Practical Review and Guidance on 1,4-Dioxane Field-Scale Biodegradation Potential and Characterization

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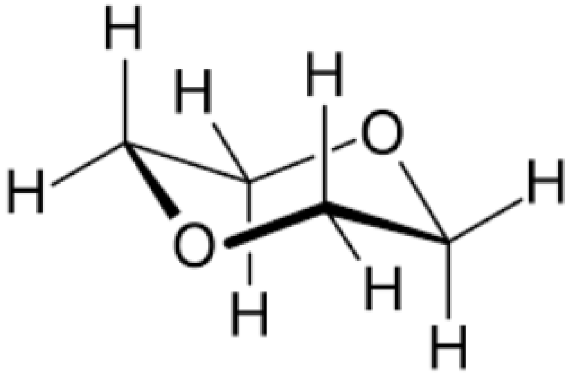
Outline

- ❖ 1,4-Dioxane Degradation Background
- ❖ Biological Tool Development
- ❖ Case Studies
- ❖ Critical Parameters for Validating MNA and In-Situ Bioremediation
- ❖ Industrial Perspectives



1,4-Dioxane Biodegradation's Evolving Background

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., 2006)

2012 •Stable Isotope Probing (SIP) study to validate 1,4-dioxane biodegradation potential in the environment (Chiang et al., 2012)

2014 •USEPA Technical Fact Sheet: "*…has not been shown to readily biodegrade in the environment*"

2014 •Biomarker development to predict biodegradation of 1,4-dioxane (Gedalanga et al, 2014)

2015 •VAFB Pilot Study: Propane Bioaugmentation and Biostimulation (Lippincott, 2015)

2016 •Impact of chlorinated solvents on biodegradation Kinetics of 1,4-Dioxane (Zhang et al., 2016)

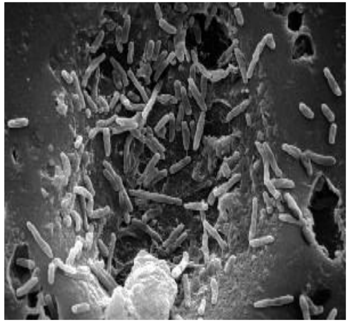
2016 •Lines of evidence framework to evaluate intrinsic biodegradation of 1,4-dioxane (Gedalanga et al, 2016)

2016 •In-situ treatment and management of 1,4-dioxane plumes: Natural attenuation may be more effective than previously thought (Adamson, 2016)

Microbial Biodegradation of 1,4-Dioxane

1,4-Dioxane and
primary substrates
(e.g., propane, THF,
toluene, phenol,
isobutane, isobutylene,
etc.)

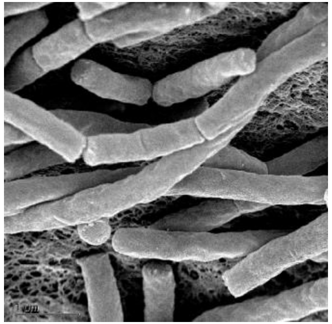
Co-Metabolism



Mycobacterium vaccae JOB5

1,4-Dioxane

Metabolism



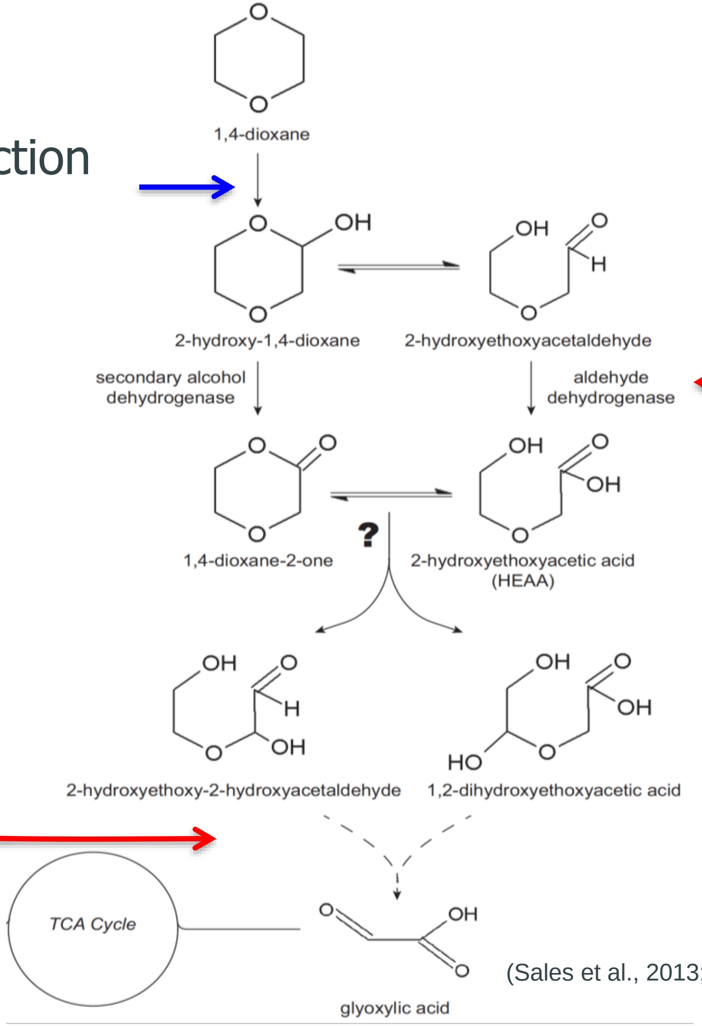
Pseudonocardia Dioxanivorans (CB1190)

