Assessment of metabolic and cometabolic 1,4-dioxane biodegradation in groundwater with complex hydrocarbon contamination.

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Outline

- Acknowledgements
- Background
- Objectives
- Biotreatability Study Approach
- Results
- Conclusions
- Next Steps

Acknowledgments

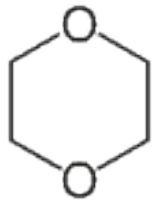
- TAUW (Germany)
  - Dr. Alfredo Pérez de Mora
  - Ludwig Immler

- SiREM
  - Jennifer Webb
  - Rachel Hallman


SiREM
BACKGROUND

1,4-Dioxane Background

- Challenges –
  - – prevalence, physical and chemical properties
    - high solubility, low volatility, and low sorptive capacity
- Aerobic metabolic and co-metabolic biodegradation well understood
- Inhibition by groundwater co-contaminants



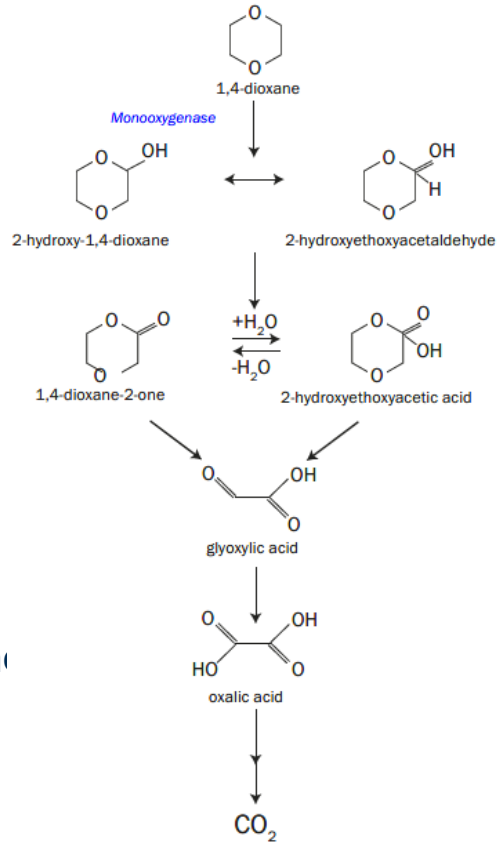
1,4-Dioxane Biodegradation

1,4-Dioxane metabolic biodegradation

- 1,4-dioxane is used as a C-source
- After inital oxidation step, further metabolites, ring opening and formation of glyoxylic acid and oxalic acid (metabolite)
- End products → H_2O + CO_2
- Preferred at concentrations >> 100 - 1,000 µg/L

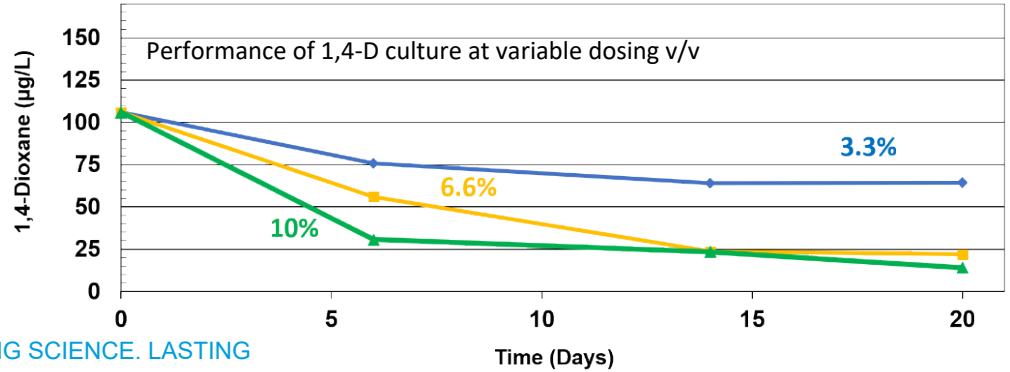
1,4-Dioxane cometabolic biodegradation

- In the presence of propane/methane
- Propane/methane-degrading bacteria can fortuitously degrade 1,4-dioxane by propane/methane monooxygenases
- First step by a different enzyme, degradation pathway remains th same thereafter
- Preferred at concentrations ≤ 1,000 -100 µg/L



