

An unintended consequence of biostimulation for treatment of chlorinated solvents:

Biologically mediated toluene production

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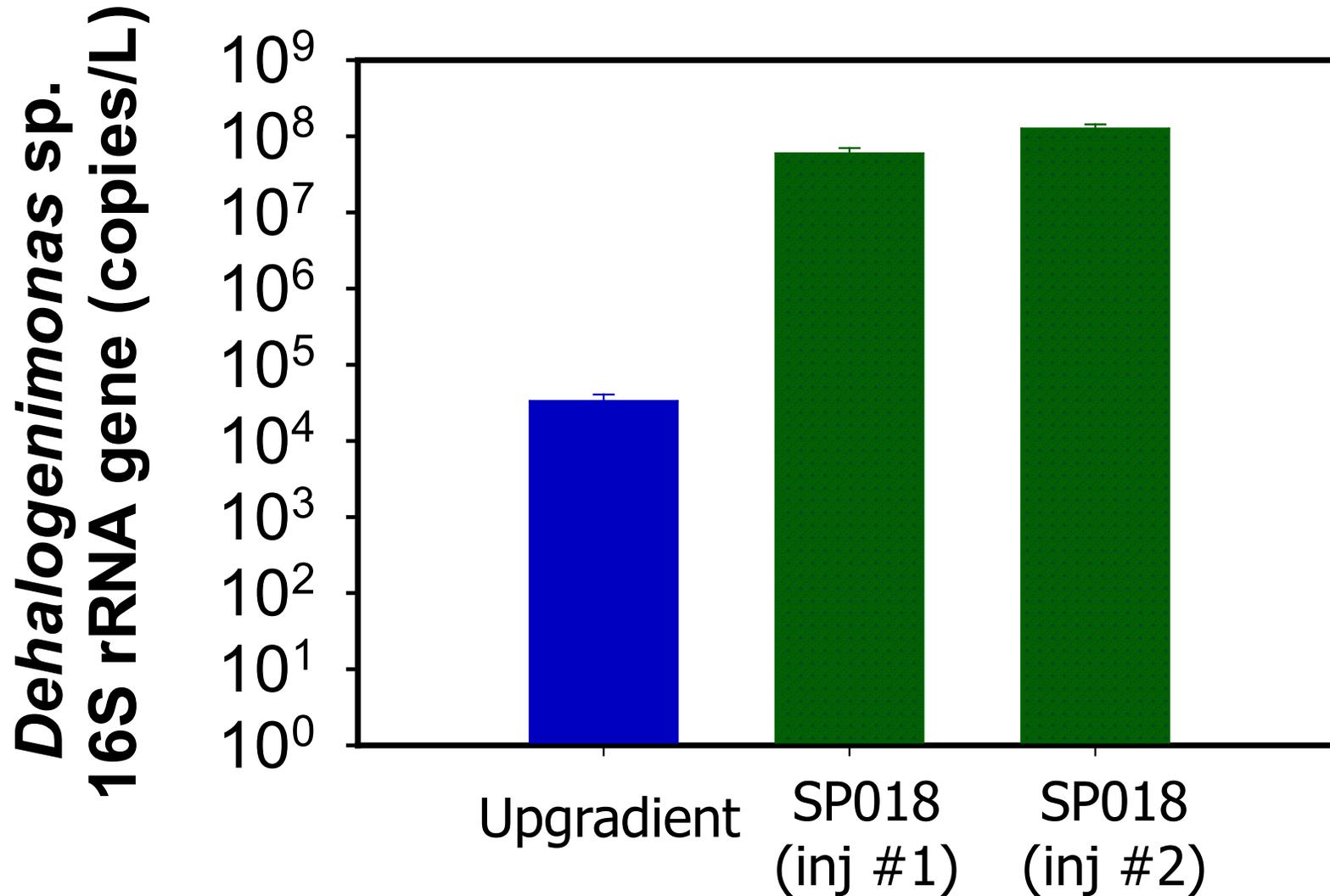
Overview

- Site background and remediation approach
- Evidence for biogenic toluene production
 - Field data
 - Laboratory enrichment cultures
 - Precursors
- Conclusions and implications

