Modeling Aerobic Cometabolism of 1,4-Dioxane and Chlorinated Solvents by Isobutane-Utilizing Bacteria

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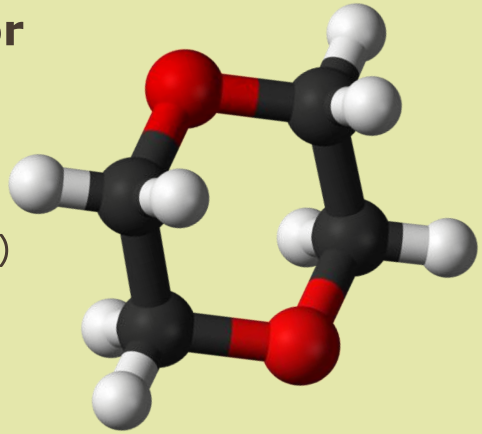
1,4-dioxane: $C_4H_8O_2$



- Detergents, resin and surfactant byproduct, **stabilizer for chlorinated solvents, 1,1,1-TCA**
- CA Geotracker database survey: 14D detected in groundwater at 32% of sites where analyzed; 95% co-contamination with chlorinated solvents (Adamson et al 2014)

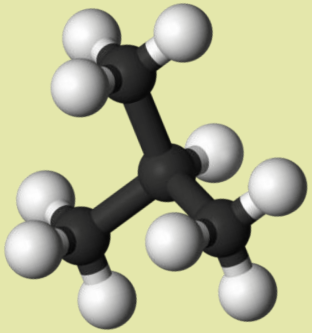
- "Likely" human carcinogen (EPA IRIS 2013)
  - $1x10^{-6}$ lifetime cancer risk in drinking water: 0.35 ppb
- State drinking water standards: 0.25 ppb (NH) to 200 ppb (IA) (Arcadis 2016)

- Miscible; log K_{ow}: -0.27; Henry's constant: $4.80x10^{-6}$ atm-cm^3/mole (ATSDR)
- Pump and treat: resistant to air stripping and activated carbon sorption
- Emerging evidence of natural attenuation, but relatively recalcitrant

Aerobic cometabolism of 1,4-dioxane

Aerobic cometabolism: use of a primary substrate (electron donor) to stimulate expression of enzymes capable of transforming both primary substrate and contaminant of interest with oxygen as the electron acceptor

- Primary substrate: Isobutane, C_4H_{10}
- Monooxygenase enzymes transform 1,4-dioxane without benefit to cell
- Model microorganism: *Rhodococcus rhodochrous* (ATCC 21198)

