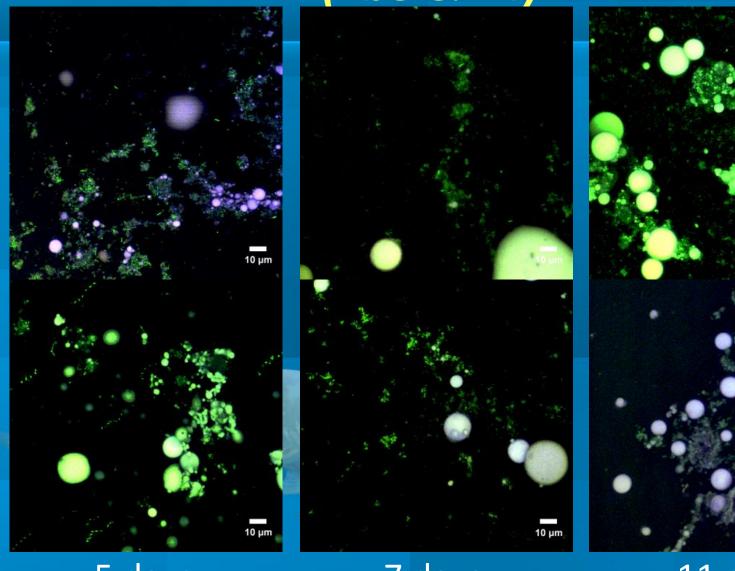
Crude oil from ONR7 Culture with corexit (Eb8 & E4)



Crude oil from ONR7 Culture with S200 (Eb8 & E4)



Crude oil from ONR7 Culture with marichem (Eb8 & E4)



5 days

7 days

11 days

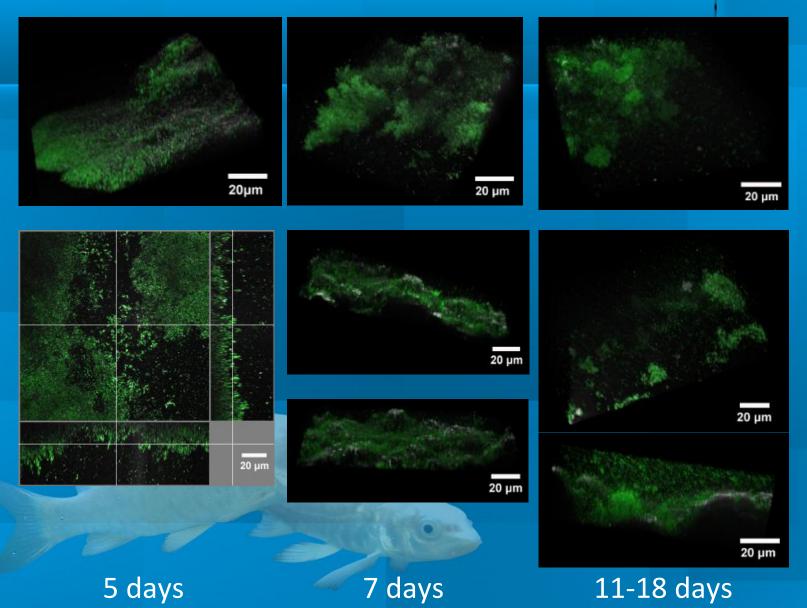
Crude oil from ONR7 Culture with marichem (Eb8 & E4)



