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Aerobic cometabolic treatment of contaminant mixtures: Are multiple primary substrates better than just one?

Dr. Michael Hyman

December 3rd, 2019

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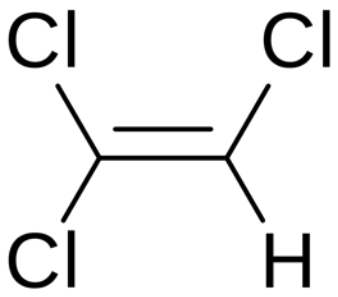
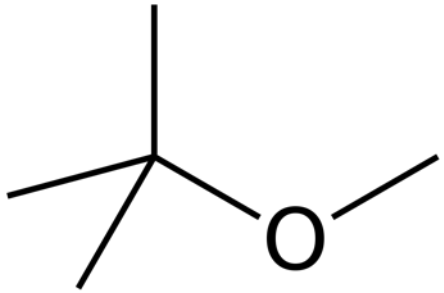
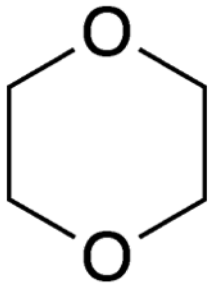
25 YEARS OF ESTCP
2019 | Enhancing DoD's Mission Effectiveness

What is Aerobic Cometabolism?

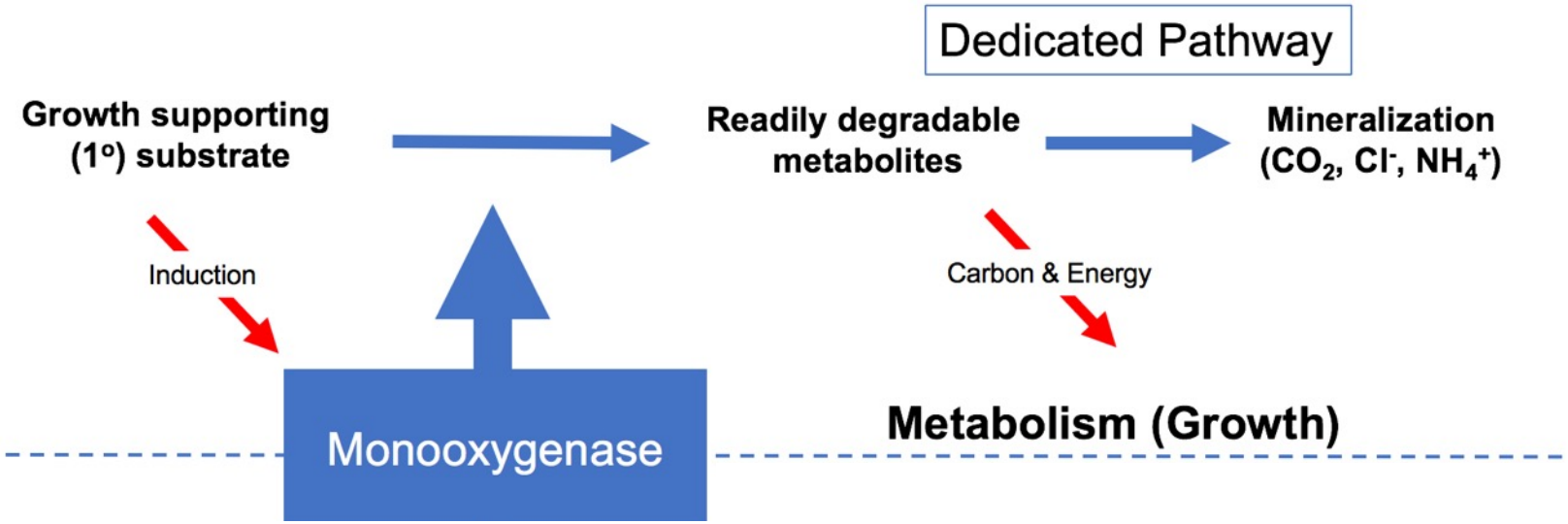
- Aerobic and low cost remediation option suitable for many small organics
- Utilizes monooxygenase enzymes for contaminant degradation
- Separates biomass generation from contaminant degradation
- Applicable to single contaminants or contaminant mixtures
- Exploits use of widely distributed microorganisms
- **Enables degradation of contaminants at ppb and ppt concentrations**