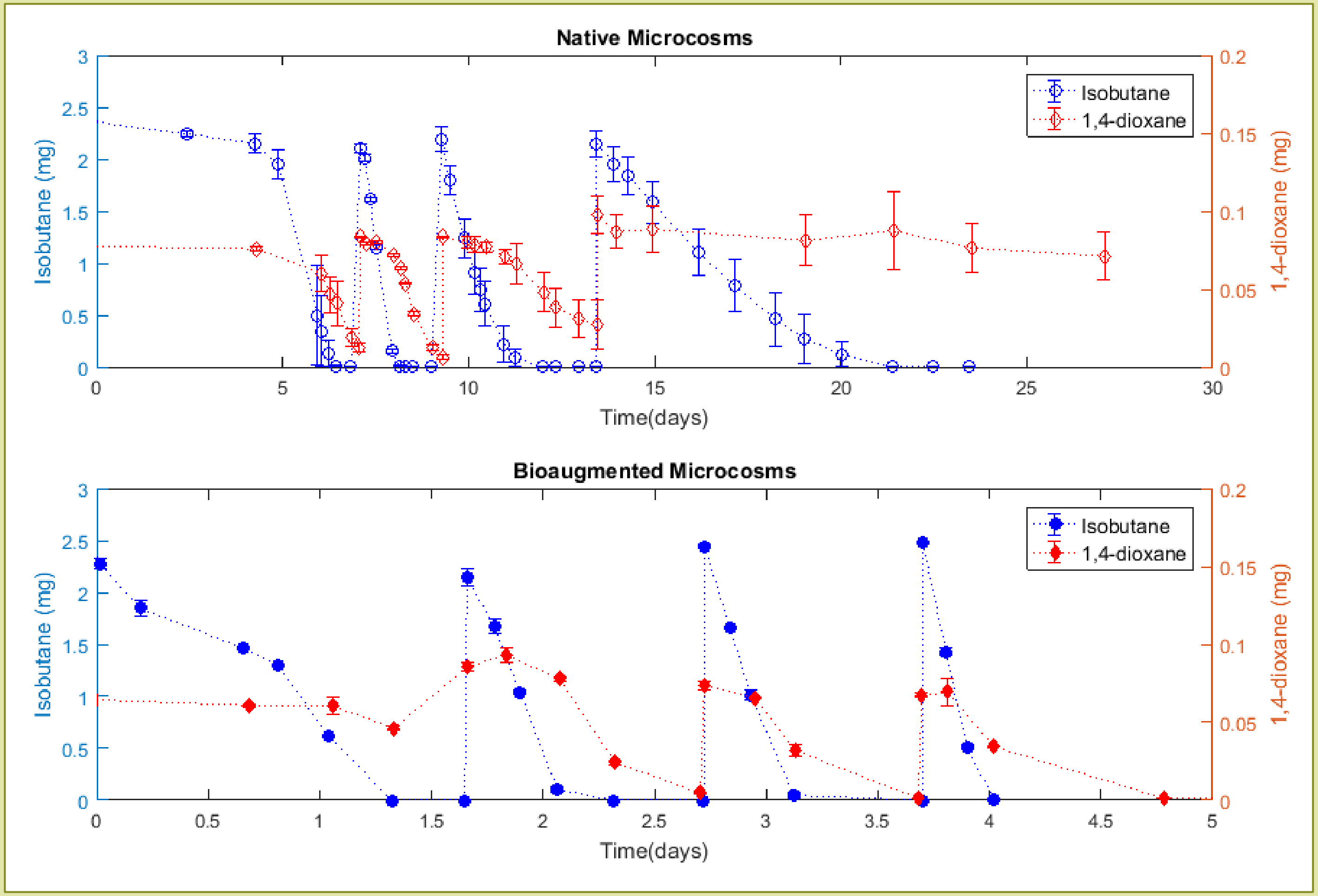


Allows for degradation of low concentrations of 1,4-dioxane

Microcosm Experiments

- Microcosms constructed from artificial groundwater and aquifer solids from Fort Carson, Colorado
  - 35% headspace, Shaken at 200 rpm, 20ºC
- Growth and biostimulation experiments
  - ***Native***: biostimulation with isobutane
  - ***Bioaugmented***: bioaugmentation with ATCC 21198 (grown as pure culture in batch) and given isobutane for growth/biostimulation
- 4 additions of isobutane and 1,4-dioxane
  - Every 2-3 days (short term experiments)
  - Every month (long term experiments)
  - Initial 1,4-dioxane concentration: 500 ppb

Short term microcosm experiments



- *Native microcosms*: biostimulation lag; decreased rates due to nutrient limitation in later additions
- *Bioaugmented microcosms*: immediate transformation; increased rates due to biomass growth in later additions

Error bars show standard error.

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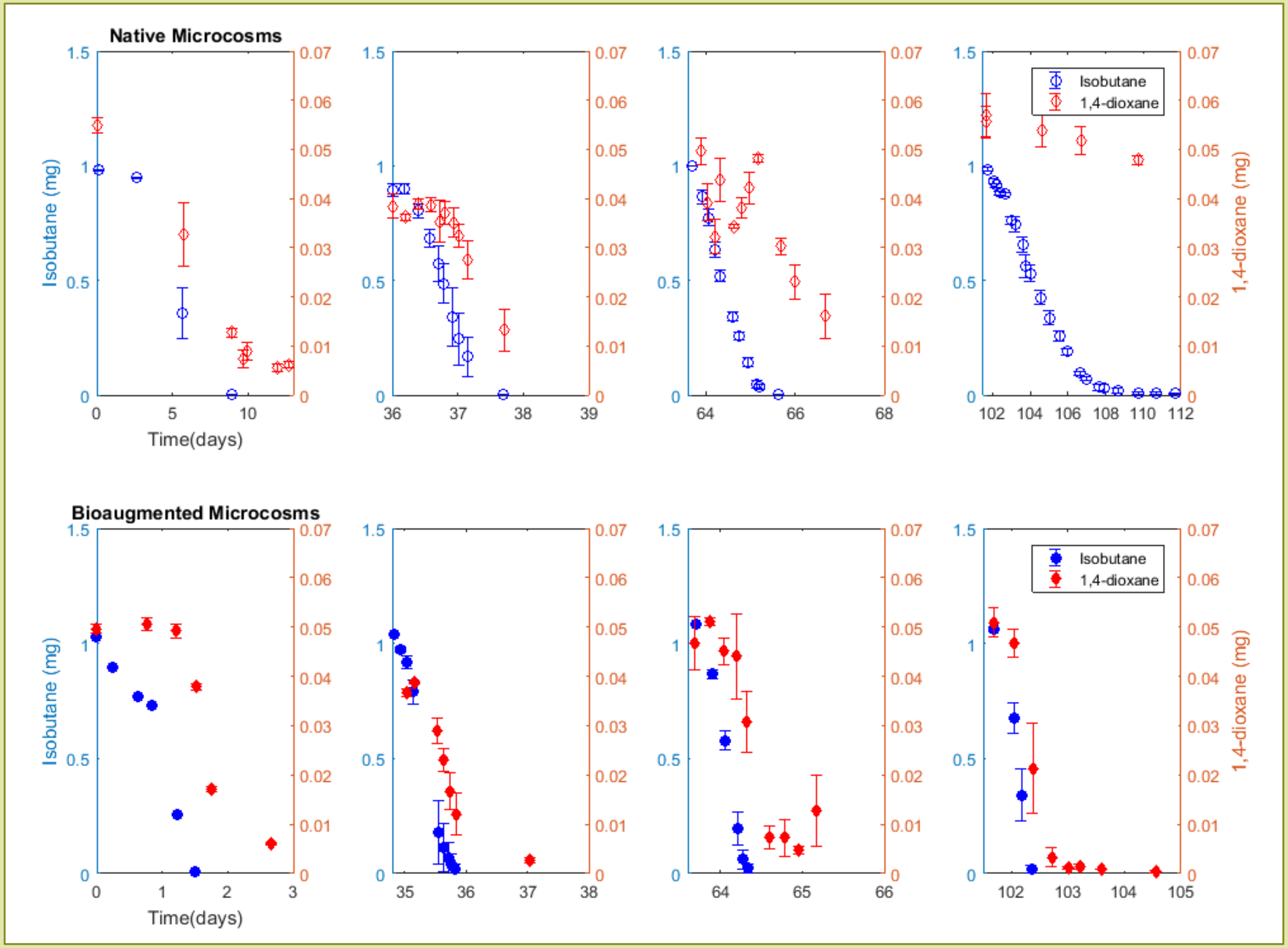
Long term microcosm experiments



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Can we model isobutane and 1,4-dioxane cometabolism in microcosms using Monod and Michaelis-Menton kinetics?