DXO-88 Bioaugmentation Culture

- Enriched from a bioreactor in 2015
- ~ 20% of population metabolic also has co-metabolizers
- Degrades 1,000 mg/L to <20 µg/L 1,4-D
- Active between pH 4 and 9
- Performs well in treatability studies
  - Both metabolically and cometabolically



DXO-88

LEADING SCIENCE. LASTING

Advanced Tools to Assess 1,4-D Bioremediation

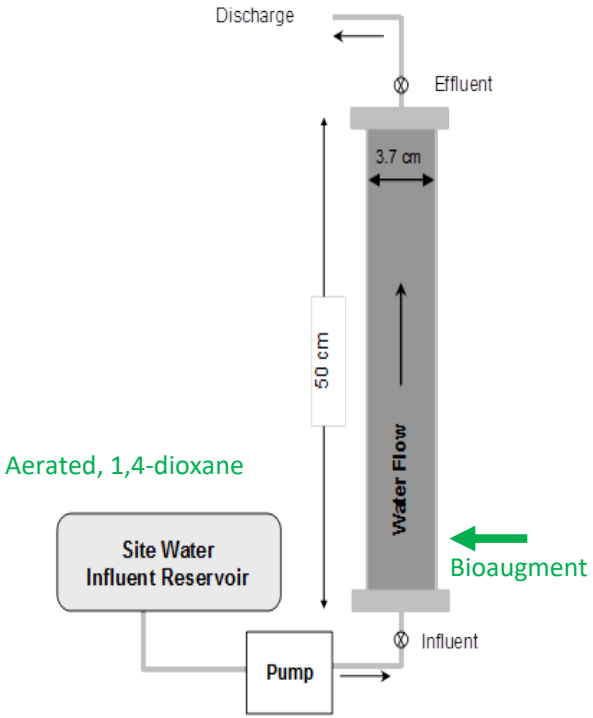
Genetic Tests: Assess if 1,4-D degraders are present/active

- Metabolic targets: dioxane monooxygenase (*dxmB*)/aldehyde dehydrogenase (*aldH)* (e.g., Gene-Trac® 1,4-Dioxane) – gene expression assays tend to correlate best to 1,4-D degradation
- Cometabolic targets – alkane gas MOs (e.g., propane [PMO]/methane monooxygenases [MMO])

Compound Specific Isotope Analysis (CSIA):

$^{13}C/^{2}H$ methods used to confirm 1,4-D degradation processes

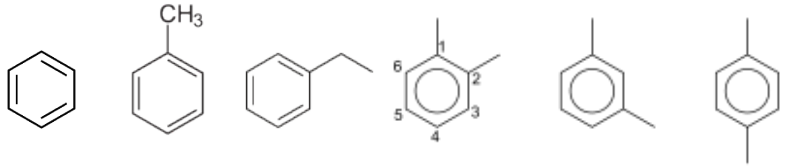
Biotreatability Studies: microcosm and column study methods confirm remediation strategies under simulated site-conditions

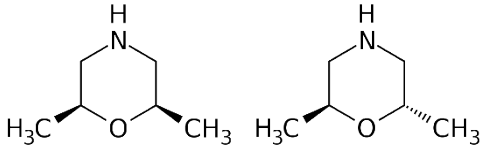


Site Background

- Groundwater hydraulic containment at chemical industrial site in Germany
- Treatment via on-site water treatment plant with discharge into mixed sewer system
- Target constituents include mixture of monoaromatic compounds