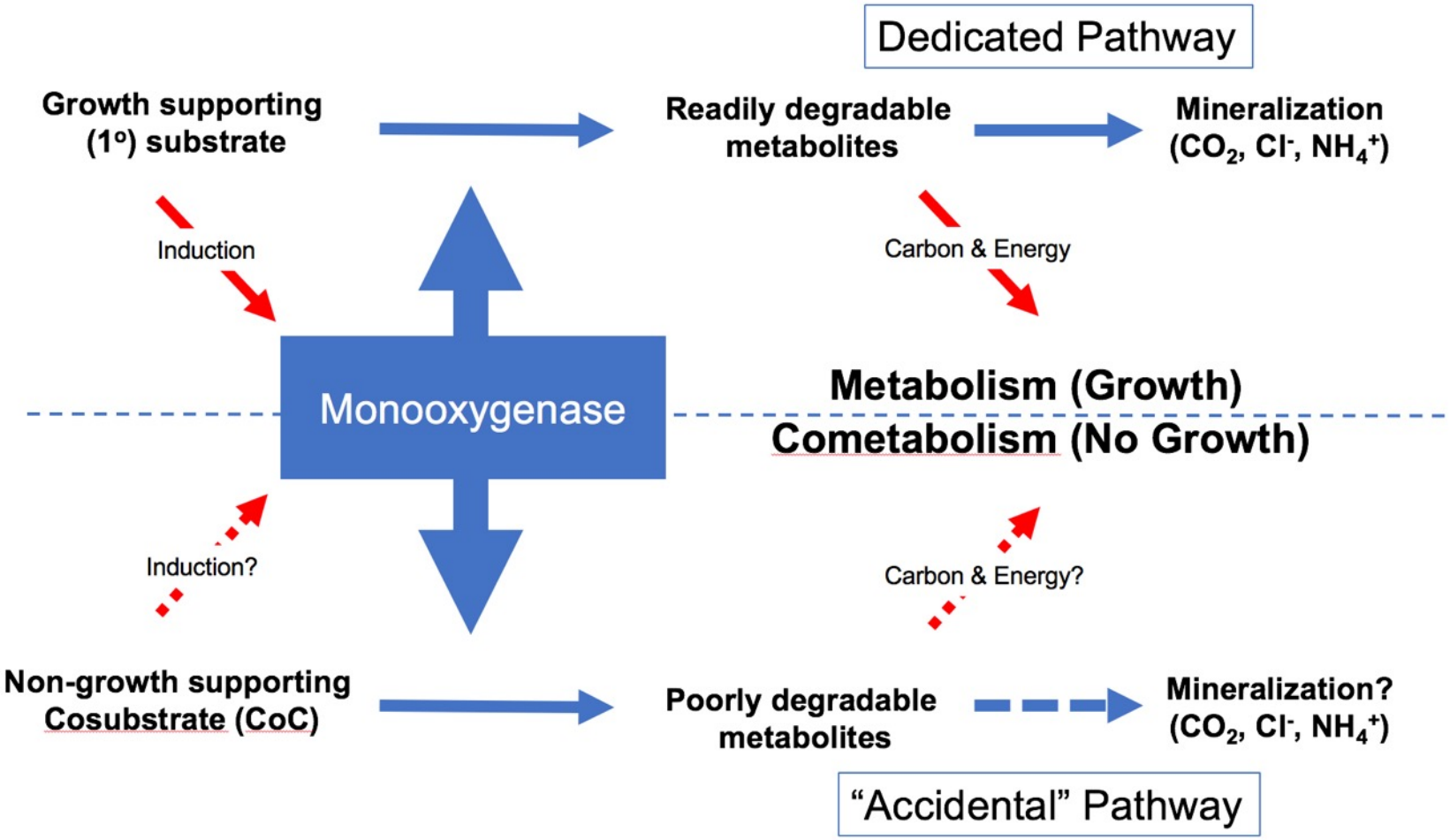




Microbial Metabolism



Microbial Metabolism *vs* Cometabolism



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Single Substrates and Cometabolism: TCE & Methane

APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Jan. 1985, p. 242–243 Vol. 49, No.1
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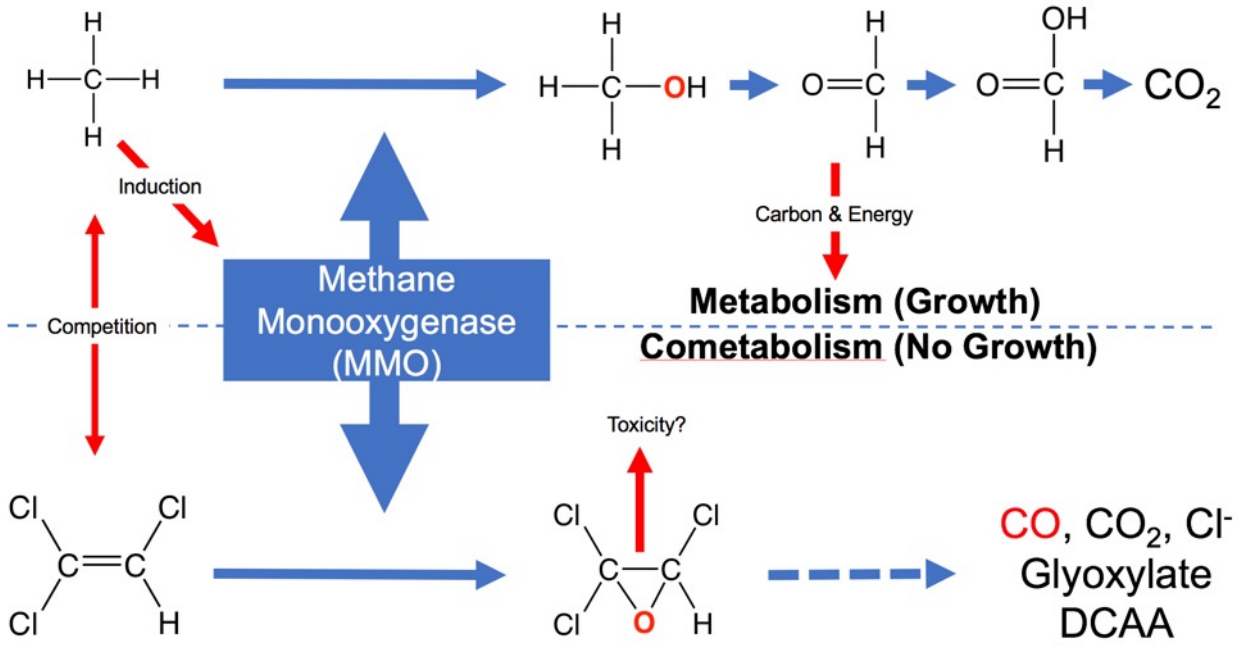
Biotransformation of Trichloroethylene in Soil

JOHN T. WILSON[1]* AND BARBARA H. WILSON[2]

Robert S. Kerr Environmental Research Laboratory, Ada, Oklahoma 74820,[1] and Environmental and Ground Water Institute, The University of Oklahoma, Norman, Oklahoma 73019[2]

Received 17 August 1984/Accepted 15 October 1984



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Mixed Substrates and Cometabolism: TCE & Methane

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*"**We exposed unsaturated soil to natural gas to enrich for methanotrophs <u>and</u> for organisms that oxidize other small alkanes present in natural gas**……..*
*A stream of air containing 0.6% natural gas by volume was passed over the head of the column. The gas was at least 96% hydrocarbons. Of the hydrocarbons, **77% was methane**, **10% was ethane**, **7% was propane**, **2.4% was n-butane**, **1% was iso-butane**, **1.5% was pentanes**, and **0.7% was hexanes**."*

