Current Knowledge of Bioaugmentation Cultures for 1,4-Dioxane Biodegradation

A5. 1,4-Dioxane Treatment Technologies

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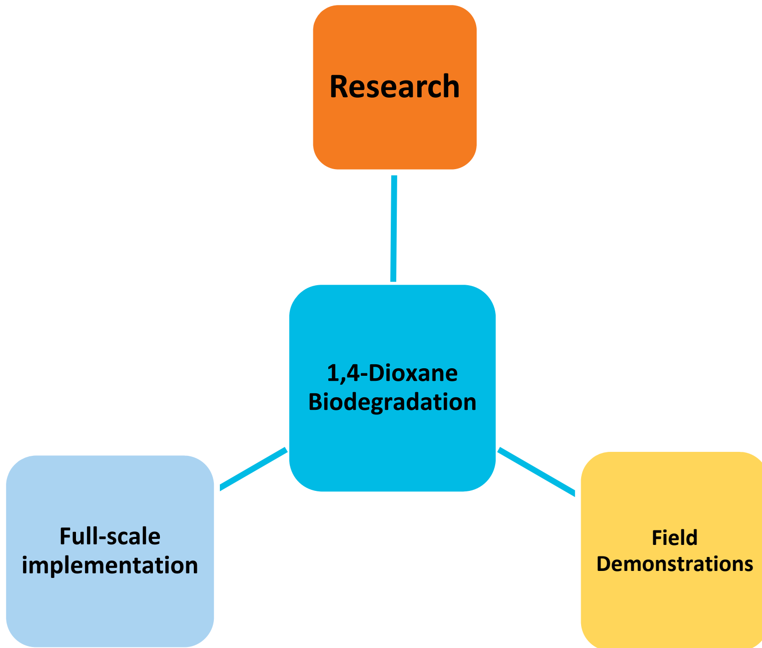
4/17/2019

Fifth International Symposium on Bioremediation and Sustainable Environmental Technologies

April 15-18, 2019
Baltimore, MD

Outline

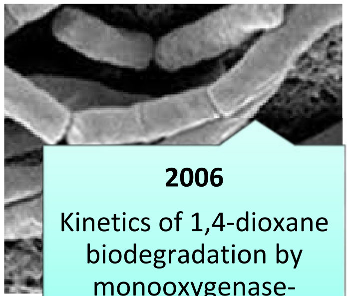
- Bioremediation trends for 1,4-dioxane
- New findings on known 1,4-dioxane biodegrading cultures
- New 1,4-dioxane biodegradation cultures
- Implications of research data on field demonstrations



1,4-Dioxane Biodegradation Milestones

1995

USEPA 1,4-Dioxane Fact Sheet "…*__1,4-Dioxane is not expected to be biodegrade in water__*."

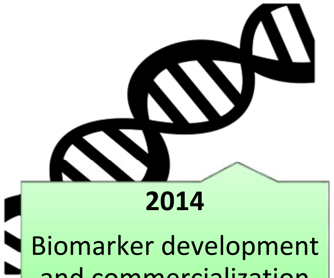


2006

Kinetics of 1,4-dioxane biodegradation by monooxygenase-expressing bacteria (Mahendra et al)

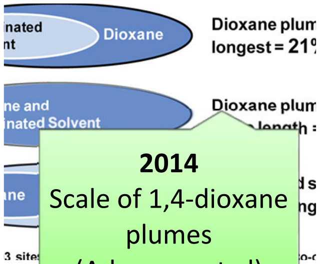
2012

Stable isotope probing to verify 1,4-dioxane biodegradation potential under field conditions (Chiang et al)



2014

Biomarker development and commercialization (Mahendra research group, AECOM and MI)

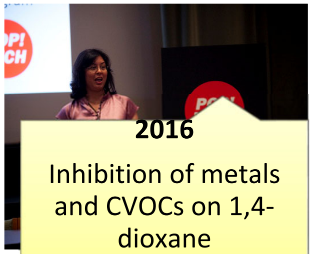


2014

Scale of 1,4-dioxane plumes (Adamson et al)