BTEX

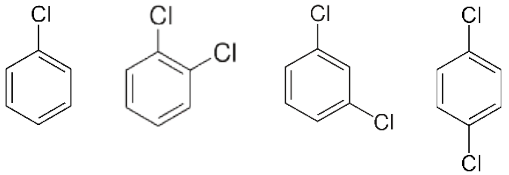


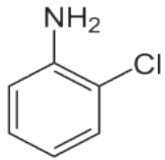


2,6-Dimethylmorpholin

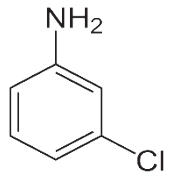
1,4-Dioxane

Chlorinated benzenes

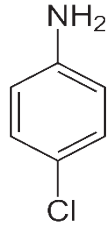




2-Chloroaniline



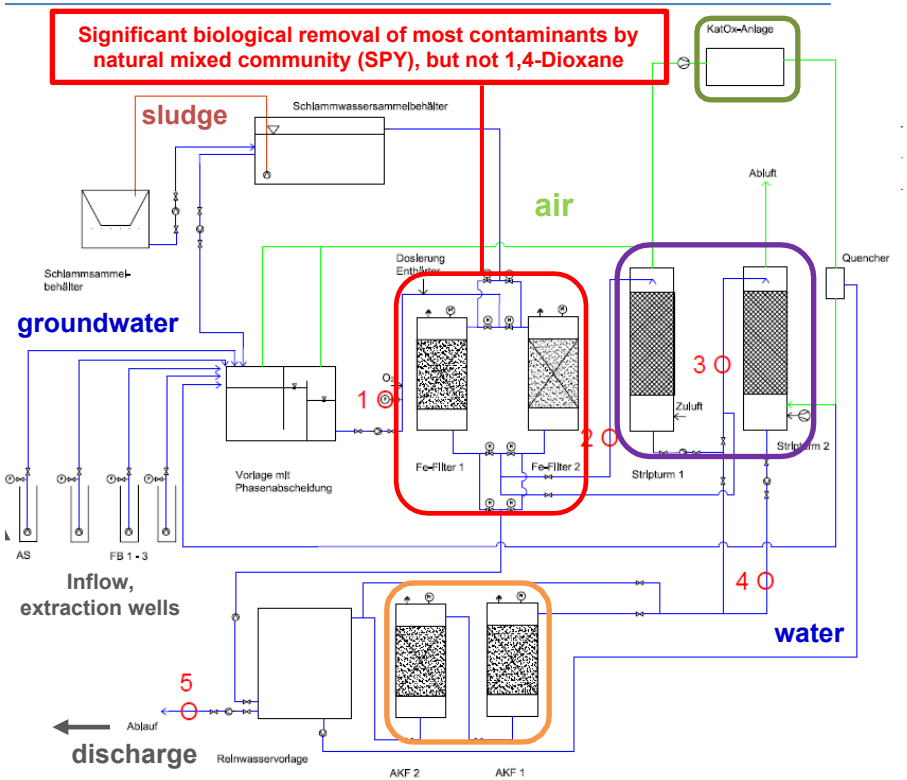
3-Chloroaniline



4-Chloroaniline

Water Treatment Plant - Process Diagram

Groundwater field parameters: pH (7-8), Temperature (15-20°C), EC (700-800 µS/cm), DO (3-6 mg/L), redox (150-200 mV)



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OBJECTIVES

Study Objectives

1) Evaluate incorporating 1,4-dioxane biodegradation into an existing on-site mixed biological-physical-chemical water treatment unit to treat a complex mixture of organic groundwater contaminants (monoaromatic hydrocarbons)
2) Improve current knowledge of mixed natural community (SPY) in the bioreactor (key players and their function)
3) Evaluate the potential of the mixed natural community (SPY) + DXO-88™ to biodegrade monoaromatic hydrocarbon mix at “low/high” concentration ranges

