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COLLEGE OF ENGINEERING | School of Chemical, Biological, and Environmental Engineering

Single-Well Push-Pull Tests to Assess Aerobic Cometabolism of Isobutene as a Surrogate for 1,4-Dioxane

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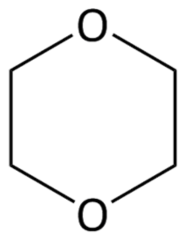
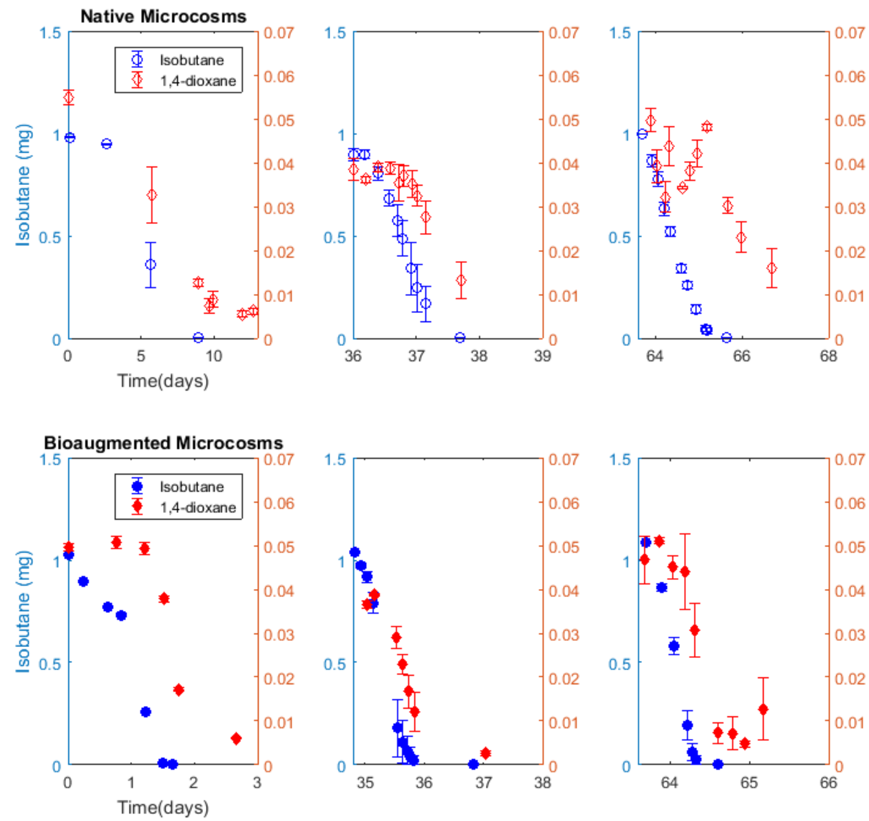
Aerobic cometabolism of 1,4-dioxane

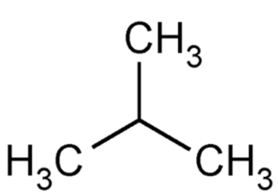


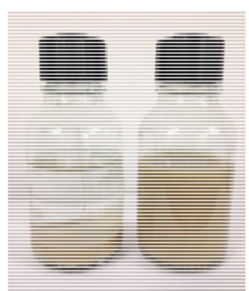
- 1,4-Dioxane:
  - Cyclic ether
  - Widespread groundwater contaminant due to use as stabilizer for 1,1,1-trichloroethane
  - Median maximal plume concentration: 365 ppb (Adamson, 2014)
  - Resistant to conventional pump and treat remediation strategies
- Aerobic cometabolism: *use of a primary substrate (electron donor) to stimulate expression of enzymes that transform both primary substrate and contaminant of interest with oxygen as the electron acceptor*