Different monooxygenases with different capabilities are stimulated by most of these individual hydrocarbons

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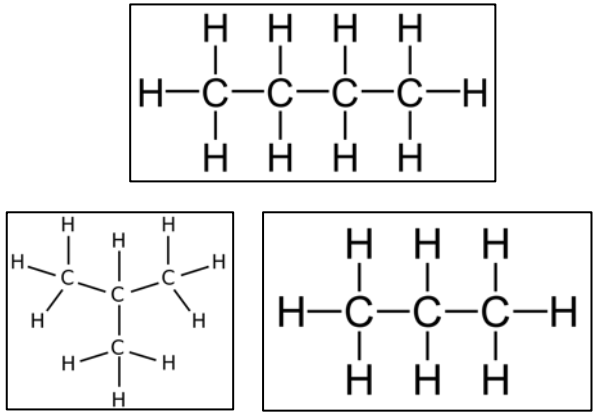
More Mixed Substrates and Cometabolism: TCE & Butane (*circa* 1995)



3.2. Mixtures

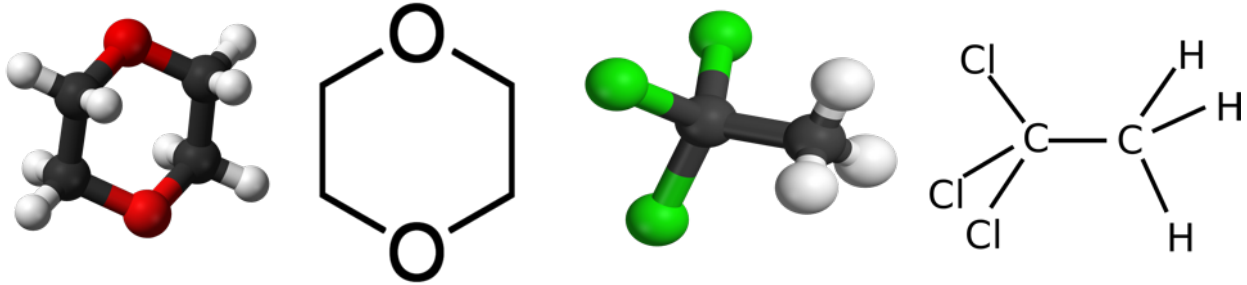
butane			**30-60%**
CAS number: 106-97-8	EC number: 203-448-7		
Contains no other substances or impurities which will influence the classification of the product.			
isobutane			**10-30%**
CAS number: 75-28-5	EC number: 200-857-2		
Contains no other substances or impurities which will influence the classification of the product.			
propane			**10-30%**
CAS number: 74-98-6	EC number: 200-827-9	REACH registration number: 01-2113486944-21-0000	
Contains no other substances or impurities which will influence the classification of the product.			

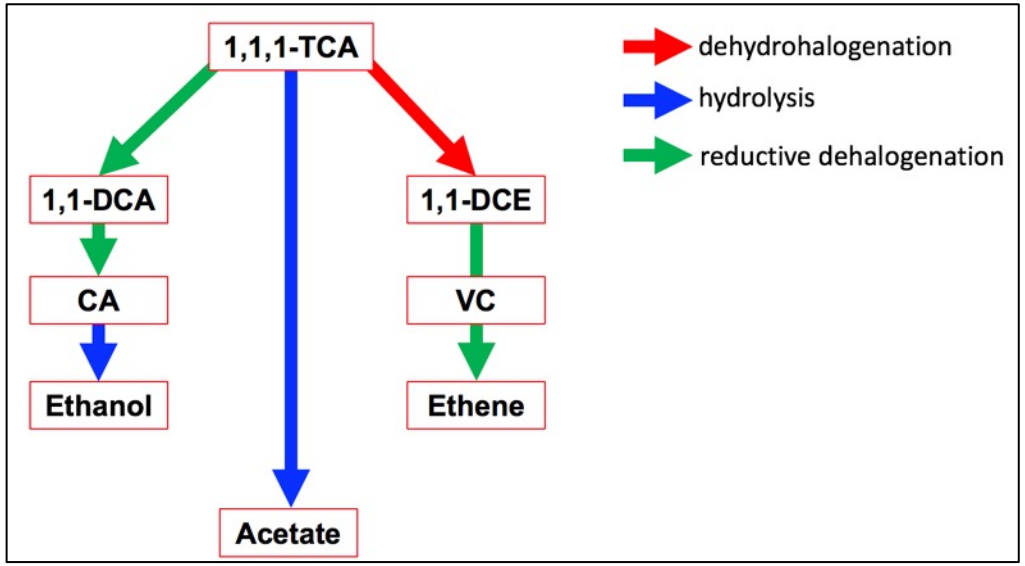
Butane monooxygenases

- **BMO** (butane monooxygenase) is structurally similar to **sMMO** and is not widely distributed.
- **BMO** oxidizes *n*-butane and some CAHs
- **PrMO** (propane monooxygenase) is found in many bacteria, including non-alkane oxidizers
- **PrMO** has limited cosubstrate range including *n*-butane and NDMA.
- **SCAM** (Short-chain alkane monooxygenase) is found in many gaseous (C_2-C_5) alkane-metabolizing bacteria
- **SCAM** oxidizes many CoCs including 1,4-dioxane (14D), MTBE and some CAHs