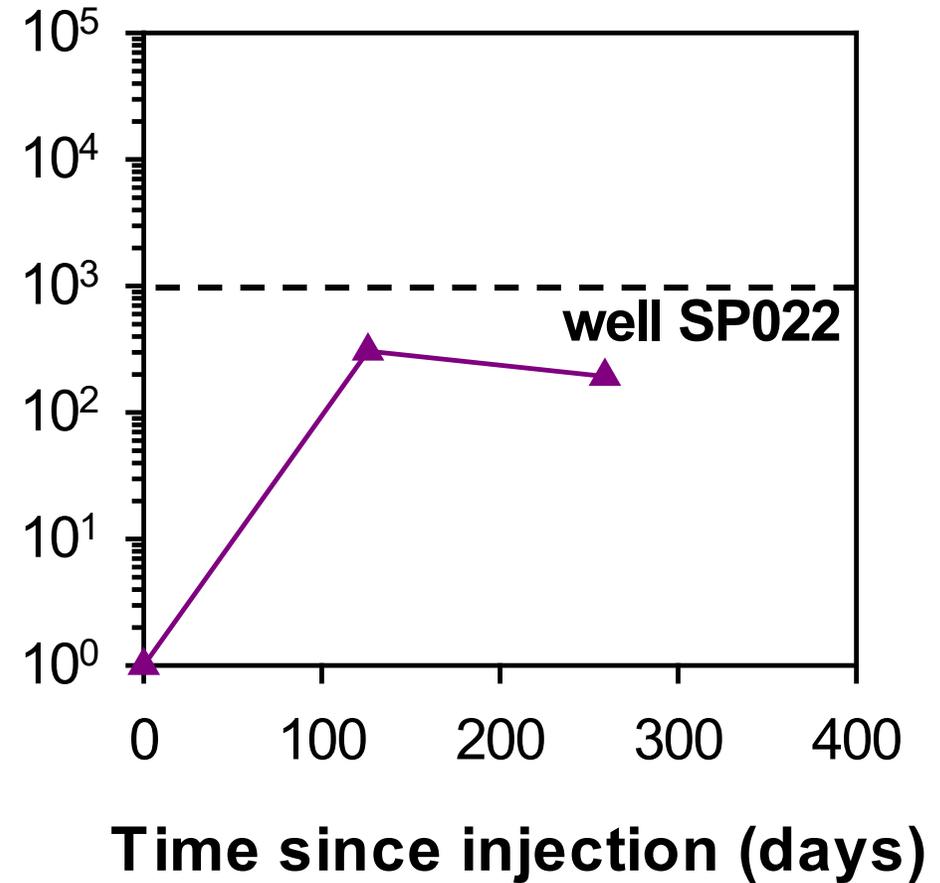
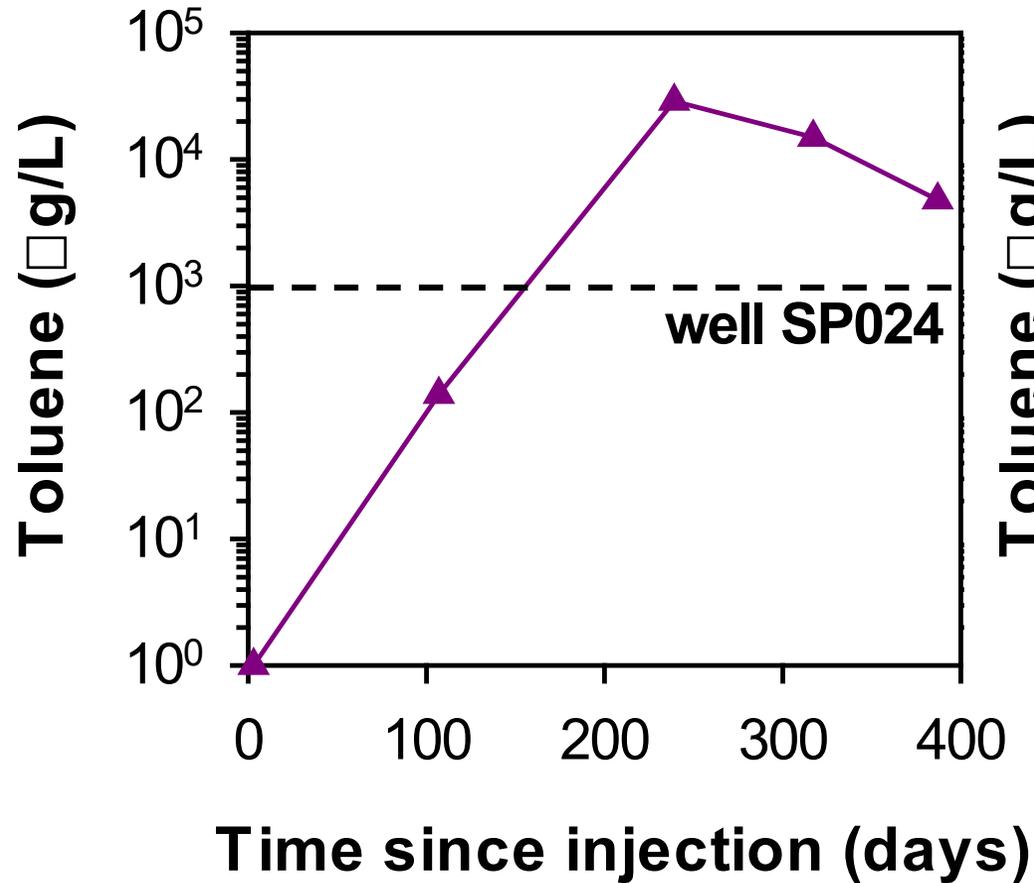
Site history and remedial approach