$$\frac{dS_G}{dt} = \frac{-K_{max,\,S_G} * S_G * X}{K_{s,\,S_G} + S_G}$$

$$\frac{dS_C}{dt} = \frac{-K_{max,S_C} * S_C * X}{K_{s,S_C} + S_C + \frac{K_{s,\,S_G} * S_G}{K_I}}$$

$$\frac{dX}{dt} = Y * \frac{dS_G}{dt} - bX = \frac{Y * K_{max,\,S_G} * S_G * X}{K_{s,\,S_G} + S_G} - bX$$

S_G—growth (primary) substrate

S_C—cometabolic substrate

X—biomass concentration

K_{max}—maximum rate of substrate utilization

K_s—half saturation constant

K_I—inhibition constant

Y—biomass yield from primary substrate consumption

b—first order decay rate, biomass

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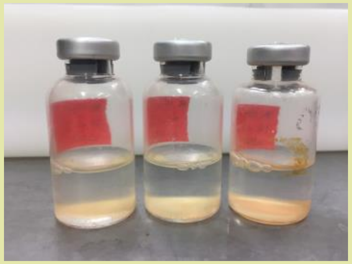
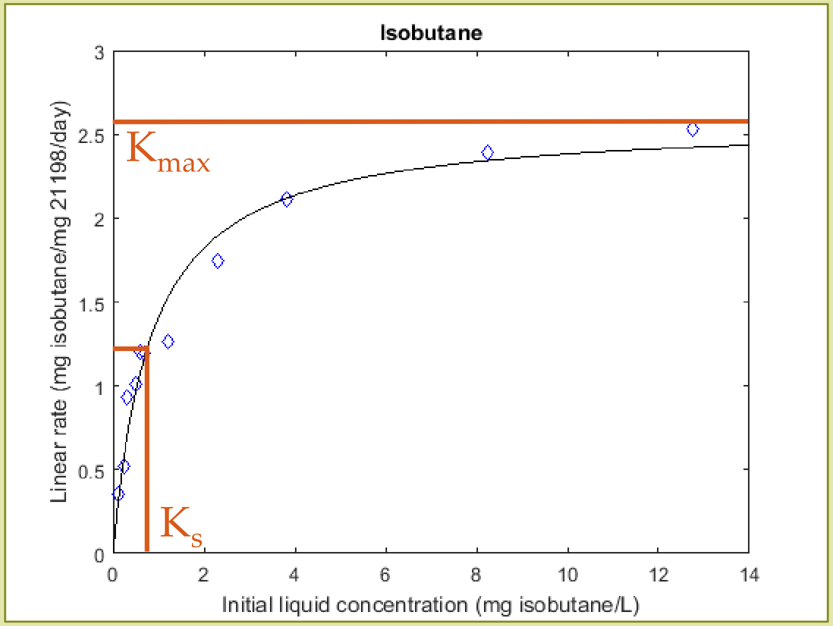
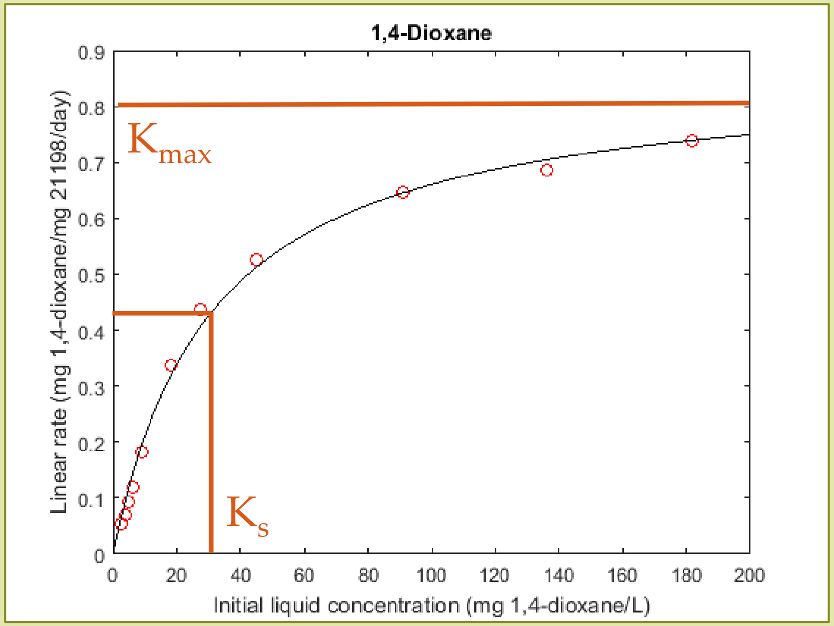
Parameter Determination: Monod Curves

- Rapid, pure culture resting cell tests
- Initial, linear rates determined for a range of isobutane and 1,4-dioxane concentrations