BIOTREATABILITY STUDY APPROACH

1,4-Dioxane Treatability Tests

Treatability Tests

1. Phase: Preliminary Test ✓

2. Phase: Batch tests multiple treatments (✓)

3. Phase: Column test

Evaluation

Planning of remedial measures for Hotspot T3.1

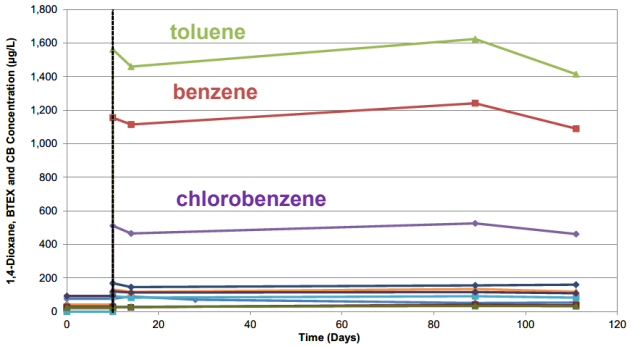
effluent water
(just 1,4-Dioxane)

influent water / blended water (influent + hotspot)

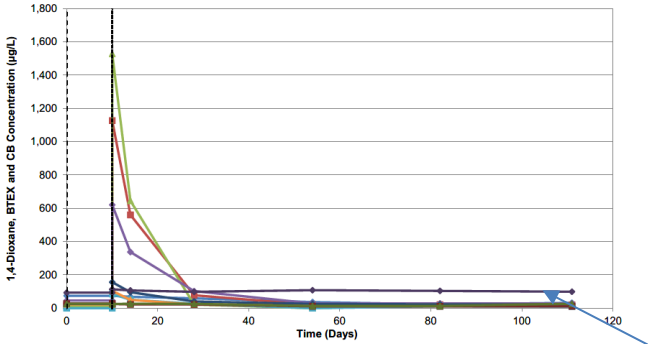
	Variant	water	1,4-Dioxane	other contaminants (partially spiked)	Description	N
1	Inflow sterile control (control)	Inflow	~70µg/L	**influent water** BTEX (100-1.000 µg/L) CB (500 µg/L), DCB (100 µg/L); 2,6 DMM (1.500 µg/L); CA (400-2.000 µg/L)	autoclaved; treated with $HgCl_2$ and NaN_3 (Natriumazide)	2
2	Inflow bioaugmented (bioaug.)	Inflow	~70µg/L		biostimulated with O_2 und Propane; Bioaugmented with DXO-88™	2
3	blended water (Inflow+T3.1) sterile control (control)	Inflow WTP new + Hotspot T3.1 (5:1)	300 - 400µg/L	**blended water** BTEX (100-3.000 µg/L) CB (700 µg/L), DCB (150 µg/L); 2,6 DMM (8.000 µg/L); CA (400-4.000 µg/L)	autoclaved; treated with $HgCl_2$ and NaN_3 (Natriumazide)	2
4	blended water (Inflow+T3.1) bioaugmented (bioaug.)	Inflow WTP new + Hotspot T3.1 (5:1)	300 - 400µg/L		biostimulated with O_2 and later with Propane; Bioaugmented with DXO-88™	2

Monoaromatics and 1,4-Dioxane Treatability Test Results

1) Influent sterile-control (1,4-D ~70 µg/L)



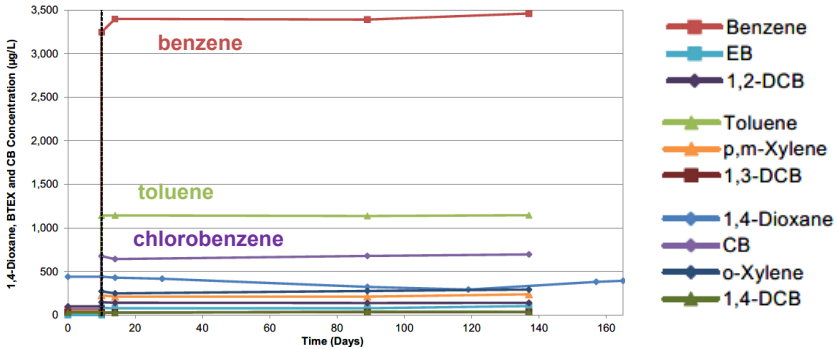
2) Inflow bioaugmented (1,4-D ~ 70 µg/L)