1,4-dioxane transformation by isobutane-utilizing microorganisms

- Primary substrate: isobutane
- Monooxygenase enzyme oxidizes 1,4-dioxane
- Model bacteria: *Rhodococcus rhodochrous* ATCC 21198
- Biostimulation and bioaugmentation in aquifer microcosms







Single-well push-pull tests

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- MW2 at the OSU motor pool
- 2”, 13’ deep, clay aquifer
- Injection via peristaltic pump:
  - Bromide (KBr)—tracer
  - Nitrate (KNO_3)—inorganic nutrient
  - Isobutane—primary substrate
  - Hydrogen peroxide—oxygen
- Extraction via 1L bailer

Site Constraints

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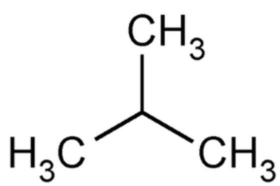
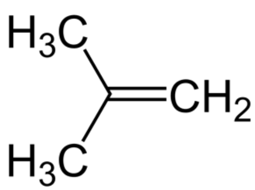
- Active lot
- Unproductive wells
  - Maximum injection/extraction rate in MW2: 400 mL/min
- 40 ppb arsenic
- **No 1,4-dioxane present!**

1,4-Dioxane Surrogate: Isobutene

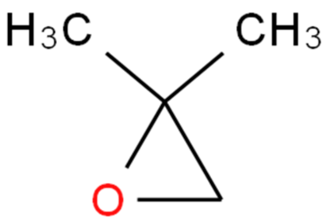
- No growth of isobutane-utilizers on isobutene
- Monooxygenase enzyme transforms isobutene (like 1,4-dioxane)
- Formation of epoxide: isobutene oxide



Isobutane



Isobutene



Isobutene oxide

Push-Pull Tests in MW2

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Well test in MW2	Date Test Started	Volume Injected (L)	Injection Rate (mL/min)	Injection Concentration (mg/L)				
				Isobutane	Isobutene	Nitrate	Bromide	Hydrogen Peroxide
Transport	09/26/17	23	260	7.7		29.3	63.7	
Biostimulation 1	10/26/17	39	270	1.8		19.8	80.6	~100
Biostimulation 2	11/07/17	33	245	4.3		12.5	95.7	~100
Biostimulation 3	11/15/17	32	300	5.7		8.7	61.3	~100
Biostimulation 4	11/29/17	22	300	6.0		10.4	80.7	~100
Biostimulation 5	02/09/18	36	450	2.4		7.3	107.0	~100
Cometabolism 1 (Isobutene alone)	02/16/18	36	300		0.8	7.7	167.9	~100
Biostimulation 6	03/16/18	35	220	2.5		6.0	100.2	~100
Cometabolism 2 (Isobutene and isobutane)	03/28/18	25	240	2.5	1.1	8.1	110.1	~100

Analytical Methods

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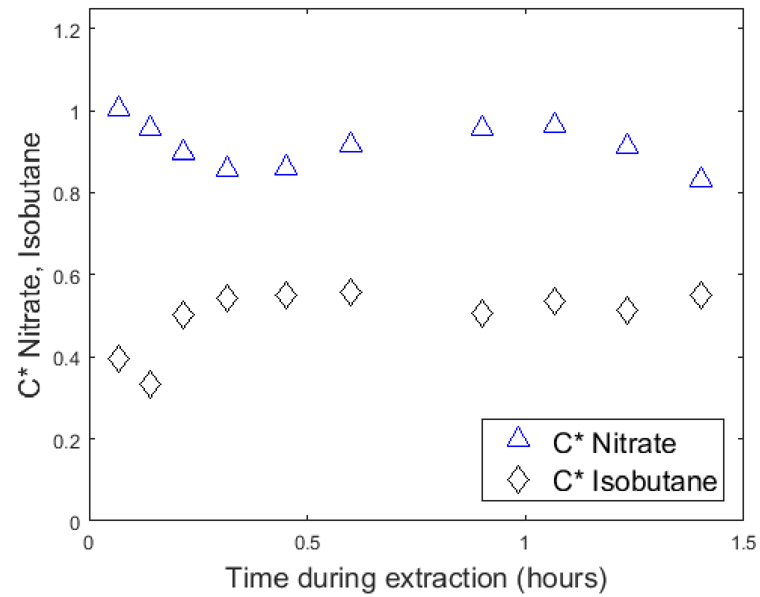
- *Isobutane* and *Isobutene*: headspace analysis, GC-FID or GC-MS
- *Isobutene Oxide*: liquid analysis, GC-MS with heated purge and trap
- *Bromide* and *Nitrate*: liquid analysis, IC
- *Oxygen*: liquid analysis, YSI dissolved oxygen probe
- *Hydrogen Peroxide*: liquid analysis, Indigo test strips