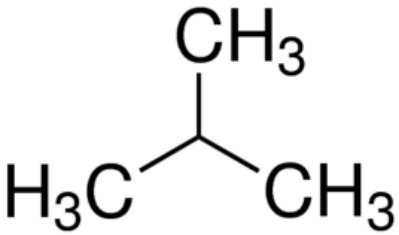
1,4-Dioxane and its Co-contaminants

- 14D was historically used as stabilizer for 1,1,1-TCA, but not TCE
- Often present at low concentrations (≤100 ppb) in large diffuse plumes
- 1,1,1-TCA abiotically degrades to 1,1-DCE and/or acetate and is biological reduced to 1,1-DCA and CA
- TCE (and its daughter products such as *cis*-DCE) are frequently detected with 14D in solvent-impacted groundwater

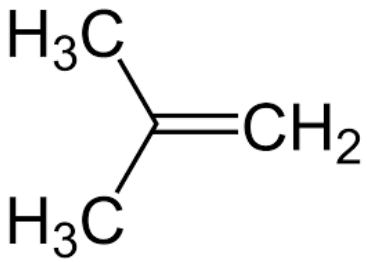




Degradation of 1,4-Dioxane by Branched Hydrocarbon-Utilizing Bacteria (ER-2303)

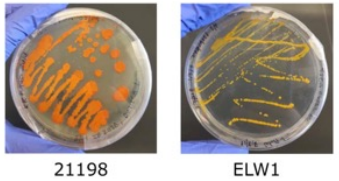


Isobutane



Isobutylene

- Isobutane (2-methylpropane) and isobutylene (2-methylpropene) are simplest branched hydrocarbons
- Neither gas had been previously tested as a potential stimulant for *in situ* cometabolic biodegradation
- Focus on model organisms including
  - ***Mycobacterium* sp. ELW1 (Isobutylene)**
  - ***Rhodococcus rhodochrous* ATCC 21198 (Isobutane)**



- Recent reports have focused on ATCC 21198
  - 2D-CSIA study of 14D degradation (Bennett *et al*. 2017)
  - Kinetics of 14D degradation in microcosms (Rolston *et al*. 2019)

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Which organisms can cometabolically degrade 100 ppb 1,4-Dioxane?

SDIMO Group	Growth Substrate	Enzyme	14D	
			≤100 ppb	≥100 ppm
1	Toluene	T3/4MO	**?**	Yes
	Alkenes	AlkMO	**No**	Yes
2	Toluene	T2MO	**No**	Yes
	Phenol	PH	**No**	?
3	Methane	sMMO	**No**	Yes (?)
	n-Butane	BMO	**No**	?
4	Alkenes	AMO	**No**	?
5	Propane	PrMO	**No**	No
	Tetrahydrofuran	THFMO	**Yes**	Yes
6	C_2-C_4 Alkanes	SCAM	**Yes**	Yes

- Bacteria that metabolize 14D (*e.g.* CB1190) are largely ineffective at degrading low, environmentally relevant concentrations of 14D (≤100 ppb).
- Most model strains expressing SDIMOs do not degrade 14D, even if reported to degrade higher concentrations
- In batch tests all bacteria (10 strains) grown on isobutane degraded 14D to ≤2 ppb. Several to below 0.15 ppb LOD.
- Genome analyses indicate 14D degradation consistently associated with short chain alkane monooxygenase (SCAM).

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A “typical” 14D-contaminated site?
ESTCP ER-201733