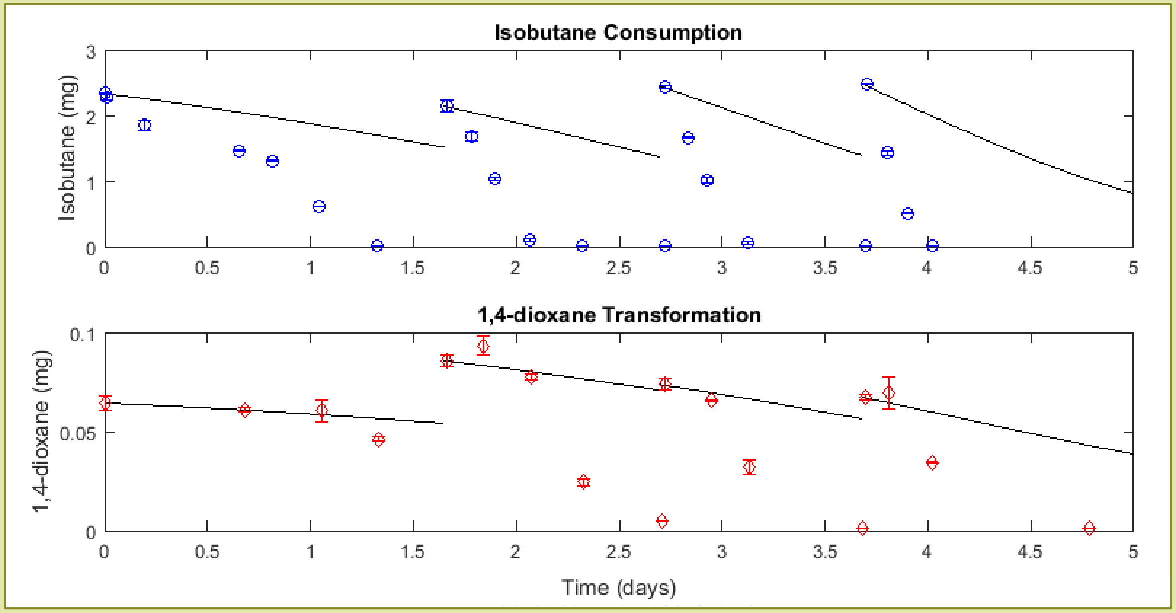






Modeling Microcosm Data: Bioaugmented, short term

Parameter	Estimated Value
K_{max}, isobutane (mg/[mg TSS*day])	2.57
K_s, isobutane (mg/L)	0.83
K_{max}, 1,4-dioxane (mg/[mg TSS*day])	0.87
K_s, 1,4-dioxane (mg/L)	31
K_I (mg/L)	3
Y (mg/mg)	0.8
b (1/day)	0.1



- Poor fit
- Model is too slow

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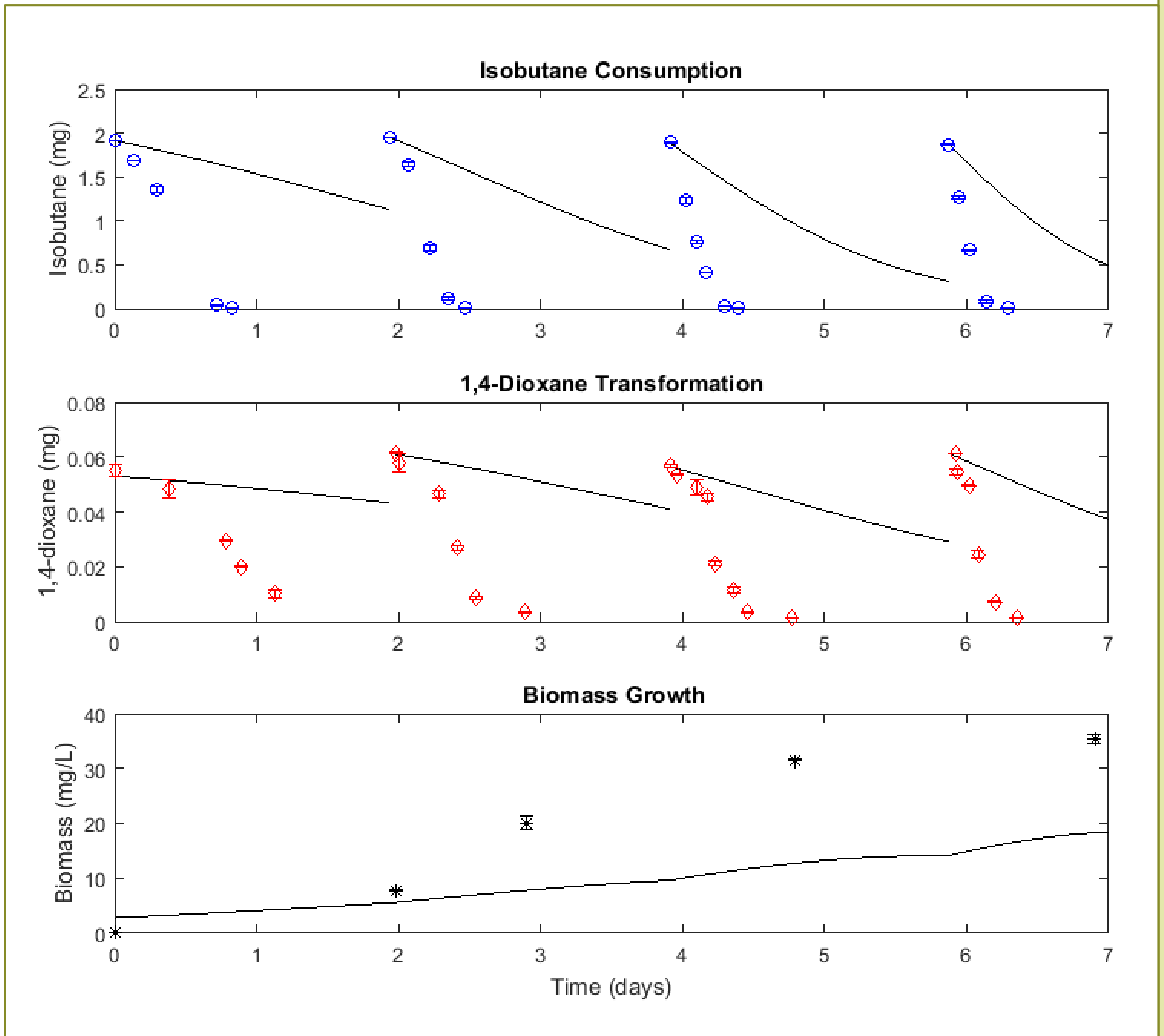
"Microcosm Scale", Pure Culture Growth Experiment



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K_s, isobutane (mg/L)	0.83
K_{max}, 1,4-dioxane (mg/[mg TSS*day])	0.87
K_s, 1,4-dioxane (mg/L)	31
K_I (L/mg)	3
Y (mg/mg)	0.8
b (1/day)	0.1
Xo (mg/L)	2.78