Transport test

- Injection: 23 L
- Continuous extraction: 50 L

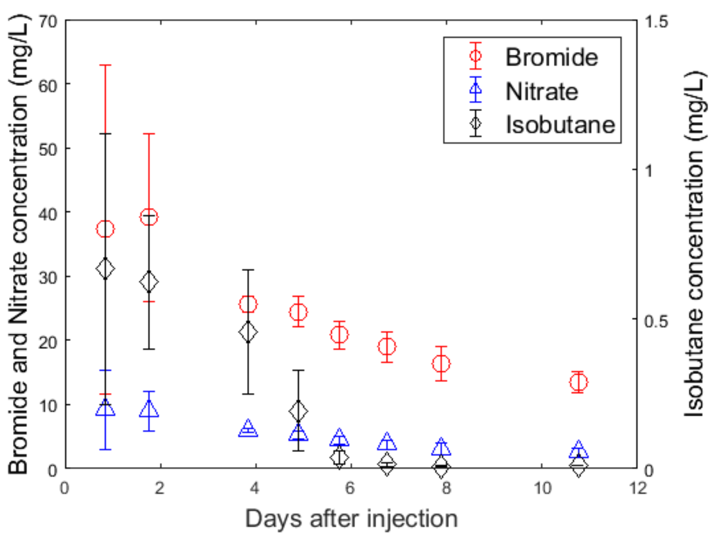
$$C^* = \frac{C/C_0}{Br/Br_0}$$



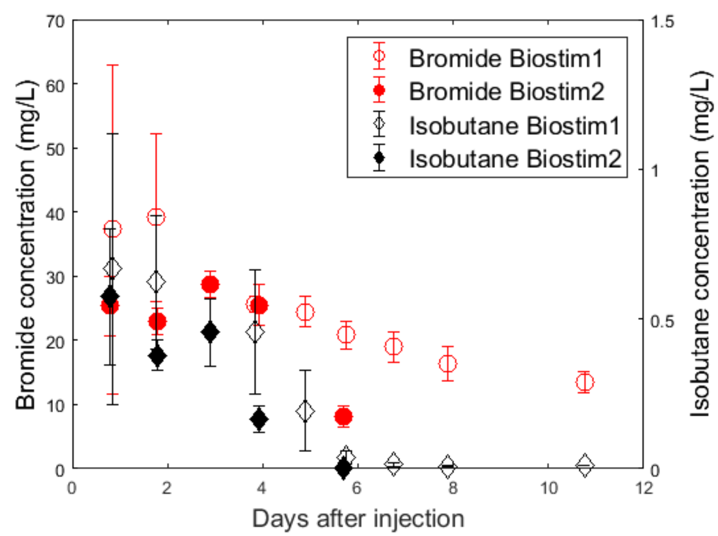
Isobutane Biostimulation

- Injection solution: Isobutane, nitrate, bromide, hydrogen peroxide
- Natural drift tests: injection followed by daily extraction of 4L