Shallow Aquifer 40’ BGS

Neutral pH

380-470 ppb 1,4-dioxane

440-470 ppb 1,1-DCE

760-770 ppb TCE

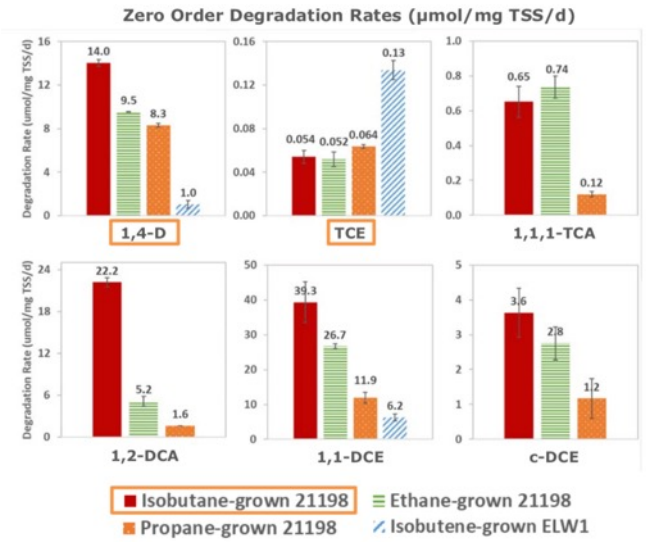
370-380 ppb *cis*-DCE

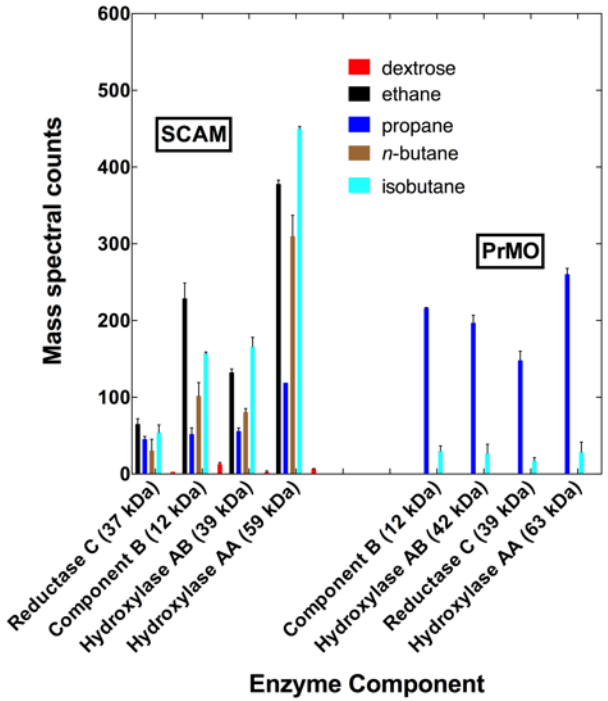
4.9-5.2 ppb CF

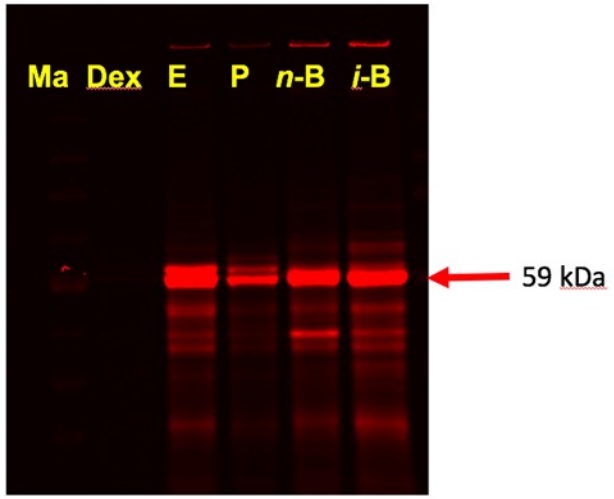
Can isobutane with other gases be used to promote cometabolic degradation of typical contaminants in the field?

Batch tests with 21198 & ELW1

- 21198 degrades 14D faster than ELW1, ELW1 degrades TCE faster than 21198
- 21198 growth substrate strongly impacts most CAH transformation rates
- Proteomics shows SCAM abundant in isobutane-grown cells, low in propane-grown cells. Highest levels of PrMO in propane-grown cells.







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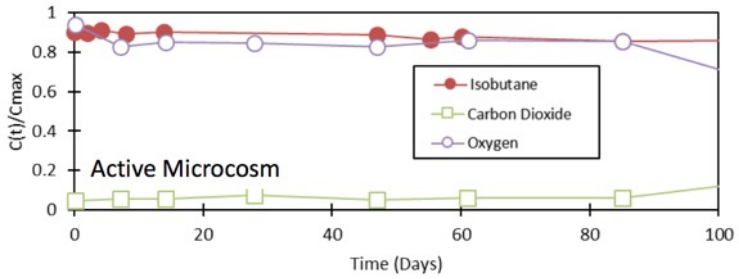
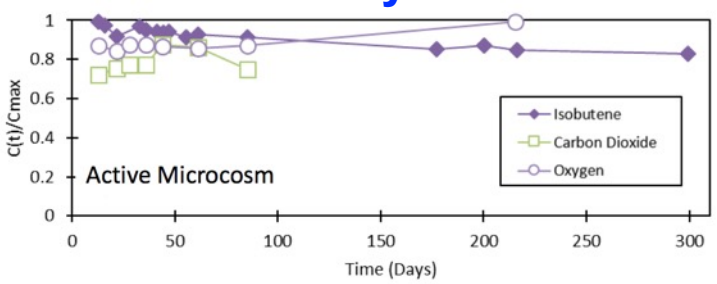
Microcosms and Biostimulation

- No isobutane, isobutylene, or propane uptake after 100 days using site water. Nutrient (N&P) availability not an issue.

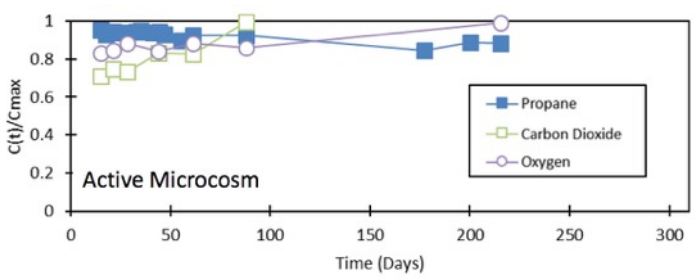
Isobutane



Isobutylene

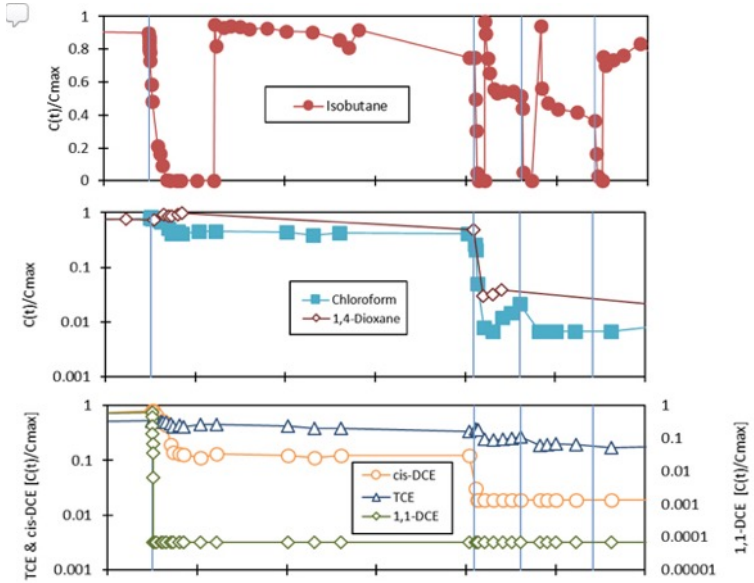


Propane

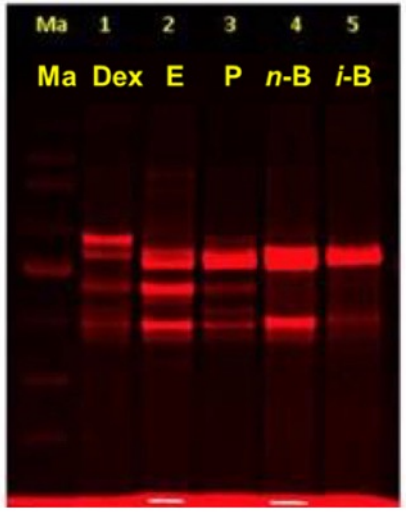


- Sparging of groundwater to remove CAHs enabled both isobutane and isobutylene to promote rapid microbial growth.
- **High concentrations of 1,1-DCE (~450 ppb) likely inhibitory due to formation of reactive and toxic epoxide.**