Pathways

MO catalyzed reaction

Potential ALDH catalyzed reaction

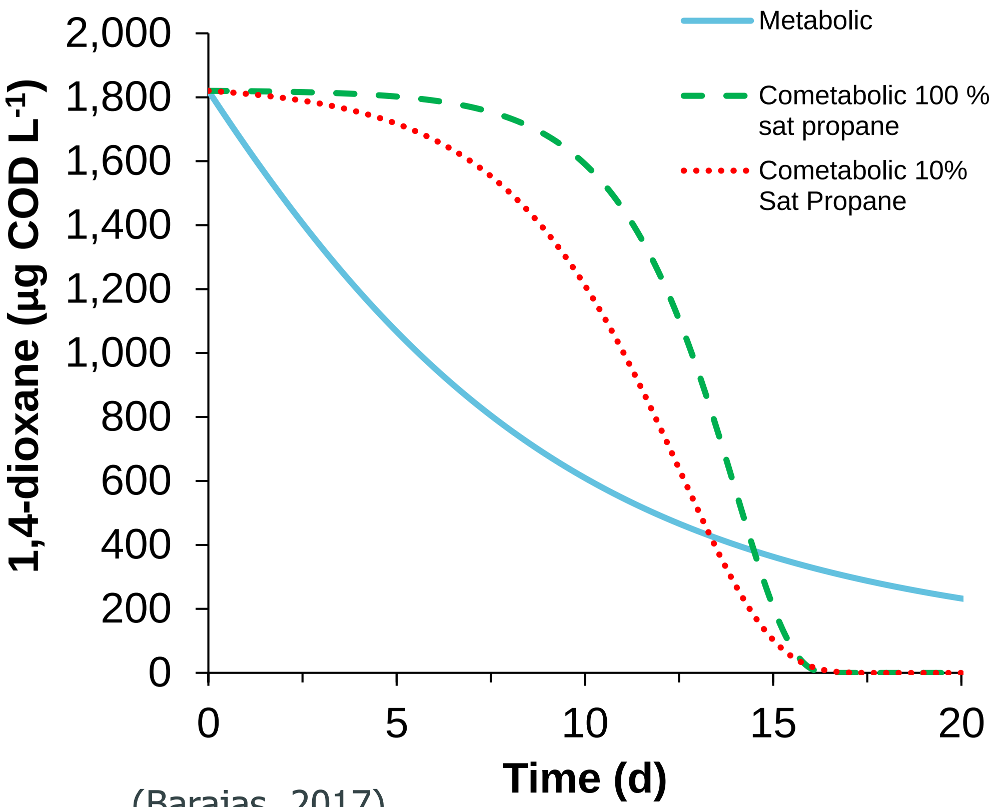
Potential ALDH catalyzed reaction



(Sales et al., 2013; Grostern et al., 2012; Mahendra et al., 2007)

Microbial Biodegradation of 1,4-Dioxane

- Co-inhibition between 1,4-dioxane and propane (cometabolism)
- Microbial performance slowed down when O_2 < 2 mg/L
- Cometabolism is better when 1,4-dioxane < 10 mg/L
- Lessons learned
  - Replication is key
  - Variability on kinetics



(Barajas, 2017)