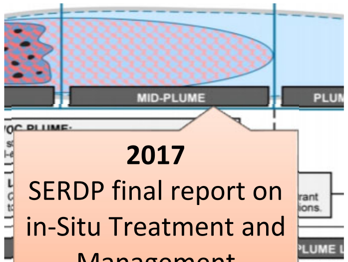
2015

In-situ cometabolic biodegradation pilot study (Lippincott et al)



2016

Inhibition of metals and CVOCs on 1,4-dioxane biodegradation (Multiple research groups)



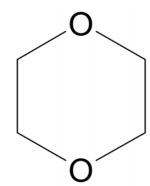
2017

SERDP final report on in-Situ Treatment and Management Strategies (Adamson et al)

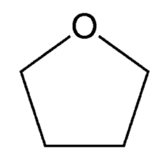
New Facts about 1,4-Dioxane

- It remains as contaminant of emerging concern
- Not much new regulatory development comparing to PFAS
- We now believe 1,4-dioxane is biodegradable!
- People start to ask if 14D is next PFAS?
- ITRC forms 1,4-dioxane team in 2019

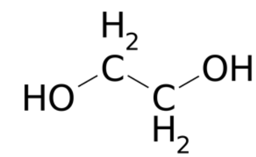
- Contaminants that we typically run into at a 14D contaminated site



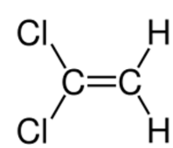
1,4-dioxane



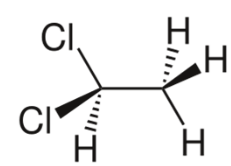
THF



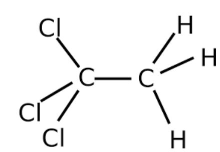
Ethylene Glycol



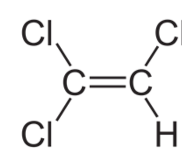
1,1-DCE



1,1-DCA



1,1,1-TCA



TCE

Remediation Trends

Lines of Evidence

- Biomarkers
- CSIA
- Kinetics and transport modeling

Technology Demonstrations

- Monitored natural attenuation
- In-situ cometabolic biodegradation of 14D
- In-situ metabolic bioaugmentation of 14D
- Ex-situ bioreactors
- Sequential bioremediation of CVOCs then 14D

Methane as Primary Substrate

- **Yes**
  - Methane and methanotrophs are ubiquitous
  - Its ubiquities may explain natural attenuation of 14D
  - Cometabolic transformation of dioxane was observed for monooxygenase expressing strains that were induced with methane, propane, tetrahydrofuran, or toluene (Mahendra and Alvarez-Cohen, 2006)
  - Multiple sites show the 14D plume shrinkage and detections of MOB and sMMO

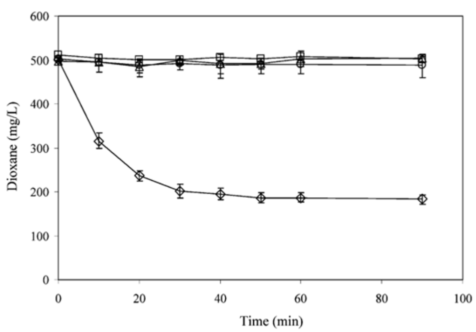


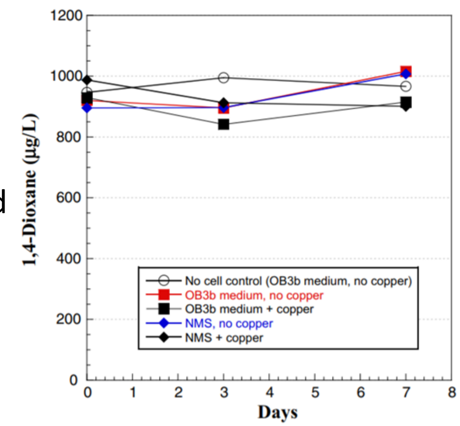
FIGURE 2. Dioxane transformation by *Methylosinus trichosporium* OB3b (~160 mg protein/L). ◇, cells expressing soluble methane monooxygenase (sMMO); △, cells expressing sMMO exposed to acetylene; ○, cells expressing particulate MMO; □, abiotic control. The error bars represent the range of triplicates.