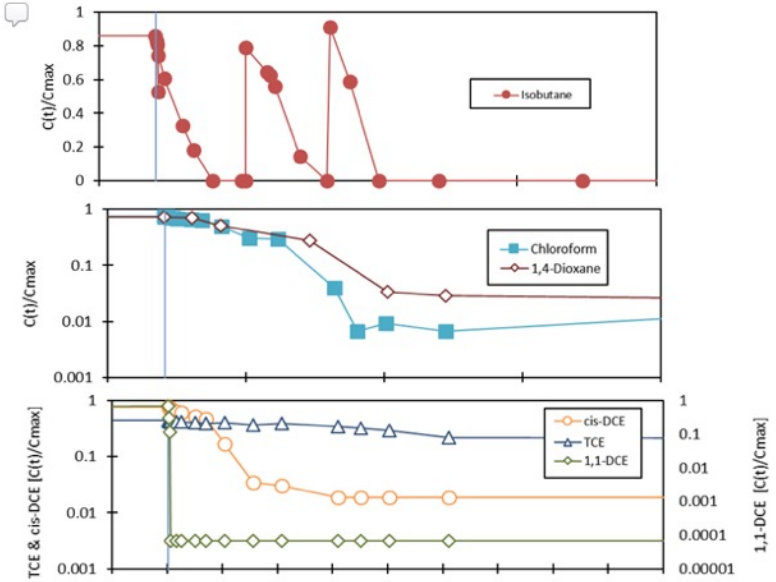
Bioaugmentation



21198



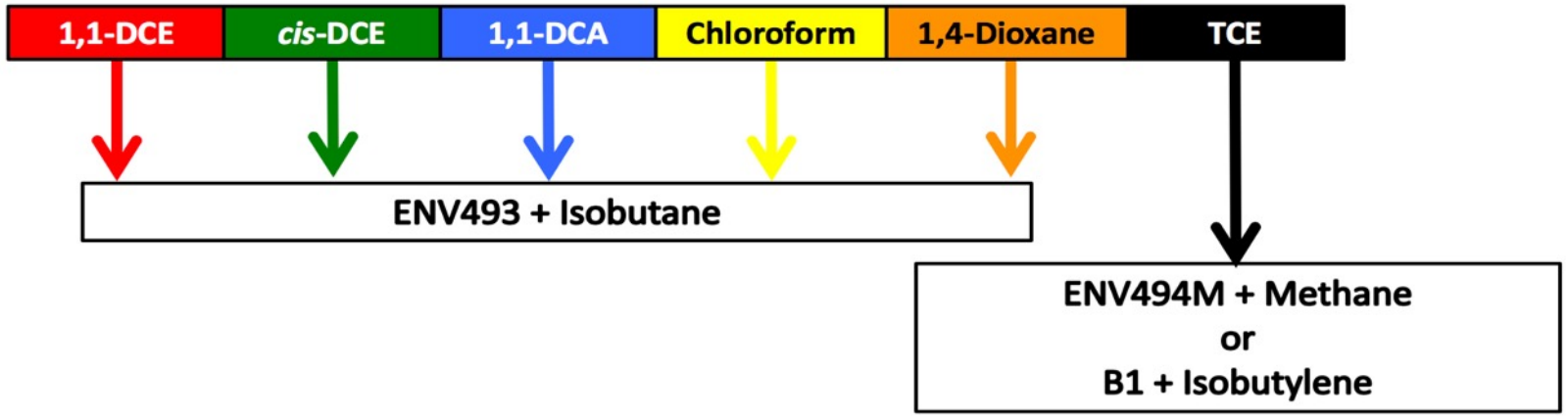
ENV493 ABL



ENV493

- 21198 rapidly removes 1,1-DCE but continued activity requires multiple additions of organism. TCE is not effectively degraded. Genome and ABL indicate SCAM is active enzyme
- ENV493 is a isobutane-metabolizer isolated from site. Removes 1,1-DCE and other contaminants while retaining activity. TCE is also not effectively degraded. ABL indicates SCAM