Biostimulation 1



Biostimulations 1 & 2



Isobutane Biostimulation

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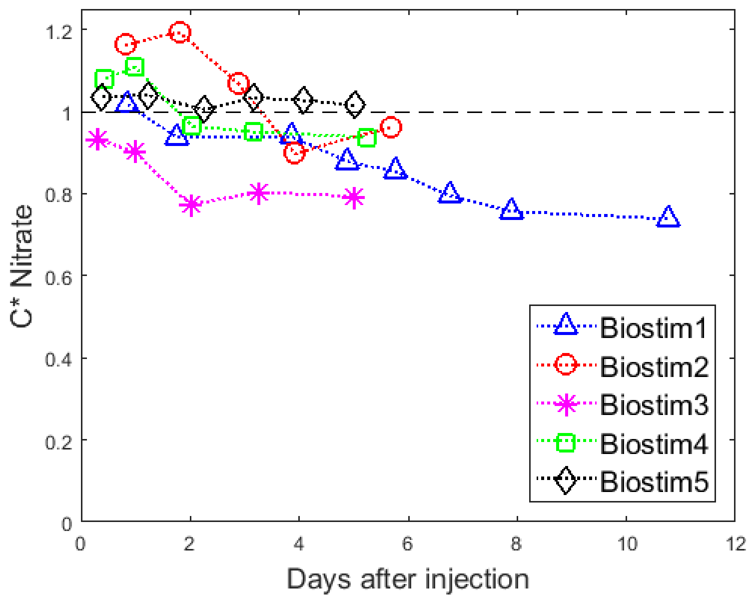

$$C^* = \frac{C/C_0}{Br/Br_0}$$

Well test in MW2	Date Test Started	Days since previous test
Biostimulation 1	10/26/17	
Biostimulation 2	11/07/17	12
Biostimulation 3	11/15/17	8
Biostimulation 4	11/29/17	14
Biostimulation 5	02/09/18	72

Isobutene Cometabolism Test 1

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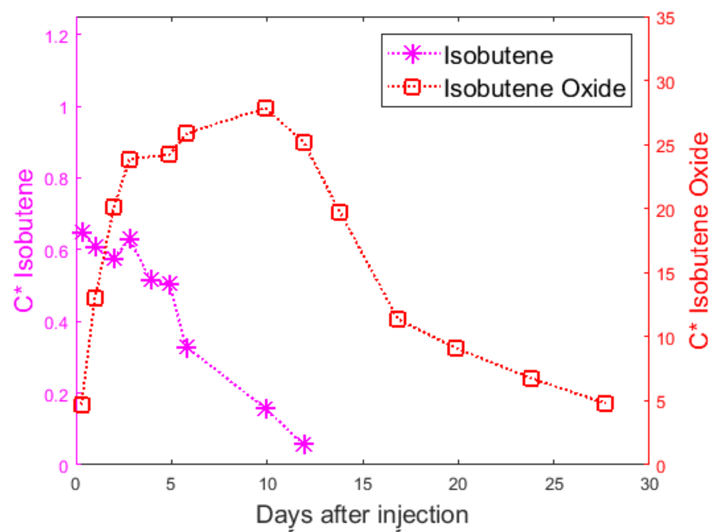
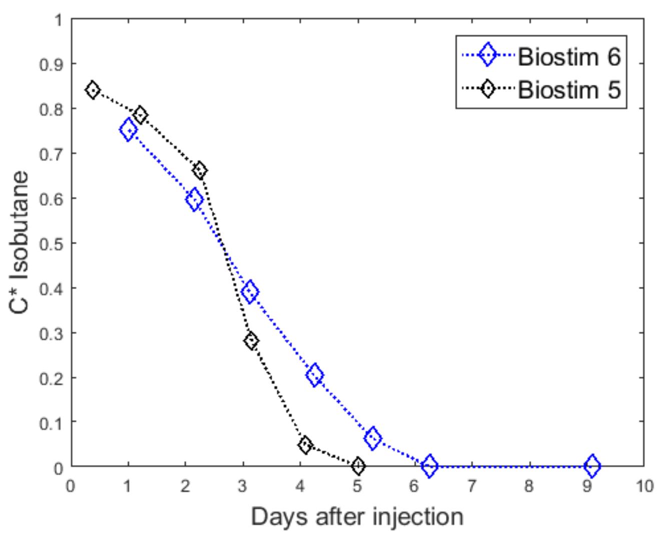
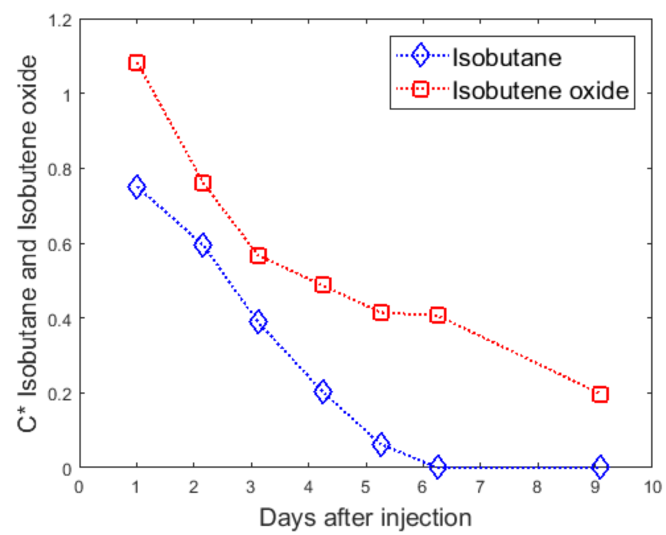