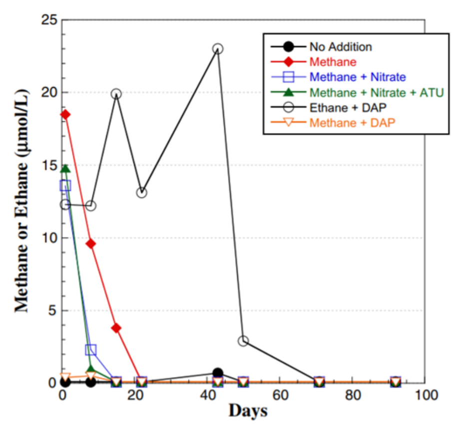
Well ID	Sampling Date	Pilot Study Area	del13C	del2H
M-69	7/13/2016	Methane Biostimulation	-30.34	-76.34
M-93	7/13/2016	Upgradient of Methane Biostimulation	-30.02	-83.66
M-98	7/19/2016	ERD	-30.39	-99.24
M-101	7/13/2016	ERD	-31.63	-103.68
M-104	7/13/2016	Sidegradient (background) of ERD	Not analyzed, 1,4-dioxane conc too low	

Exhibit 9. CSIA results for 1,4-dioxane

Characterizing In-Situ Methane-Enhanced Biostimulation Potential for 1,4-Dioxane Biodegradation in Groundwater (Sadeghi et al., 2016)

- **No**
  - Methane did not appreciably stimulate the biodegradation of 14D in aquifer microcosms or in methane-oxidizing mixed cultures enriched from two different aquifers
  - Ethane may serve as a better substrate to enhance 1,4-D degradation in aquifers





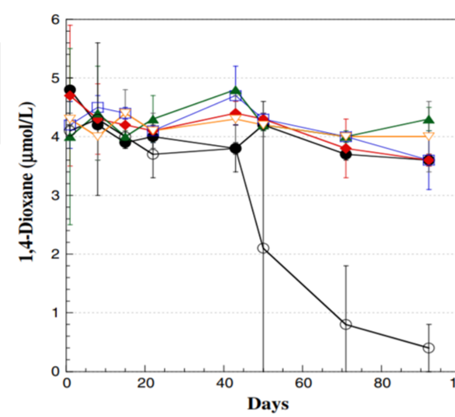


Fig. 1 Biodegradation of methane or ethane (top panel) and 1,4-dioxane (bottom panel) in microcosms prepared from MBAFB. Error bars in the bottom panel represent the standard deviation from duplicate samples

(Hatzinger, 2017)

Propane as primary substrate

VAFB Pilot Study

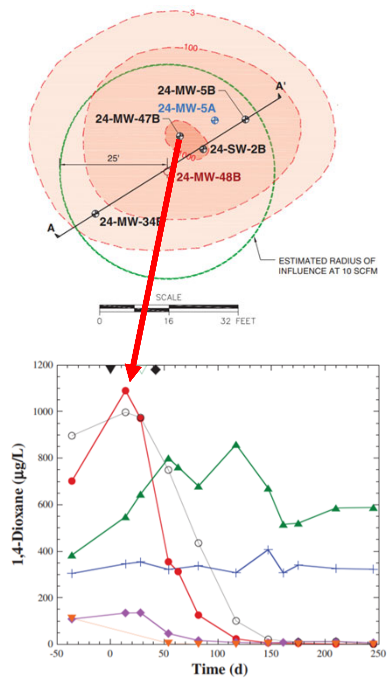
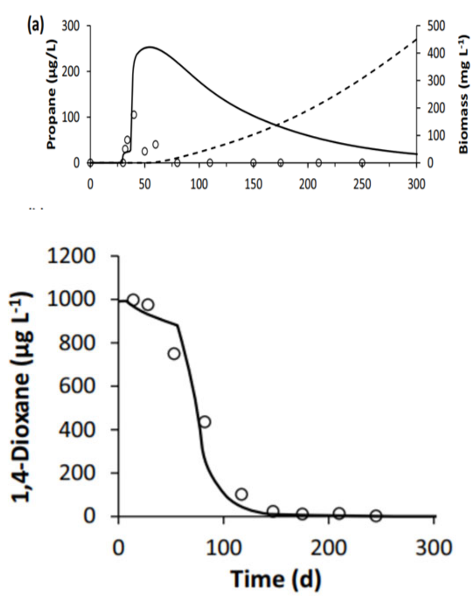