- DNAPL-impacted site in southeast Louisiana. Migrating plume
- Contaminants: Chlorinate alkanes (1,2-DCA, 1,2-DCP, 1,1,2-TCA)
Chlorinated alkenes (PCE, TCE, and vinyl chloride)
- Microbial characterization: *Dehalogenimonas* and *Dehalococcoides* present
- Enhanced attenuation (biostimulation)
 - Agricultural feed grade cane molasses
 - In some injections, also bicarbonate

Post-injection qPCR results



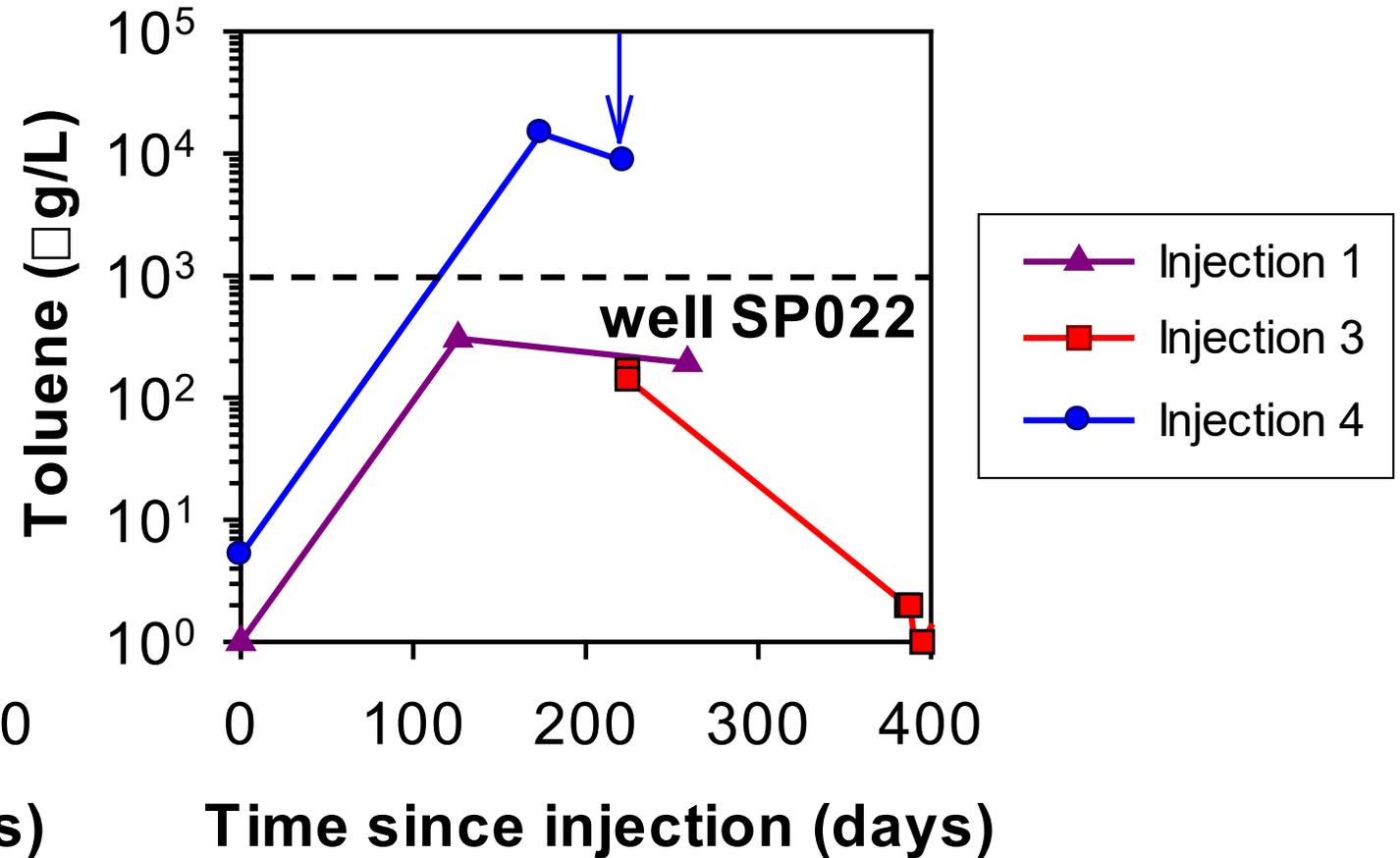
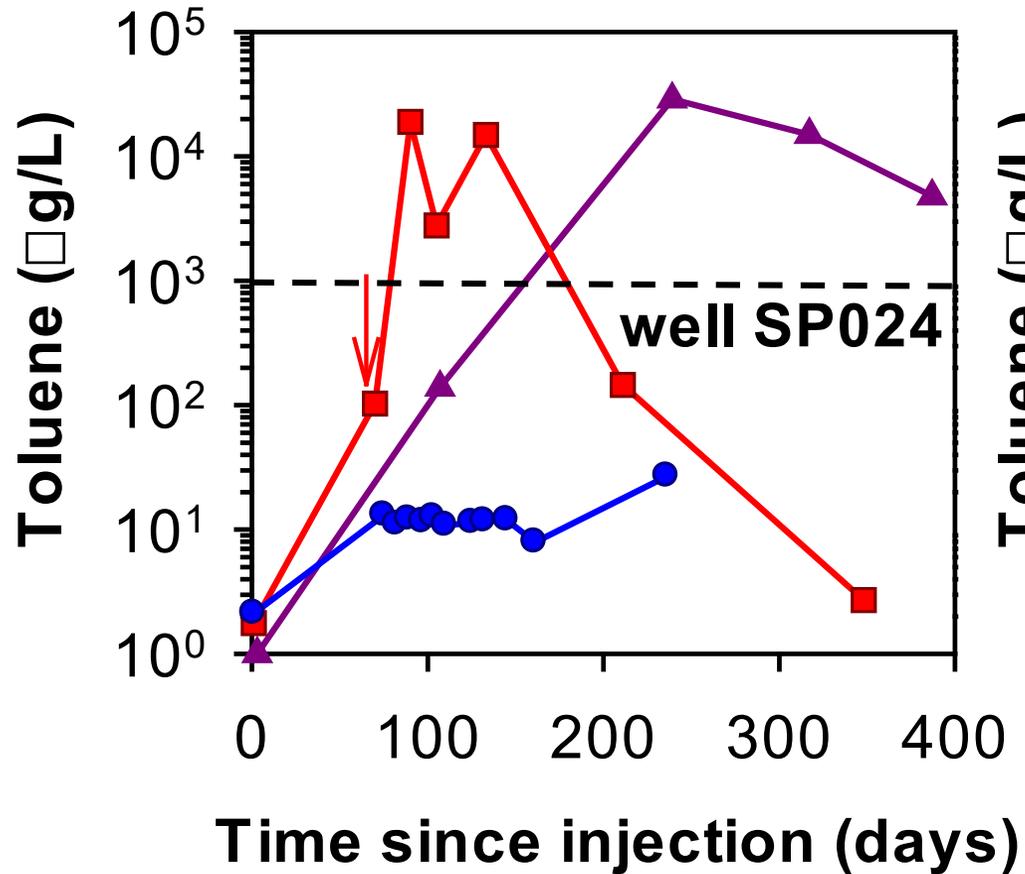
Toluene observations: Field site