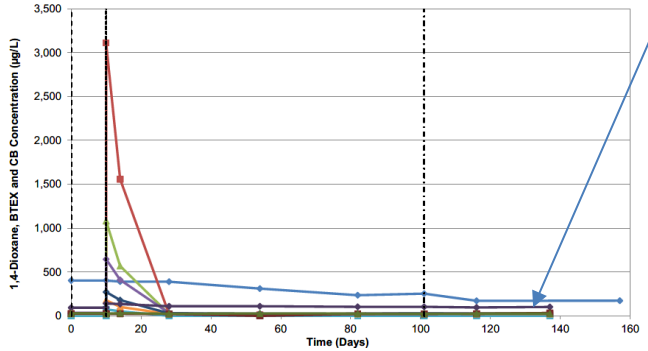
Successful degradation of most substances, including chloranilines and 2,6-DMM (not shown)

Limited biodegradation of 1,2-Dichlorobenzene

3) Blended sterile control (1,4-D 300-400 µg/L)

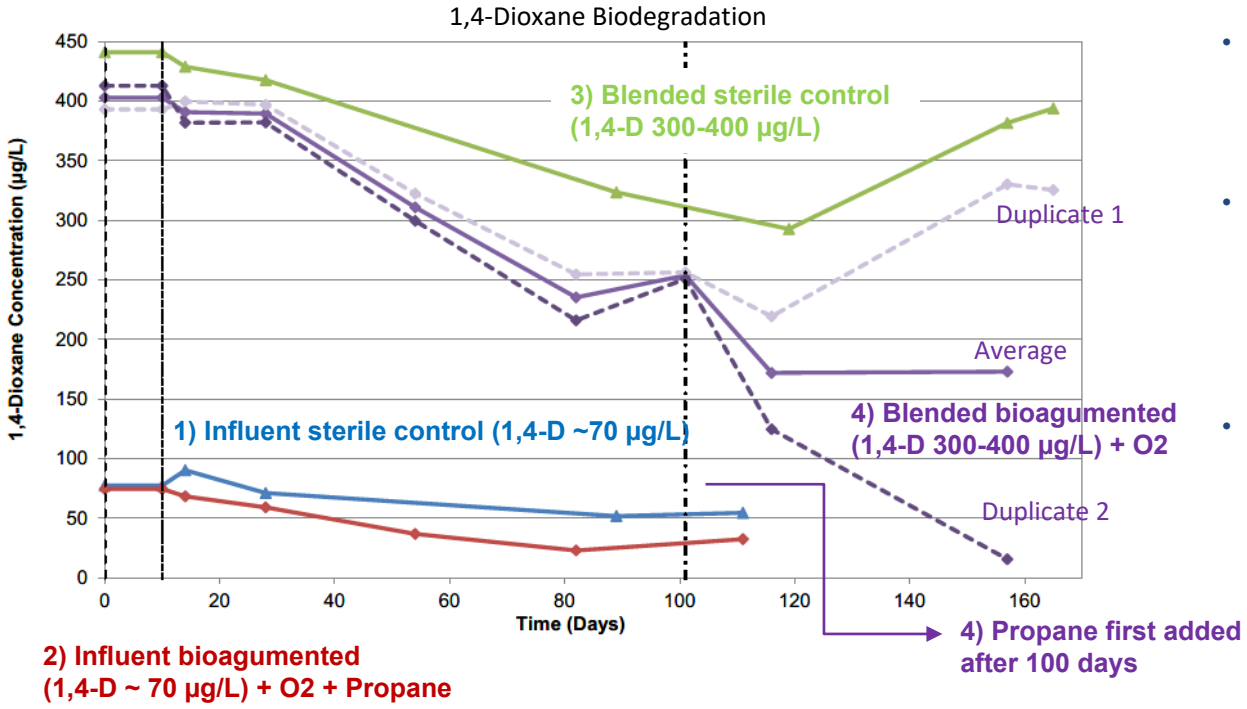


4) Blended bioaugmented (1,4-D 300-400 µg/L)