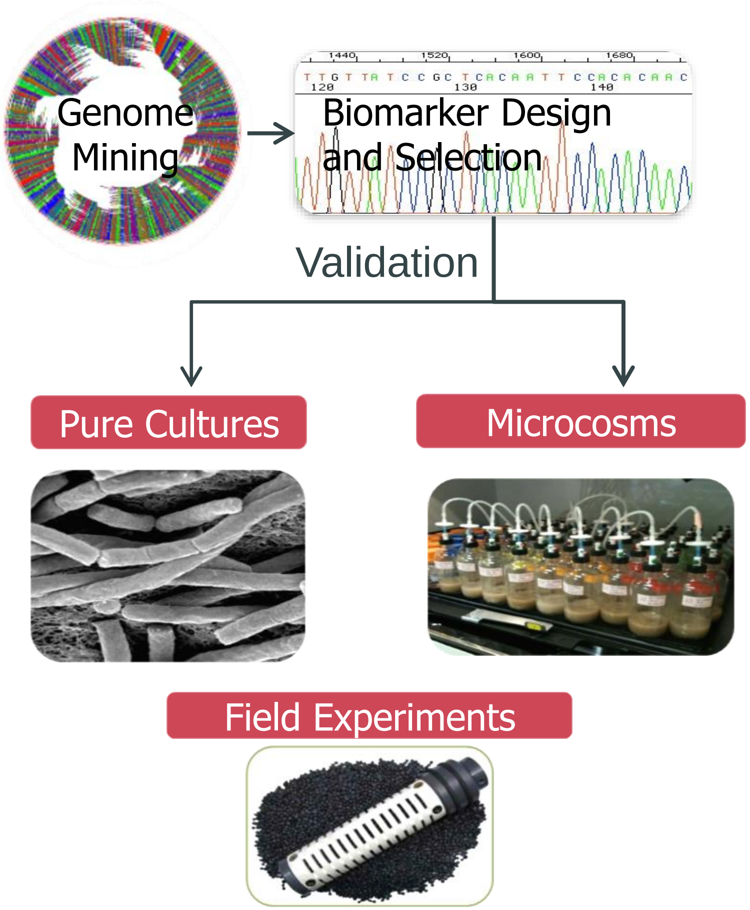
Biomarker Development

- Biomarkers were validated in pure cultures, in microcosms, and in diverse samples directly collected from the field
- DXMO and ALDH constitutes the best marker set for dioxane aerobic biodegradation (Gedalanga *et al*, 2014)
- Recommended biomarkers for analysis:
  - DXMO
  - ALDH
  - DXMO mRNA
  - ALDH mRNA
  - PPO
  - RMO
  - PHE…

DXMO, ALDH → Indicative of presence of dioxane degrading bacteria

DXMO mRNA, ALDH mRNA → Confirms expression of enzymes, which are directly linked to rates and extent of dioxane bioremediation

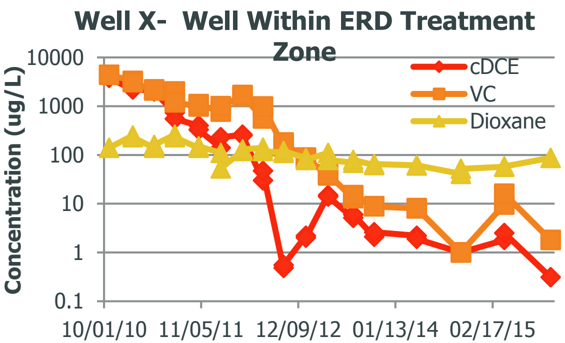
PPO, RMO, PHE… → Cometabolic biodegradation can only be verified indirectly using nucleic acid-based biomarkers

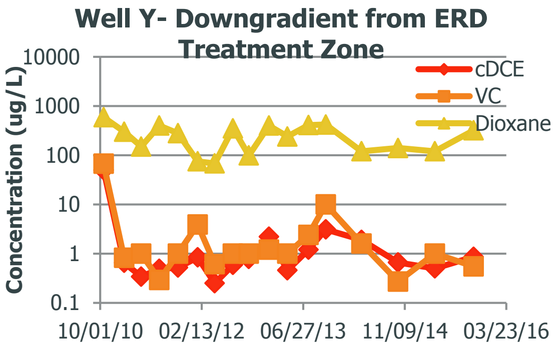


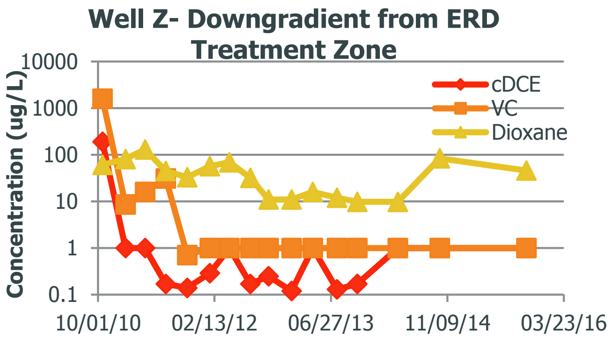
Biomarker Applications – Propane Biostimulation