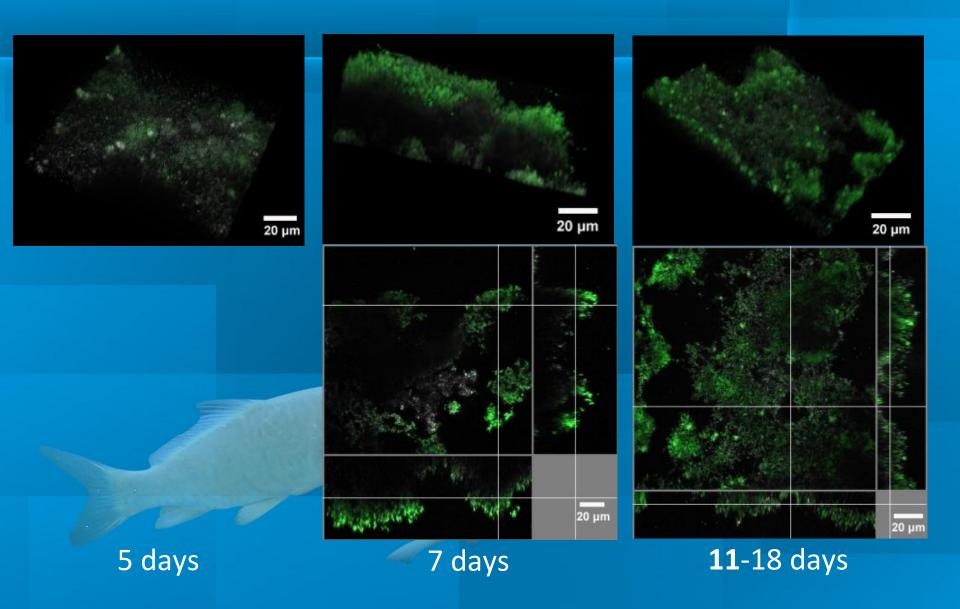
14 days

18 days

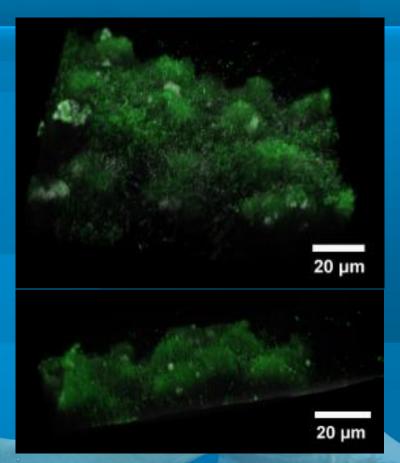
C20 from ONR7 Culture (Eb8 & E4)

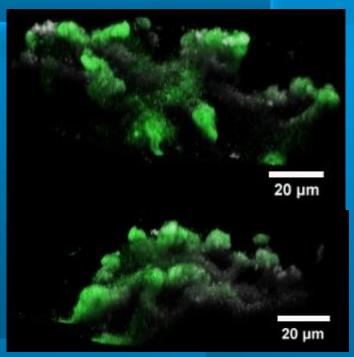


C20 from ONR7 Culture with Rha (E4)



C20 from ONR7 Culture with Rha (Eb8)





5 days

7 days

Concluding Remarks

- Degradation kinetics: saturated fraction is degraded faster & more extensively than the aromatic fraction
 - Alkanes: C15>C20>C25>C30>C35
 - PAHS: fluorene > dibenzothiophene > phenanthrene > chrysene
- Pristane and Phytane use as conserved internal markers in biodegradation index is unreliable as they are also degraded.
- Control treatment had no significant hydrocarbon degradation.
 Microbial growth and hydrocarbon breakdown are dependent on nutrients & other additives.
- An increase in q_s in agreement with an increase in μ implies a growth associated degradation rate.
- The ability of biosurfactants to emulsify oil and making it available to hydrocarbon degraders is crucial as a bioremediation strategy.

Concluding Remarks

- ineffectiveness of inorganic nutrients (NPK) in enhancing microbial growth and thus facilitate hydrocarbons degradation has been proved in Seawater 1 experimental set. On the contrary inorganic nutrients usually being washed out when applied in seawater perform better when applied to sand almost equally to ULR performance (Sand 1&2 experimental sets).
- It needs to be stressed out that although *Alcanivoracaceae* as investigated was the dominant family at early stage of the experiment especially in treatments with inorganic nutrients, *Pseudomonadaceae* was the dominant family at the late stage of the experiment especially in treatments with biosurfactant (rhamnolipids).

Concluding Remarks

- CLSM investigation revealed that bacteria are organized into clusters forming strings, star and grape like shapes of bacteria and fine oil droplets bridging each other with EPS.
- Moreover gradual dissolution of C20 droplet is encouraged in both consortia (especially in the presence of rhamnolipids).
- The combination of ABA-bioaugmentation with biostimulation could be a promising strategy to speed up bioremediation in cases where there is lack of both nutrients and indigenous degraders (at early times).

Acknowledgments







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"Unravelling and exploiting Mediterranean Sea microbial diversity and ecology for Xenobiotics' and pollutants' clean up" – ULIXES FP-7 PROJECT No. 266473



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PROGRAMME IKYDA 2012

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Thank you for your attention!