- Injection solution: Isobutene, nitrate, bromide, hydrogen peroxide
  - Resting cell test
- Isobutene decreases relative to bromide tracer
- Isobutene transformation was slower than isobutane transformation from recent biostimulation test
- Isobutene oxide formed

Biostimulation Test 6

- Immediately after isobutene cometabolism test
- Injection solution: Isobutane, nitrate, bromide, hydrogen peroxide, **30 ppb isobutene oxide**
- Isobutane consumption not significantly slower than in biostimulation 5
- Decrease of isobutene oxide relative to bromide tracer – biotically driven





Isobutene Cometabolism Test 2

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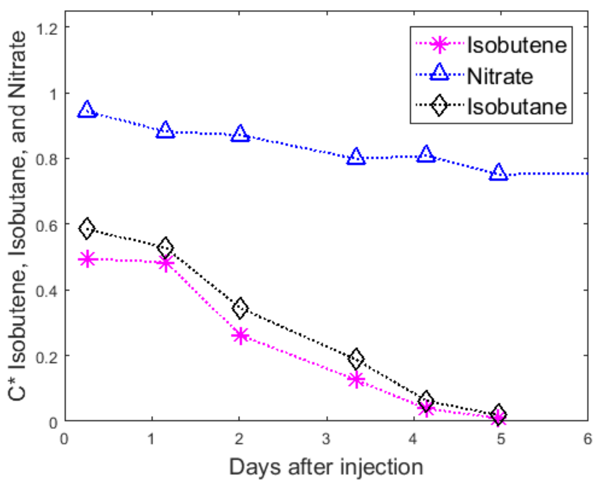
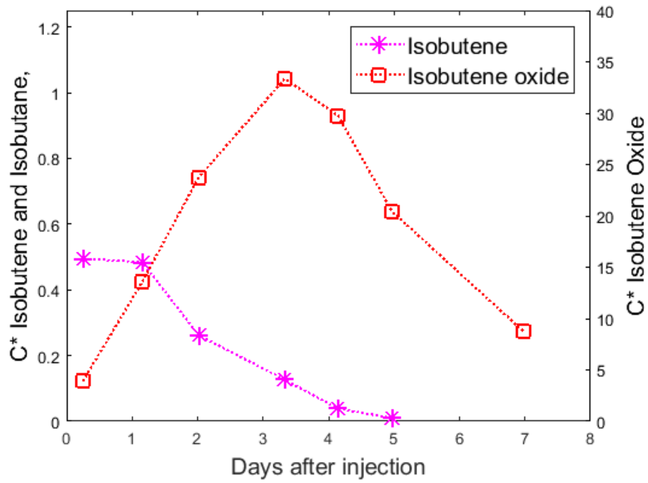
- Active cell test– primary substrate present
- Injection solution: Isobutane, isobutene, nitrate, bromide, hydrogen peroxide
- No inhibition of isobutene transformation by isobutane (unlike 1,4-dioxane)