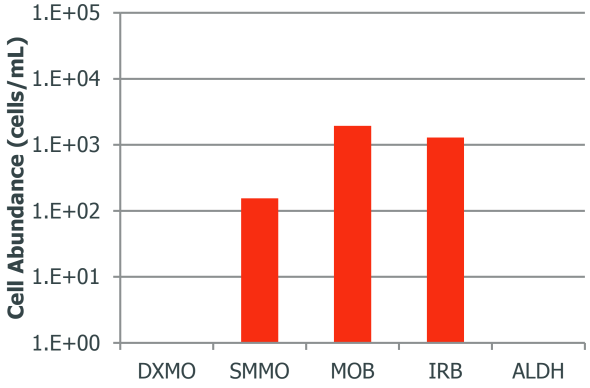
Propane Biostimulation Pilot Study Well				
Days	-244	28	117	245
1,4-Dioxane (ug/L)	**1070**	**975**	**101**	
1,1-DCE (ug/L)	283	14.6	<1	
cis-1,2-DCE (ug/L)	12.6	5.8	<1	
TCE (ug/L)	24.4	6.1	0.97 J	
PCE (ug/L)	34.2	6.8	1.59	
Propane (ug/L)	2.8J	<6	<6	
DO (mg/L)	0.56	3.63	9	
pH	6.3	6.38	6.39	
ORP (mV)	65.5	44	38.3	
Groundwater (cells/mL)				
PPO	2.27E+04	7.13E+04	6.37E+02	1.11E+04
DXMO	**<9.1**	**1.34E+01**	**1.50E+00**	**3.2 J**
EBAC		1.24E+05	1.61E+04	2.28E+06
Biotrap Beads (cells/bead)				
PPO	7.15E+04	1.23E+06	1.74E+04	7.01E+04
DXMO	**<50**	**6.41E+01**	**6.94E+01**	**226 J**
EBAC			2.33E+06	6.79E+06

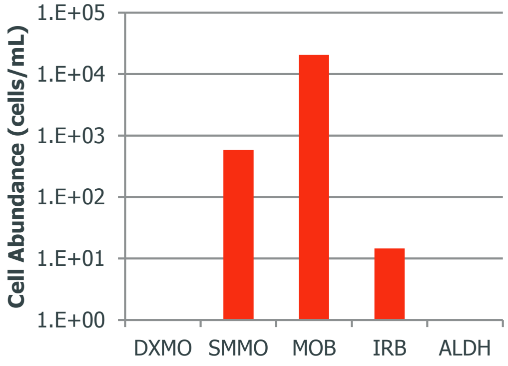
Biomarker Applications – Dioxane Monitoring Only

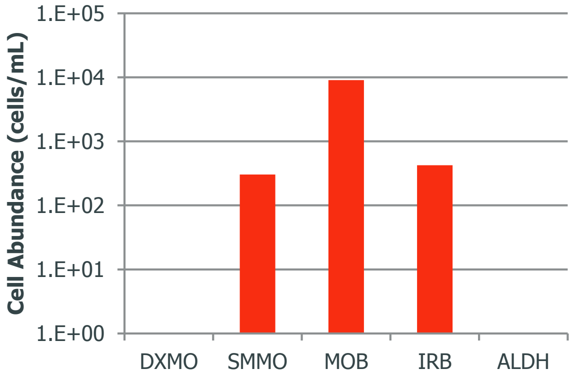












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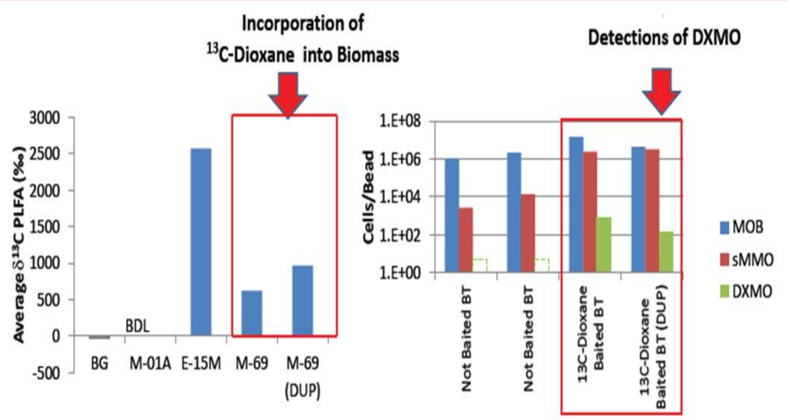
Reducing Conditions with High Methane Concentrations (~10ppm)