Figure 6. 1,4-Dioxane concentrations in groundwater at the demonstration plot monitoring wells—24-MW-48B (sparge; ▾), 24-MW-47B (●), 24-SW-2B (▴), 24-MW-5B (○), 24-MW-34B (◆), and 24-MW-5A (control; +). Symbols on the top of the graph designate the beginning of air sparging (▾), the beginning of propane addition (▿), and bioaugmentation (◆).

Lippincott et al., 2015

Simulation of in situ biodegradation of 1,4-dioxane

24-MW-47B

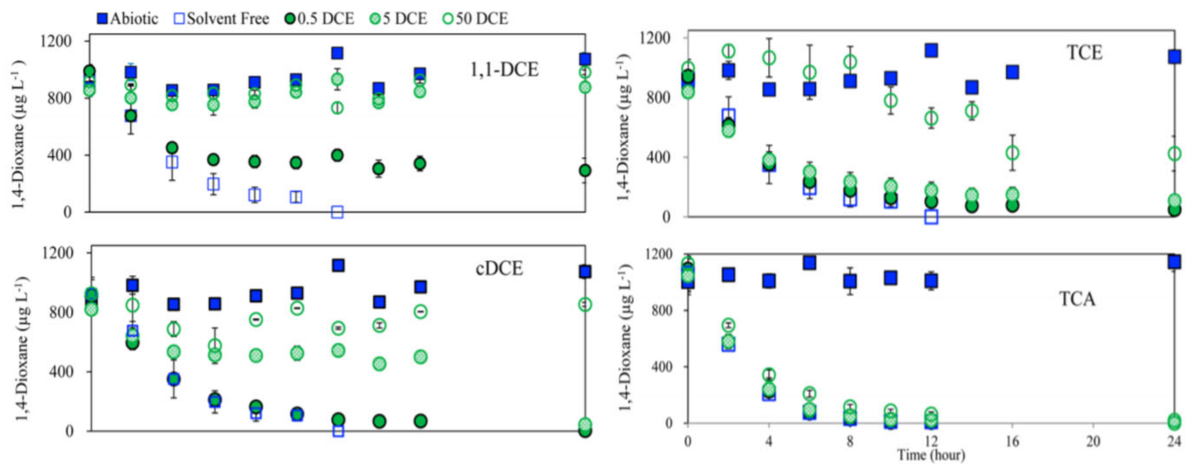


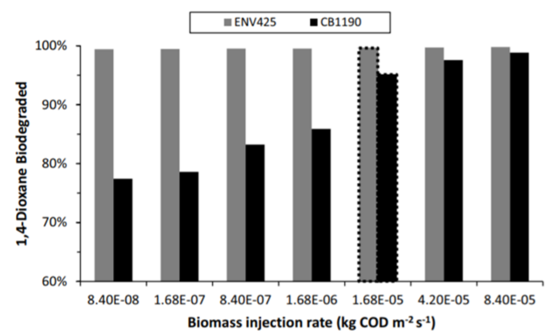
Barajas-Rodriguez, 2019

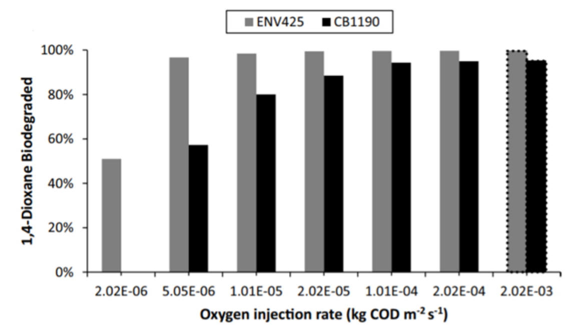
- Very promising
- Verified by multiple microcosm studies and other field demonstrations
- First field demonstration at VAFB involved in-situ bioaugmentation with additions of propane, diammonium phosphate and ENV425 culture
- Kinetics (Barajas-Rodriguez, 2018, 2019)
  - Aerobic biodegradation kinetics for 1,4-dioxane
  - Simulation of in situ biodegradation of 1,4-dioxane
- Groundwater recirculation and ex-situ bioreactor treatment using propane MO (Chu *et al.*, 2018)
- Novel propane MO (PH-06, encoded by the prmABCD gene cluster), which can be induced by dioxane, THF, or propane, was developed and published by Deng *et al.* (2018)