Resting vs Active Cometabolism

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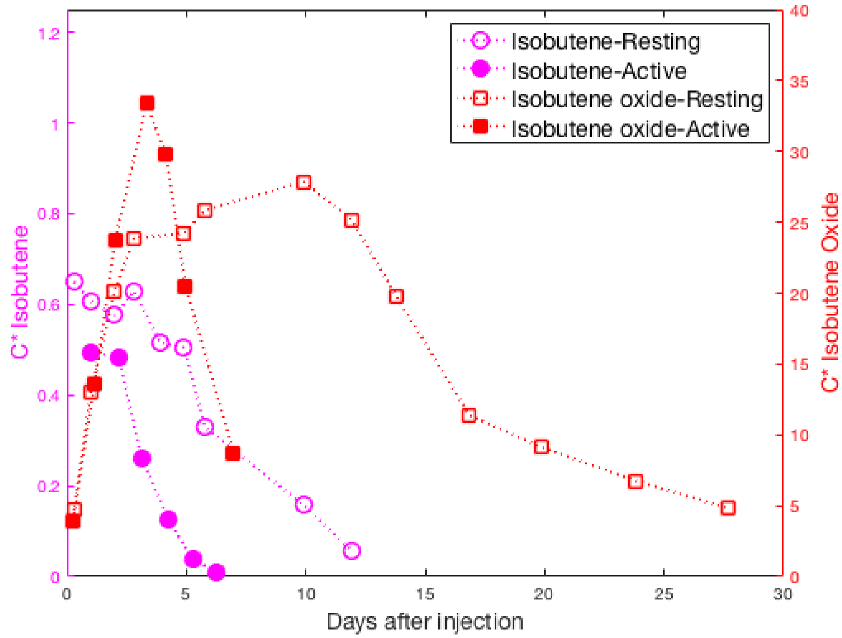
- Faster isobutene and isobutene oxide transformation in active cometabolism test
- Potential implications for 1,4-dioxane transformation products

Test	Isobutene injected (mg/L)	Maximum Isobutene oxide (mg/L)
Cometabolism 1– Resting (Isobutene alone)	0.8	0.11
Cometabolism 2– Active (Isobutane and isobutene)	1.1	0.135



Summary and Conclusions

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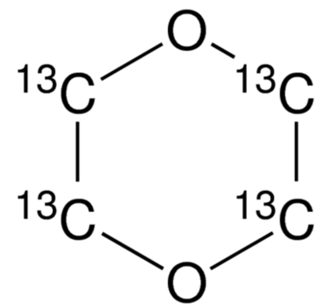
- Isobutane-utilizing microorganisms were biostimulated in situ
- In situ cometabolic activity demonstrated by transformation of isobutene to isobutene oxide
- Cometabolic transformation did not diminish activity of microbial community
- Faster isobutene and isobutene oxide transformation in the presence of isobutane—implications for 1,4-dioxane mineralization

Future Work

- Molecular analysis: qPCR for SCAM enzyme
- Push-pull tests with labeled 1,4-dioxane
- Reactive-transport modeling
- Assessment of other primary substrates



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Questions?

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