1,4-Dioxane biodegradation exclusively cometabolic

1,4-Dioxane Treatability Test Results



- **1,4-Dioxane degradation with inflow water (70 µg/L 1,4-Dioxane) occurs co-metabolically**
- **1,4-Dioxane degradation with blended water (300-400 µg/L 1,4-Dioxane) is possible co-metabolically (after propane addition); metabolic not observed**
- **1,4-Dioxane biodegradation occurs more slowly than for the other compounds**

Acclimatization of DXO-88

Compartimentalization of degradation processes

qPCR gene quantification

Baseline water for microcosms set up (effluent – Phase 1) (influent and T3.1 – Phase II)

Baseline water sampled		New WTP Inflow (cop/L)	New WTP Outflow (cop/L)	Hotspot T3.1 (cop/L)
metabolic	Dioxane Monooxygenase (dxmB)	3,00+E03	3,00+E03	3,00+E03
metabolic	Aldehyde Dehydrogenase (ALDH)	5,00+E03	3,00+E03	3,00+E03
cometabolic	Particulate Methane Monooxygenase (PMMO)	3,28E+03	(-)	3,28E+03
cometabolic	Propane Monooxygenase (PMO)	2,36+E04	(-)	4,33+E04
Total bacteria	Total Prokaryotes (PROK)	3,30+E09		2,18+E10

- No evidence for 1,4-dioxane biodegradation at the site (influent and T3.1 water)
- Also no evidence for effluent water as expected

Phase II bioagumented microcosms (influent and blended)

Treatments		Inflow bioaug. (cop/L) initial	Inflow bioaug. (cop/L) final	Blended bioag. (cop/L) initial	Blended bioag. (cop/L) final
metabolic	Dioxane Monooxygenase (dxmB)	6,92+E04	2,18+E04	1,35+E05	4,37+E04
metabolic	Aldehyde Dehydrogenase (ALDH)	6,38+E04	2,18+E04	1,37+E05	4,37+E04
cometabolic	Particulate Methane Monooxygenase (PMMO)	2,18+E04	2,18+E04	4,37+E04	4,37+E04
cometabolic	Propane Monooxygenase (PMO)	**1,82+E07**	**2,26+E05**	**2,46+E07**	**5,27+E04**

- No presence of 1,4-dioxane genes for metabolic degradation
- Bioaugmented microcosms show only co-metabolic degradation biomarkers

NGS Sequencing Data - baseline (site) water

- **Effluent representative of enrichment in sand filter (*Burkholderia, Methylonetera and Woesearchaiea*)**
- **Influent and T3.1 (Hotspot) show different community**

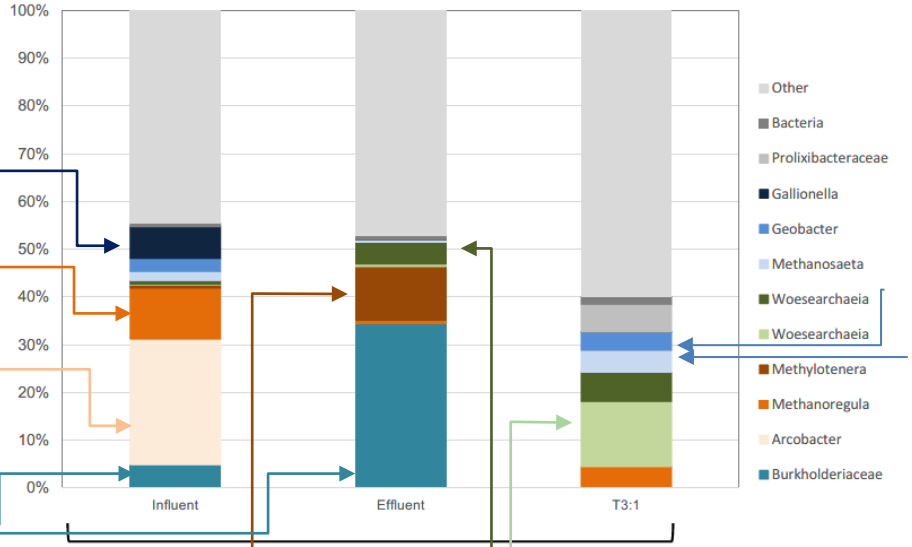
Galionella – (microaerophile, S- and Fe-oxidation)