New Understanding on CB1190 Bioaugmentation

- 14D biodegradation when CVOCs are present (Zhang et al., 2016)
    - Inhibition: 1,1-dichloroethene (1,1-DCE) > cis-1,2-diochloroethene (cDCE) > trichloroethene (TCE) > 1,1,1-trichloroethane (TCA)
    - Delayed ATP production and down-regulation of both 1,4-dioxane monooxygenase (dxmB) and aldehyde dehydrogenase (aldH) genes
- Simulations to estimate the desired 14D biodegradation (Barajas-Rodriguez et al., 2019)
    - CB1190 abundance
    - Oxygen concentrations

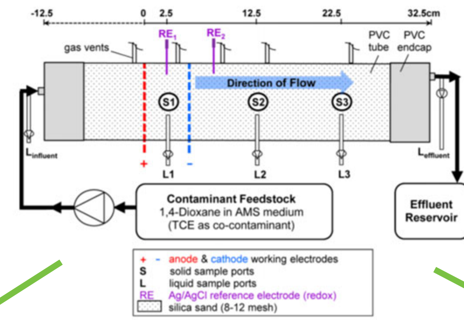




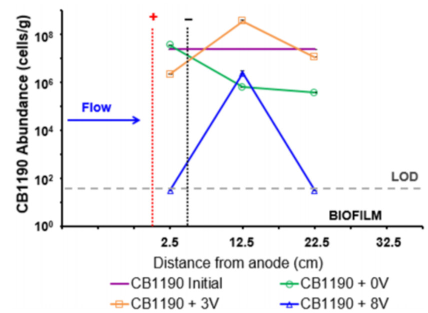


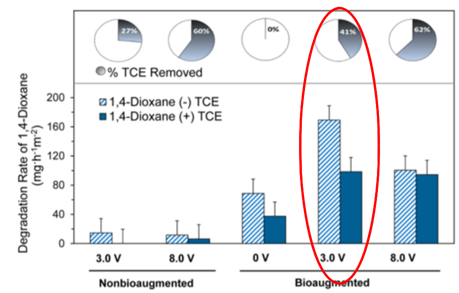
New Treatment Approaches on CB1190 Bioaugmentation

- Using flow-through electrolytic reactors equipped with Ti/IrO2-Ta2O5 mesh electrodes combining CB1190 bioaugmentation offer enhanced 14D biodegradation.
- Data confirmed microbial growth was promoted by anodic oxygen-generating reactions.

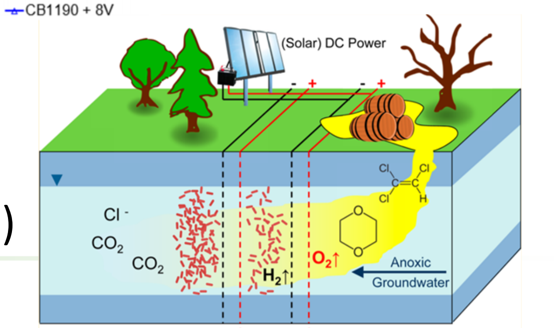


O_2, CB1190

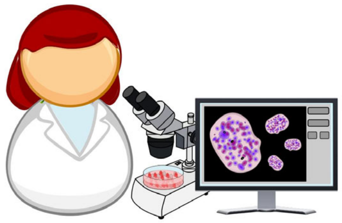




(Jasmann et al 2017)