Biomarker Applications

- Methane oxidizing bacteria and methane monooxygenase (MOB and sMMO) are ubiquitous under both aerobic and anaerobic conditions
- High MOB/sMMO abundance or high methane concentrations in the aquifer does not necessarily suggest dioxane biodegradation potential
- DXMO and ALDH have high correlation with 1,4-dioxane degradation in pure cultures
- No conclusive results on DXMO and ALDH applications for co-metabolic biodegradation systems

Other Available Tools to Validate Dioxane Biodegradation

EMD	Description (1)	Application on 1,4-Dioxane
SIP	A synthesized form of the contaminant containing a stable isotope (such as ^{13}C label) is added. If biodegradation is occurring the isotope will be taken up by the organism and detected in biomolecules (e.g., phospholipids, DNA) or respired CO_2. By tracking ^{13}C label contaminant, it is possible to obtain direct evidence of biological degradation of contaminants, and identify the degrading microorganisms. It is commercially available, but not widely used. Isotopically labeled compounds can be expensive to synthesize.	
CSIA	Compound specific isotope analysis (CSIA) can be used to gain information relevant to potential contaminant sources, extent of degradation, and comingling of contaminant plumes relevant to environmental remediation decision makers.	See table below

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	

(Sadeghi et al, 2016)

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Validation Parameters for Managing In-Situ Bioremediation

Parameter	Range	Importance	Note
Dioxane spatial and temporal data		Critical	First line of evidence
DO	>2 mg/L	Critical	No evidence on anaerobic biodegradation of dioxane
ORP	>0 mV	High	Same as above
Temperature	>20 C	High	Degradation rates are not highly sensitive to temperature, but higher temperature can increate the degradation rates
Iron		Marginal	Competing electron acceptor
Sulfate		Marginal	Competing electron acceptor
Methane		Low	No strong evidence as primary substrate. More research is needed
Ethene/Ethane		TBD	TBD as primary substrate
Propane	Detected	High	Critical for propane biostimulation
TOC	Low	High	Presence of potential competing substrate

Validation Parameters for Managing In-Situ Bioremediation

Parameter	Range	Importance	Note
MOB & sMMO		Low	Ubiquitous in the aquifer. Methane is not considered as primary substrate
DXMO & ALDH	Detected	High	Direct correlation to dioxane biodegradation. But it is not commonly detected for sites under natural attenuation or cometabolic biodegradation conditions
DXMO & ALDH	10^7 cells/L	Critical	Direct correlation to dioxane biodegradation. It is critical when metabolic biodegradation is applied
SIP		Low	Does not provide dioxane degradation rate information.
Chlorinated solvents	<5 mg/L 1,1-DCE <50 mg/L cis-1,2-DCE & TCE	Critical	Inhibition is evident
Other VOCs		High	May compete with primary substrate for cometabolic biodegradation of dioxane
Copper	<20 mg/L	Marginal	Inhibition is evident when copper concentrations are very high

Industrial Perspectives – Knowledge Gaps

- Proper and consistent standard methods to improve dioxane data quality
- Case studies assessing and demonstrating dioxane plume stability
- High resolution site characterization to improve understanding of dioxane mass storage and discharge
- Usefulness and applicability of EMDs for evaluating dioxane biodegradation
- Pilot and full-scale demonstration of in situ dioxane biodegradation
- Science and engineering of different biostimulants
- Low-cost *ex-situ* treatment technologies (e.g., bioreactor)
- Green and sustainable aspects of dioxane plume management
- Confirmation of MNA for dioxane

Dioxane Initiatives

- Verify dioxane data quality
- Metabolic microcosm studies for a highly impacted dioxane site
- Support fundamental research and development of novel technologies
  - Anaerobic biodegradation
  - Dioxane sorption on sorbents to concentrate and degrade dioxane
  - Advanced electrochemical oxidation
  - In-situ bioreactor
- Industrial alliances and working groups

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Thank You

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