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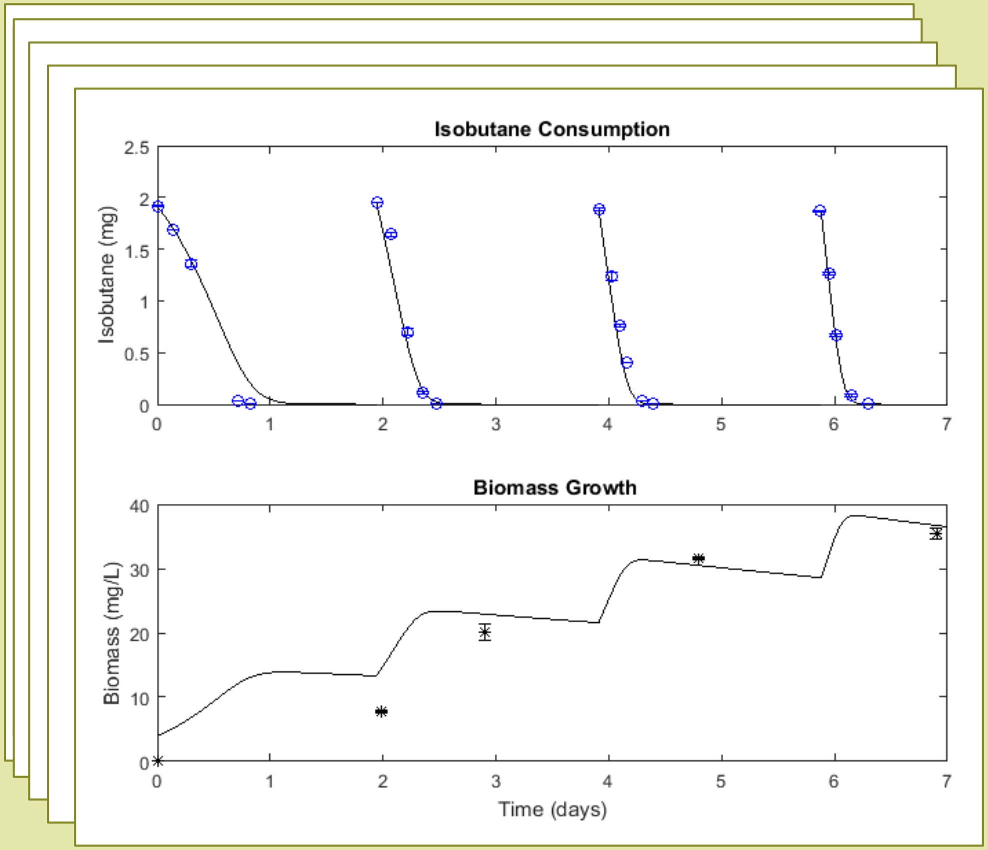
Fitting the model for isobutane (S_G) and biomass (X) data

$$\frac{dS_G}{dt} = \frac{-K_{max,S_G} * S_G * X}{K_{s,S_G} + S_G} \qquad \frac{dX}{dt} = \frac{Y * K_{max,S_G} * S_G * X}{K_{s,S_G} + S_G} - bX$$

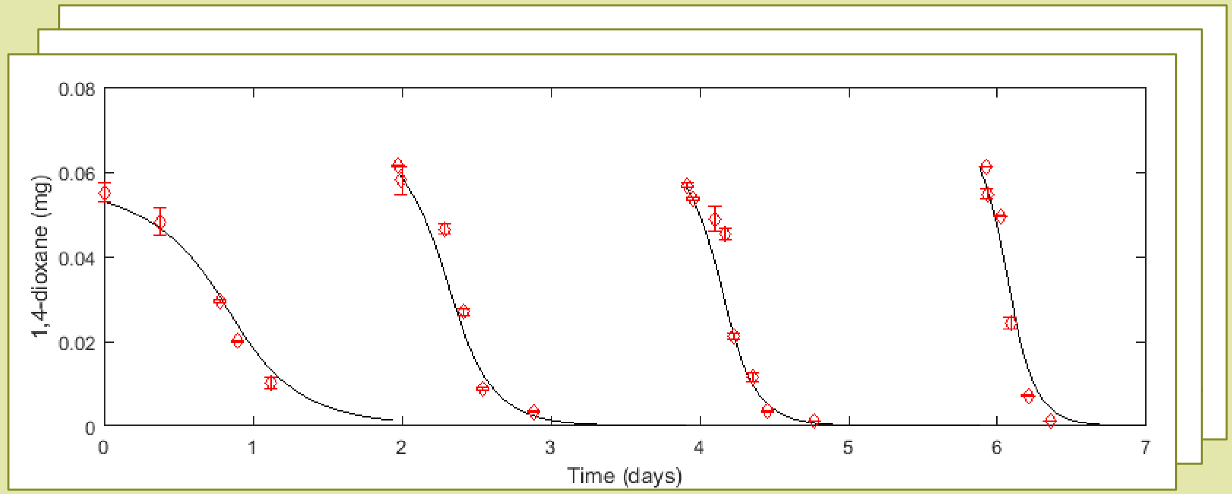


- Use K_{max} from Monod curve
- Determine K_s from low concentration isobutane rate tests: K_s:0.83→0.05
- Optimize yield and decay coefficient to fit biomass data: b: 0.1→0.06, Y: 0.8→1
- Increase K_s:0.05→0.1
- Increasing initial biomass does not have a long-term effect Xo:2.78→4.0