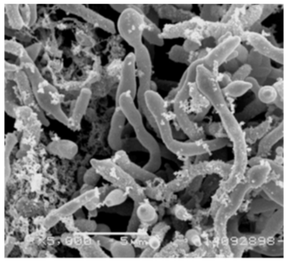
New strains for <u>metabolic</u> biodegradation of 14D



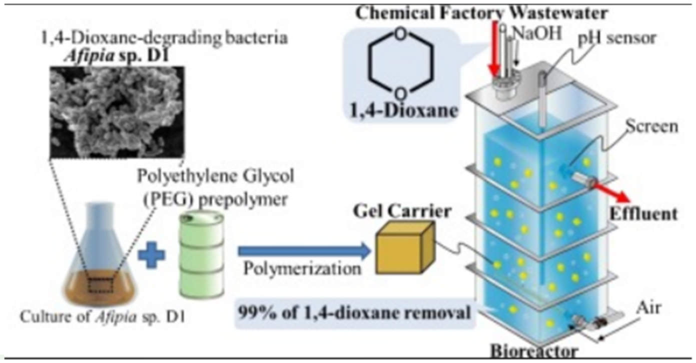
- No or limited field applications
- May or may not use environmental samples to validate 14D biodegradability
- Initial concentrations in mg/L range
- Typically evaluates
  - 14D degradation rates
  - Cell yields and growth
  - Ability to tolerate co-contaminants
  - Acclimation to wider range of Environmental conditions
  - Utilization of other substrates, which may co-occur in water to sustain cell growth
- CB1190 becomes a target strain to compare data against

New strains published on <u>metabolic</u> biodegradation of 14D
More to come!

- Strain **N23**: 99.9% 16S rRNA gene sequence similarity to P. tetrahydrofuranoxydans K1, but use 14D as sole carbon and energy source and has the highest k_{max} value reported today (Yamamoto et al.,2018)



- Rhodococcus aetherivorans **JCM 14343**: Degradation activity maintained at a wide range of temperature (5–40^{o}C) and pH (4–9) conditions. 14D degradation by this strain was inhibited in the presence of other cyclic ethers, but not by EG, 1,4-butanediol not only induced but also enhanced 14D degradation. (Inoue et al., 2018).
- A pilot-scale (120 L) bioreactor system using a gel carrier-entrapped pure bacterial strain, ***Afipia* sp. strain D1**, capable of degrading 1,4-dioxane as a sole carbon and energy source was constructed and applied to treat real industrial wastewater containing 1,4-dioxane from a chemical factory (Isaka et al 2016)



Takeaways

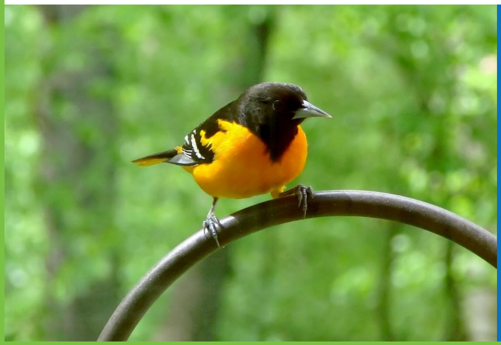
Implications of research data on field demonstrations

- More 14D degraders have been isolated from environmental matrices suggesting 14D biodegradation is probable
- In-situ bioaugmentation
  - In-situ cometabolic biodegradation of 14D using propane and propane MO is currently a mainstream approach
  - In-situ metabolic biodegradation of 14D has very limited applications due to the requirements of high 14D concentrations, CB1190 abundance and oxygen
  - 14D in an impacted plume may need to be pre-concentrated in a closed loop enriched environment for metabolic biodegradation of 14D to occur

Takeaways

Implications of research data on field demonstrations

- Ex-situ bioaugmentation
  - Both co-metabolic and metabolic approaches can be considered
  - This application still has very limited peer-reviewed publications
  - With established kinetic data, better engineering design can be done
- Generally, 14D biodegradation has not yet passed field “demonstration” phase ….
- Research
  - Culture-independent investigation using cutting edge metabolomic and proteomic methods can be conducted to identify 14D-degrading microorganisms within microbial communities in the environment



THANK YOU

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