Final Anticipated Treatment Process

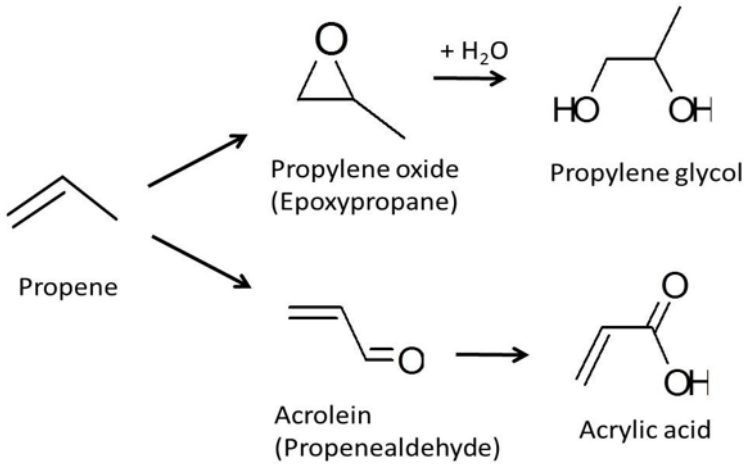


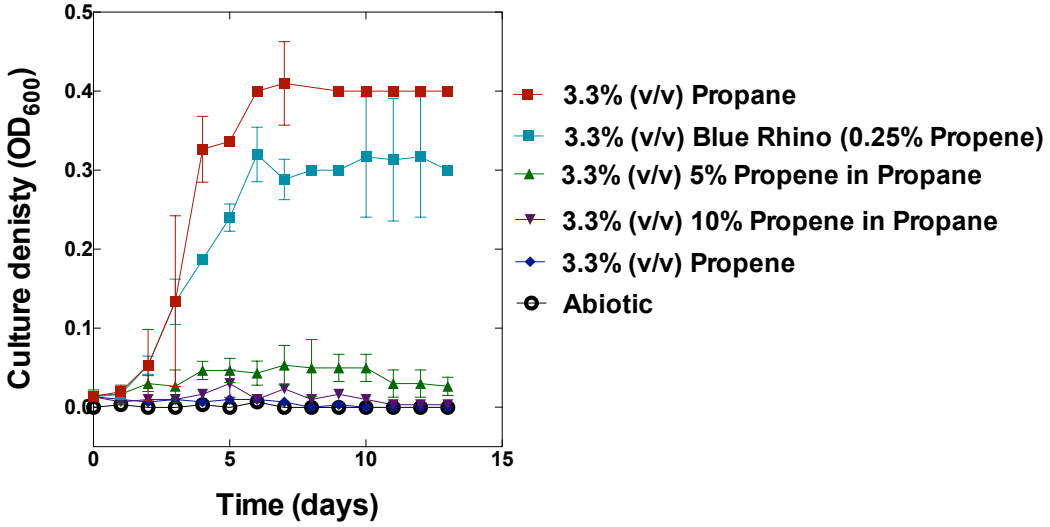
Overall, the major challenge at this site is not 14D but CAHs

- Bioaugmentation/Biostimulation with isobutane-grown ENV493 to effectively remove all major contaminants except TCE
- Subsequent Bioaugmentation/Biostimulation with either native mixed methanotrophic culture (ENV494M) or native mixed isobutylene-metabolizing culture (B1) to remove residual TCE

Fortuitous Combined Use of Alkanes and Alkenes
ER18-5168

- Consumer propane is typically HD5 (≥95% propane, ≤5% **propene**, thiol oderant)
- Propanotrophs cometabolically oxidize **propene** to potentially toxic metabolites including epoxypropane, allyl alcohol and their further metabolites.
- In pure cultures ≥1% v/v **propene** in propane strongly inhibits propanotroph growth
- In mixed cultures (*e.g. in situ in* ground water) HD5 can potentially support growth of both propanotrophs and **propenotrophs**.
- ***In situ*, does HD5 give greater monooxygenase diversity and cometabolic CoC-degrading capability than pure propane?**





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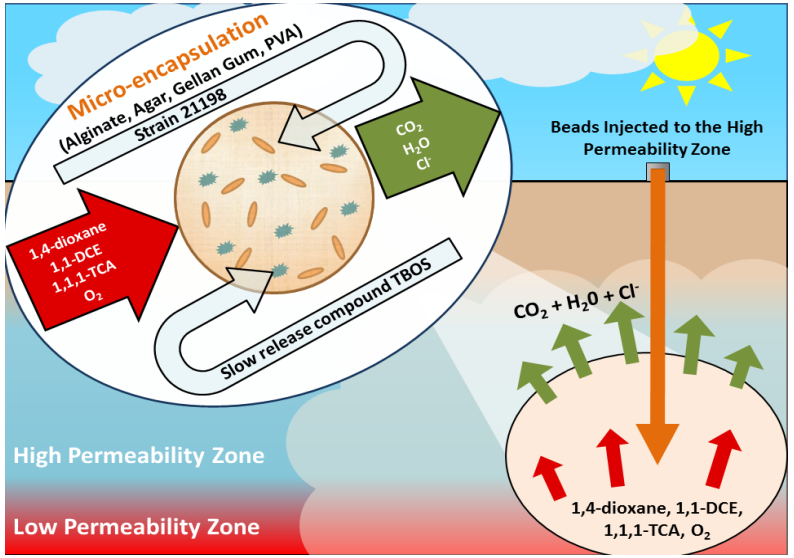
Strain 21198 encapsulated with slow release substrates (SRCs)
ER-2716

"Precision Cometabolism"

Designer Primary Substrate Mixtures

Specific Microorganisms



- Uses specific microorganisms with known monooxygenases and contaminant cosubstrate range
- Bacteria can be selected or combined to suit site-specific contaminant ranges
- Encapsulation protects bacteria from predation and adverse environmental conditions
- SRC sustains activity of encapsulated bacteria and not external organisms
- SRC avoids competition between primary growth substrate and contaminant cosubstrate
- Beads and SRC eventually decompose to harmless byproducts (dead bacteria, cellular debris, sugars, and simple common minerals)

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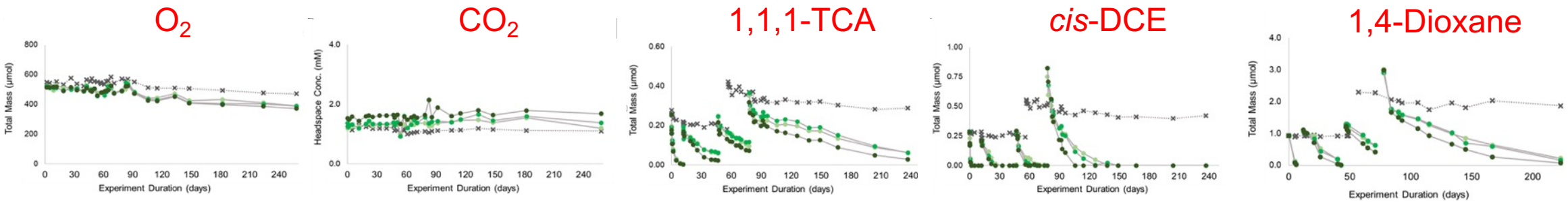
Strain 21198 + SRC in Beads in Batch

- ATCC 21198 co-encapsulated with SRC in gellan gum beads (OSU)
- Continuous degradation of 1,1,1-TCA, *cis*-DCE, and 14D for 240 days
- Transformations inhibited by C_2H_2
- Similar longevity in separate system using TCE-degrading strains