Fitting the data for 1,4-dioxane (S_C) degradation

$$\frac{dS_C}{dt} = \frac{-K_{max,S_C} * S_C * X}{K_{s,S_C} + S_C}$$

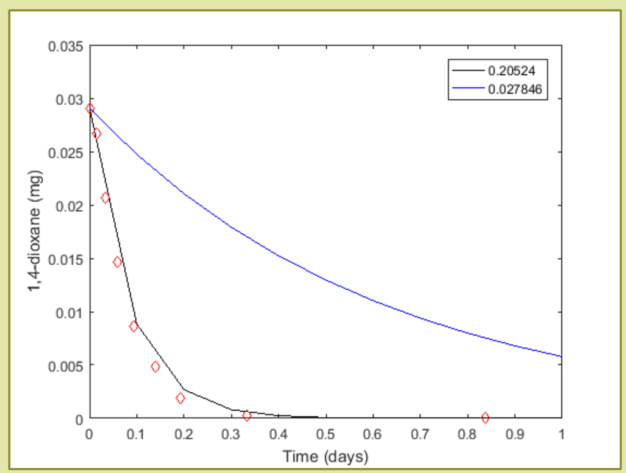
$$\frac{dS_C}{dt} = -K_{FO} * S_C * X$$



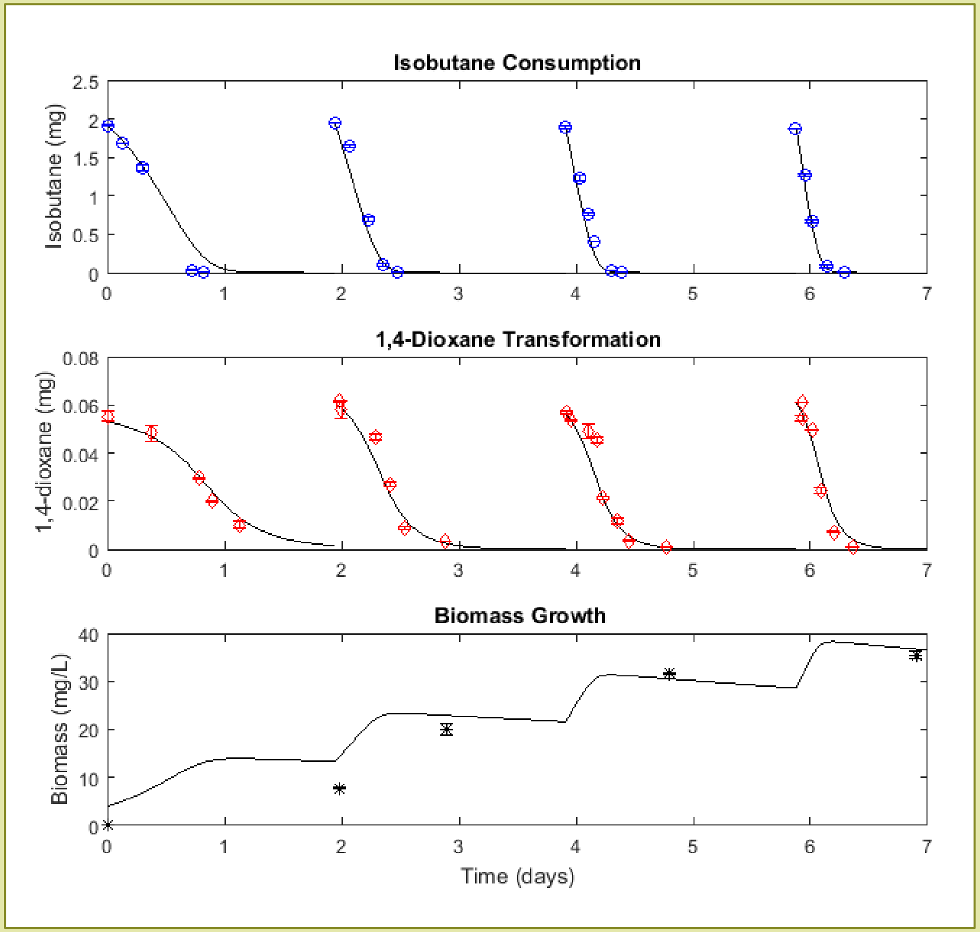
Inhibition

$$\frac{dS_C}{dt} = \frac{-K_{max,S_C} * S_C * X}{K_{s,S_C} + S_C + \frac{K_{s,S_C} * S_G}{K_I}}$$

$$\frac{dS_C}{dt} = \frac{-K_{FO} * S_C * X}{1 + K_I * S_G}$$



Updated Parameters: modeling pure culture growth and transformation



Error bars show standard error.

Parameter	Estimated Value	Fit Value
K_{max}, isobutane (mg/[mg TSS*day])	2.57	2.57
K_s, isobutane (mg/L)	0.83	0.1
K_{max}, 1,4-dioxane (mg/[mg TSS*day])	0.87	NA
K_s, 1,4-dioxane (mg/L)	31	NA
K_{FO}, 1,4-dioxane (L/[mg*day])	NA	0.20
K_I (L/mg)	3	10.5
Y (mg/mg)	0.8	1
b (1/day)	0.1	0.06
Xo (mg/L)	2.78	4.0