Methanoregula – (Archaea, methanogen)

Arcobacter – (microaerophile, S-oxidation, Perchlorate reduction, catechol degradation)

Burkholderia – (known to degrade monoaromatics, polycyclic aromatics and heterocycles)

Figure 7: Next Generation Sequencing
Top 16 Microorganisms in TAUW Site Groundwater



Methylonetera – (known to use methylated amines as N source, but also as C source (methylotrophs))

Woeserarchaeia – (salt tolerant Archae found in BTEX contaminated groundwater – mutualistic interactions with BETX degrading communities)

Geobacter – (anaerobe, dechlorinating microorganism, but not CB or DCB)

Methanosaeta – (Archaea, methanogen)

NGS - microbial community structure - microcosm results

Baseline water

Influent

T3.1

Microcosms

Bioaug. Influent

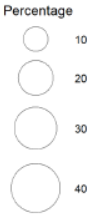
Initial

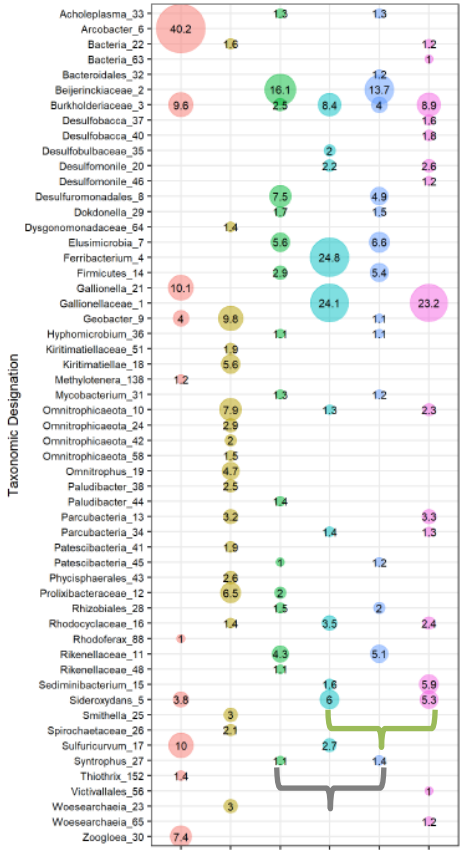
Final

Bioaug. Blended

Initial

Final





- **Microbial community composition of baseline water not well-reflected in initial composition of the bioaugmented microcosms (imprint DXO-88)**
- **Very similar initial composition of bioaugmented microcosms (imprint DXO-88)**
- **Significant and similar shift in microbial community structure from initial to final sampling event for bioaugmented microcosms (loss of DXO-88 imprint and raise of)**

Initial

OTU2 *Beijerinkiacea (15%)*
OTU3 *Burkholderiaceae (3%)*
OTU 8 *Desulfuromonadales (6%)*
OTU 7 *Elusimicrobia (6%)*
OTU 14 *Firmicutes (4%)*
OTU 11 *Rickenellaceae (5 %)*