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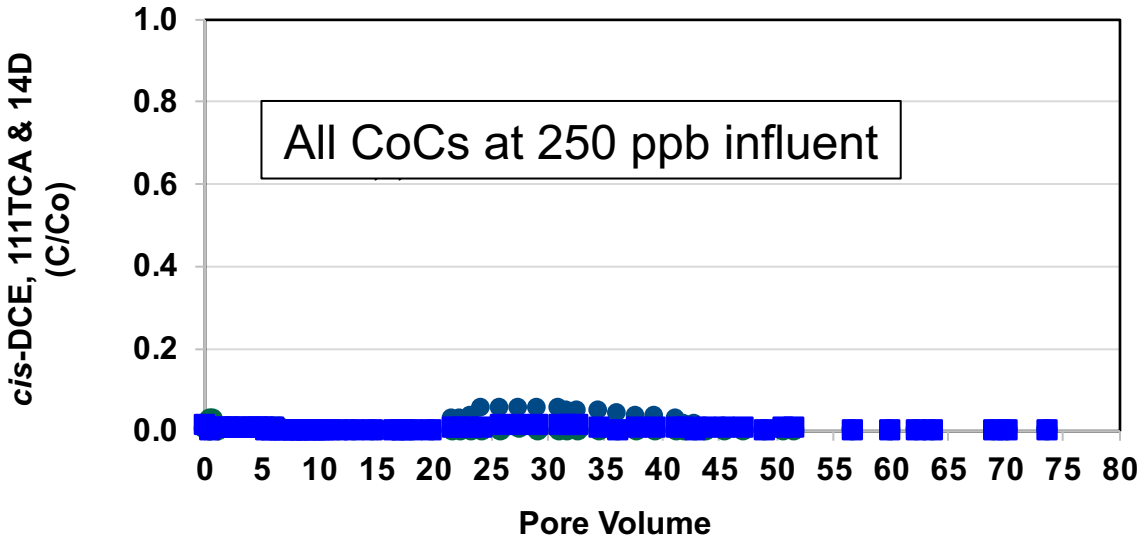
Strain 21198 + SRC in Beads in Columns

~50g beads with *R. rhodochrous* ATCC 21198 encapsulated with 10% wt/wt SRC



Compound	[Influent] (µg/L)	[Effluent] (µg/L)	% Transformed
1,4-Dioxane	250	1.1	99.6
1,1,1-TCA	250	0.2	99.9
cis-DCE	250	0.3	99.8

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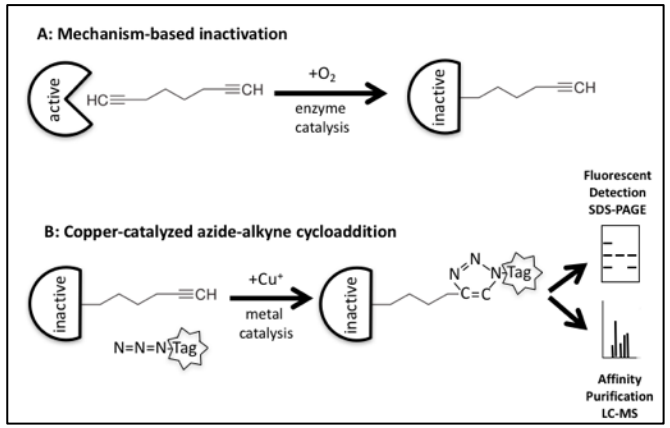
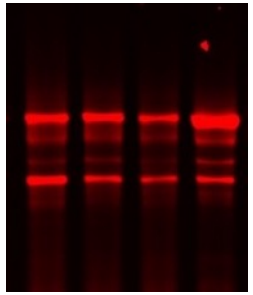
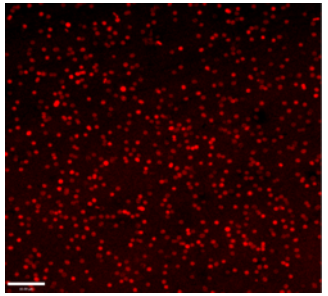
Activity-based labeling (ABL) of monooxygenases



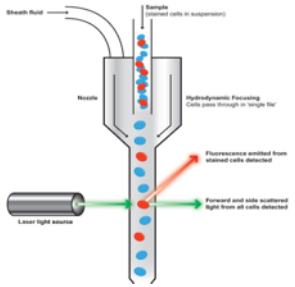
- Developed for ammonia monooxygenases (AMO) **(ER-2303)**
- Characterization of monooxygenase inducers and SRCs **(ER-2716)**
- Development to detect/identify monooxygenases in active 14D remediation projects **(ER18-5168, ER201733)**
- Application to natural attenuation of 14D **(ER -201730)**
- Potential for bead-based bioreporters for natural attenuation of CAHs



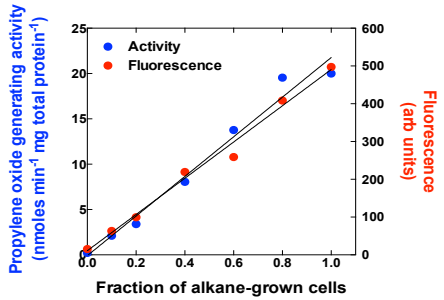
Enzyme labeling



Whole cell fluorescence



FACS enumeration



Rate estimation

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Detects/Quantifies/Identifies active enzyme, not proxy like gene or transcript

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Conclusions

- Use of multiple primary substrates for aerobic cometabolism can overcome issues raised by complex contaminant mixtures.
- Primary substrates can be added either sequentially or concurrently.
- Approach is facilitated by genome-enabled analyses of non-specific monooxygenases and their catalytic activities in model and native bacteria.
- “Precision bioremediation” is enabled using purposefully designed primary substrate mixtures and/or encapsulated specific microorganisms.
- Recently developed tools such as ABL that focus on enzyme activity rather than gene/transcript abundance can potentially provide direct estimates of ongoing rates of biotransformation and identification of key enzymes.

Acknowledgements

SERDP

ER-2303

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ER-2716

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ER18-5168

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ER-201730

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