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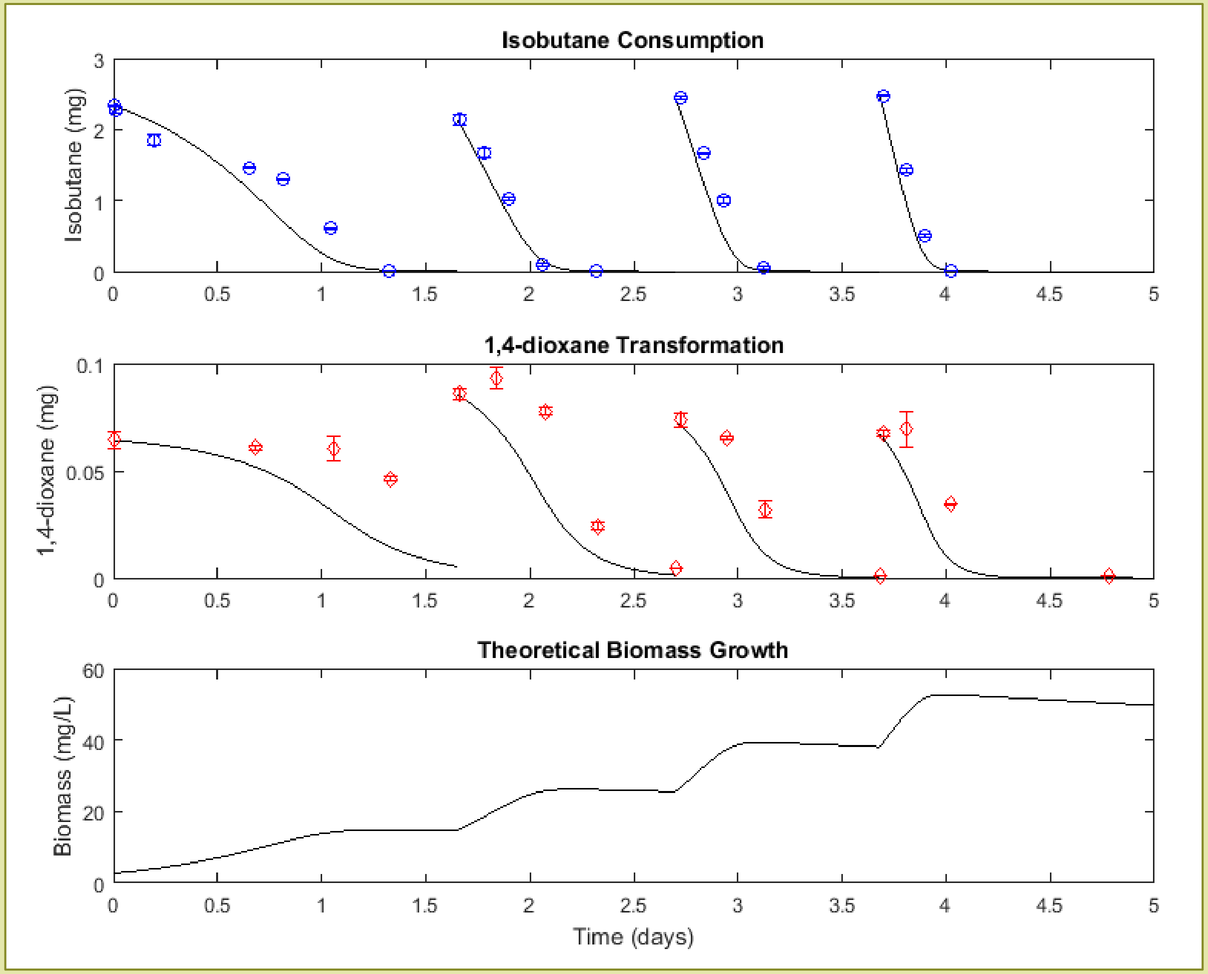
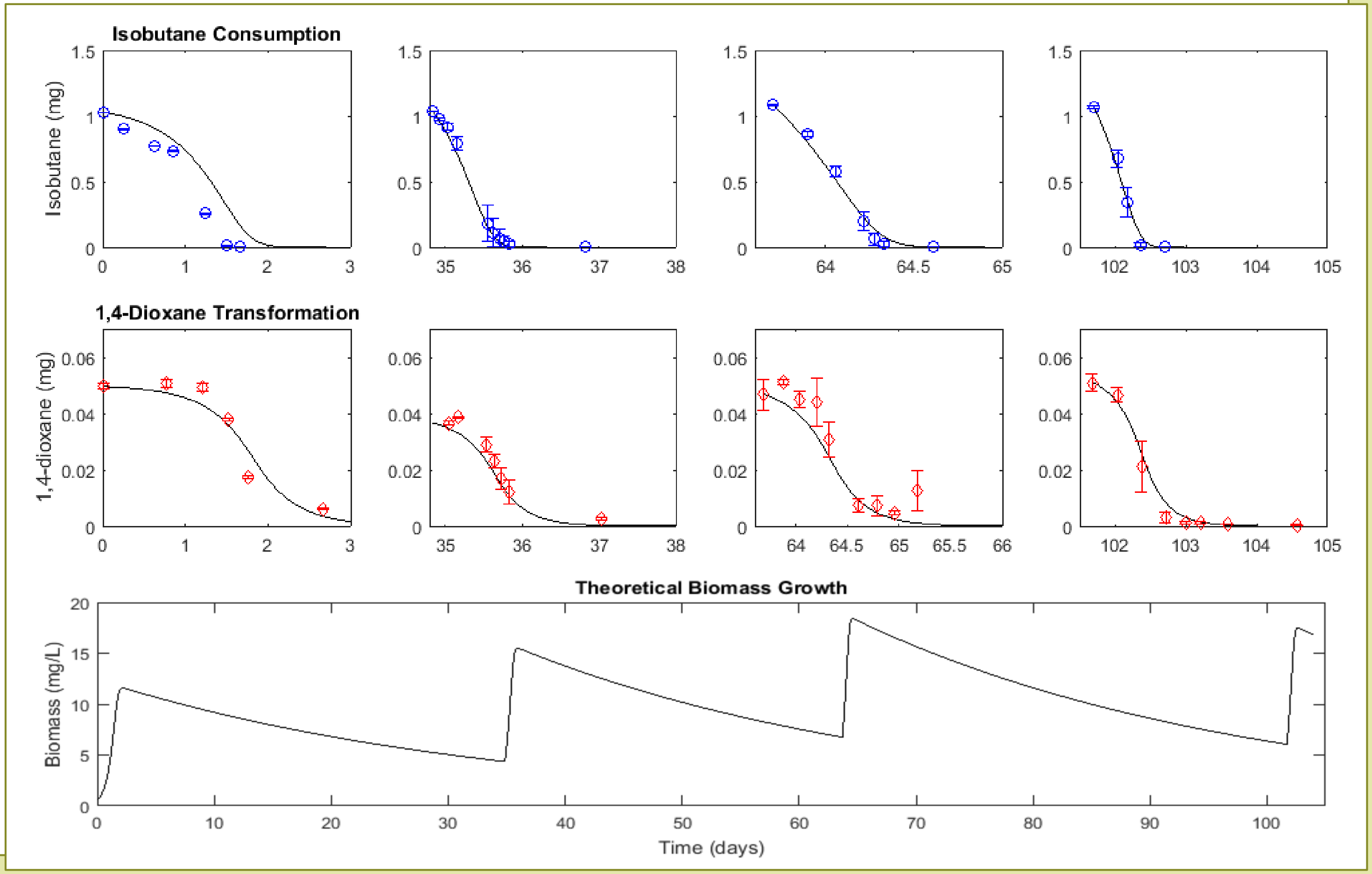
Modeling microcosm data with updated parameters