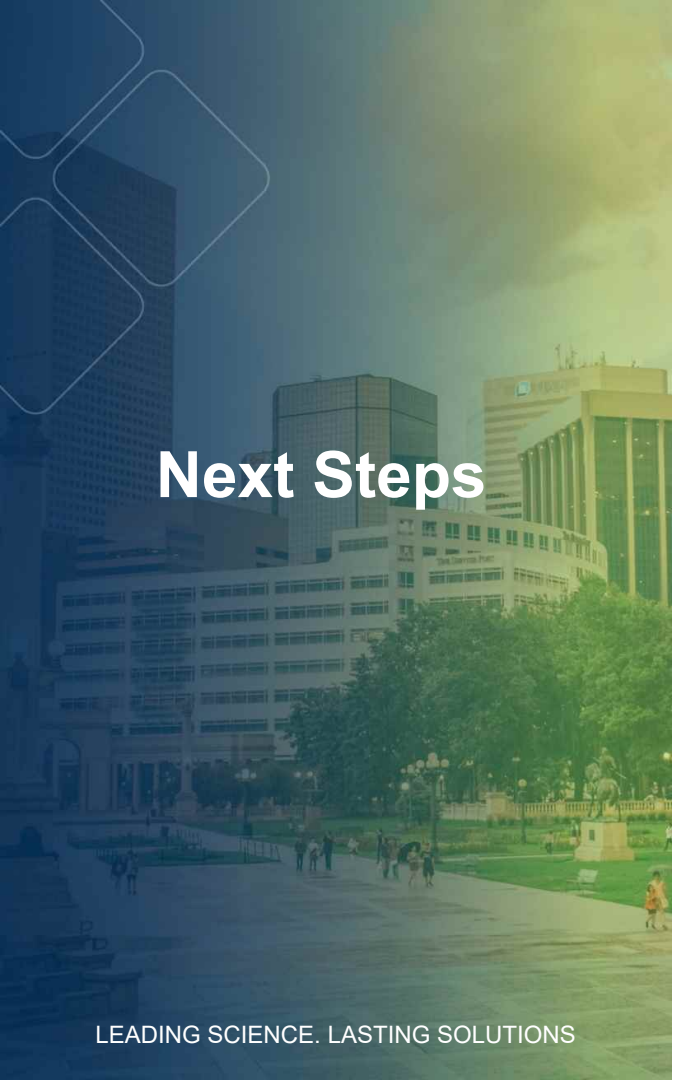
Final

OTU3 *Burkholderiaceae (9%) (biodegrader)*
OTU 1 *Galionellaceae (24%)*
OTU 4 *Ferribacterium (25%, only influent)*
OTU 5 *Sideroxydans (6%)*

Conclusions

- **The Preliminary Test – Phase 1 – (no co-contaminants) identified that cometabolism should be considered in the Biotreatability Study at 1,4-Dioxane <100µg/L**
- **The Biotreatability Test – Phase 2 – (co-contaminants) indicated that 1,4-Dioxane removal was only possible cometabolically at both 300-400 µg/L and <100µg/L**
  - potential for bioaugmentation with DXO-88™ and propane in presence of other Site contaminants may not be inhibitory to their degradation
- **Complete biodegradation of co-contaminants by the natural mixed community (SPY) is possible (exception 1,2-dichlorobenzene)**
  - with main biodegrader in mixed community likely ***Burkholderia* OTU**
- **Spatial separation of biodegrading activities by DXO-88TM and SPY may be necessary if 1,4-Dioxane biodegradation occurs co-metabolically**

TAUW



Next Steps

- **Enrich and characterize "Bioreactor-WTP" SPY culture for degradation potential for BTEX, chlorobenzene, dichlorobenzenes, chloroanilines and 2,6-dimethymorpholine**
- **Implement a 2-stage column test**
  - 1st stage - SPY culture
  - 2nd stage - DXO-88 culture
- **Incorporate 1,4-dioxane degradation process in the WTP**
  - subsequent connection of a gravel filter downstream of the AK filters
  - with possible addition of propane/methane and enrichment of the DXO-88 mixed culture)

SiREM

QUESTIONS?

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