- Bioaugmented
- Short term

Error bars show standard error.

Modeling the long term microcosm experiment



Error bars show standard error.

- Bioaugmented
- Reduced b by 50%: 0.06→0.03 day^{-1}

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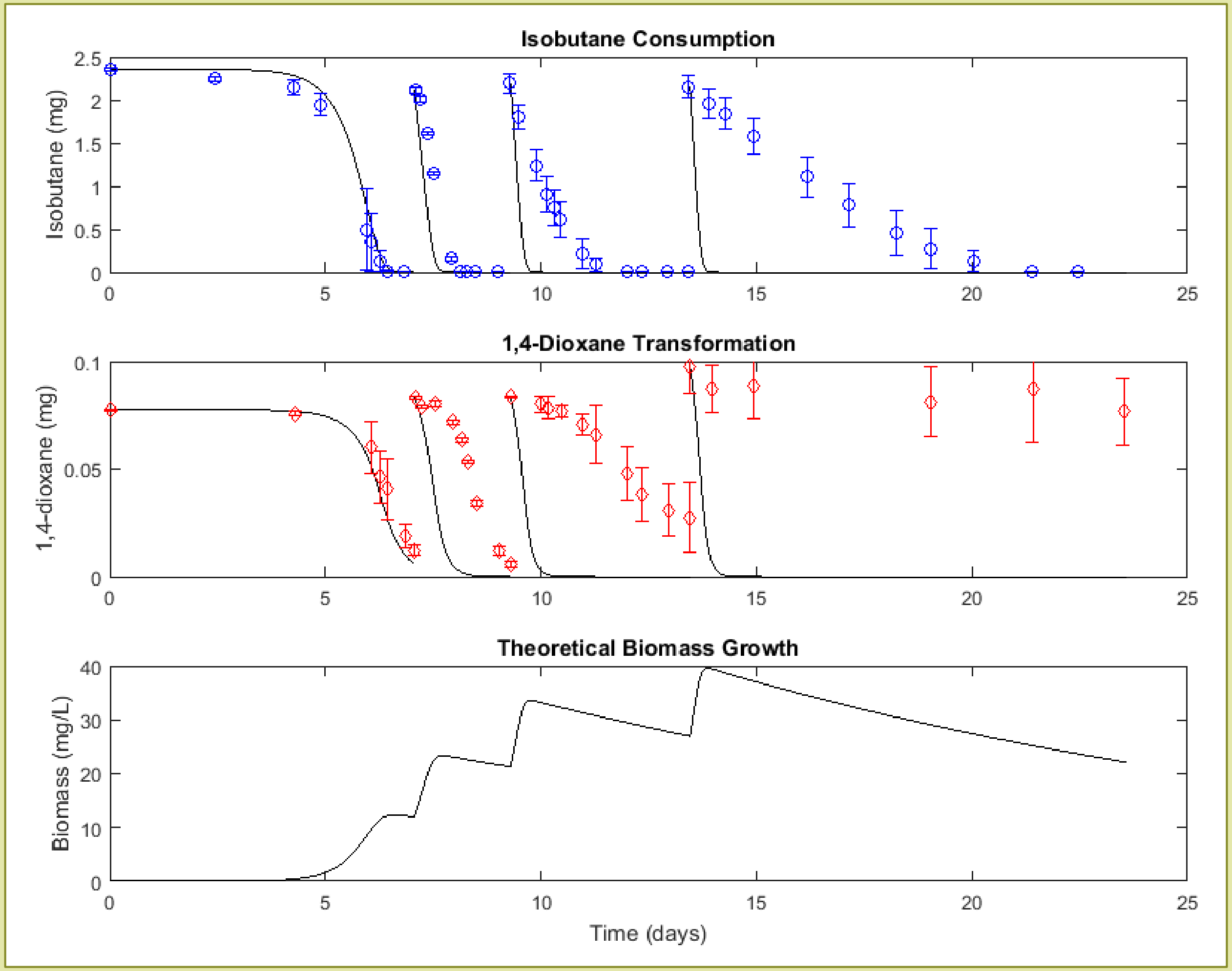
Modeling microcosm data with updated parameters



Error bars show standard error.

- Native
- Short term
- Reduced initial biomass concentration to 0.0001 mg/L to fit biostimulation lag

- Nutrient limitation needs to be incorporated into the model

Summary

- Isobutane is an effective primary substrate to stimulate cometabolic transformation of 1,4-dioxane in Fort Carson aquifer solids
- Bioaugmentation with ATCC 21198 results in sustained transformation of 1,4-dioxane in microcosms over 100 days
- Estimation of model kinetic parameters should reflect environmentally relevant concentrations
- Simplified Monod/Michaelis-Menten model fits short- and long-term experiments
- Nutrients are an important limiting condition for cometabolism

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Questions?

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