Search for the origin

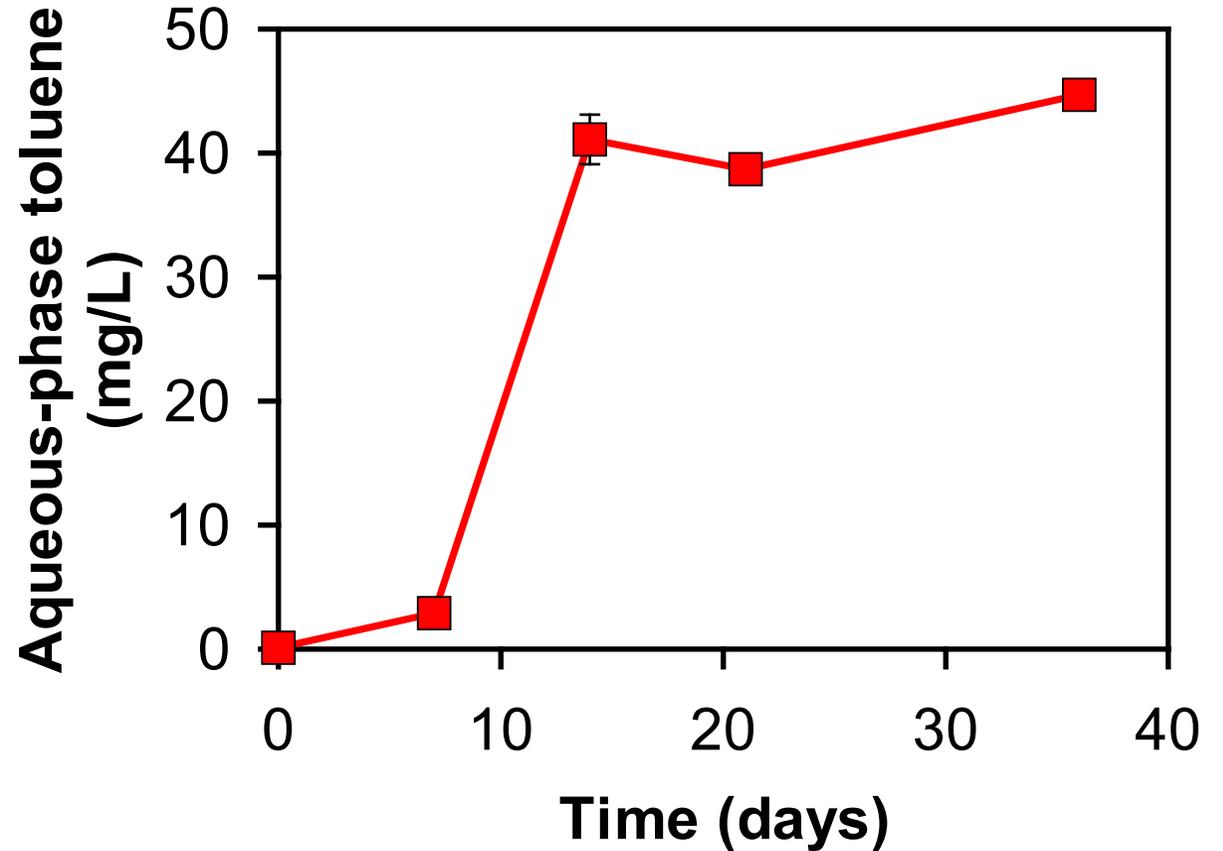
- Benzene (B) and ethylbenzene (E) consistently below detection
- No anthropogenic aromatic hydrocarbon source identified
- A plausible biological source?
 - *Clostridium aerofotidum* (Pons *et al.*, 1984)
 - *Tolomonas auensis* (Fischer-Romero *et al.*, 1996)
 - Sewage sludge and lake sediment enrichment cultures (Mrowiec *et al.*, 2005; Zargar *et al.*, 2016)

Field Site: More substrate injections, more toluene observations

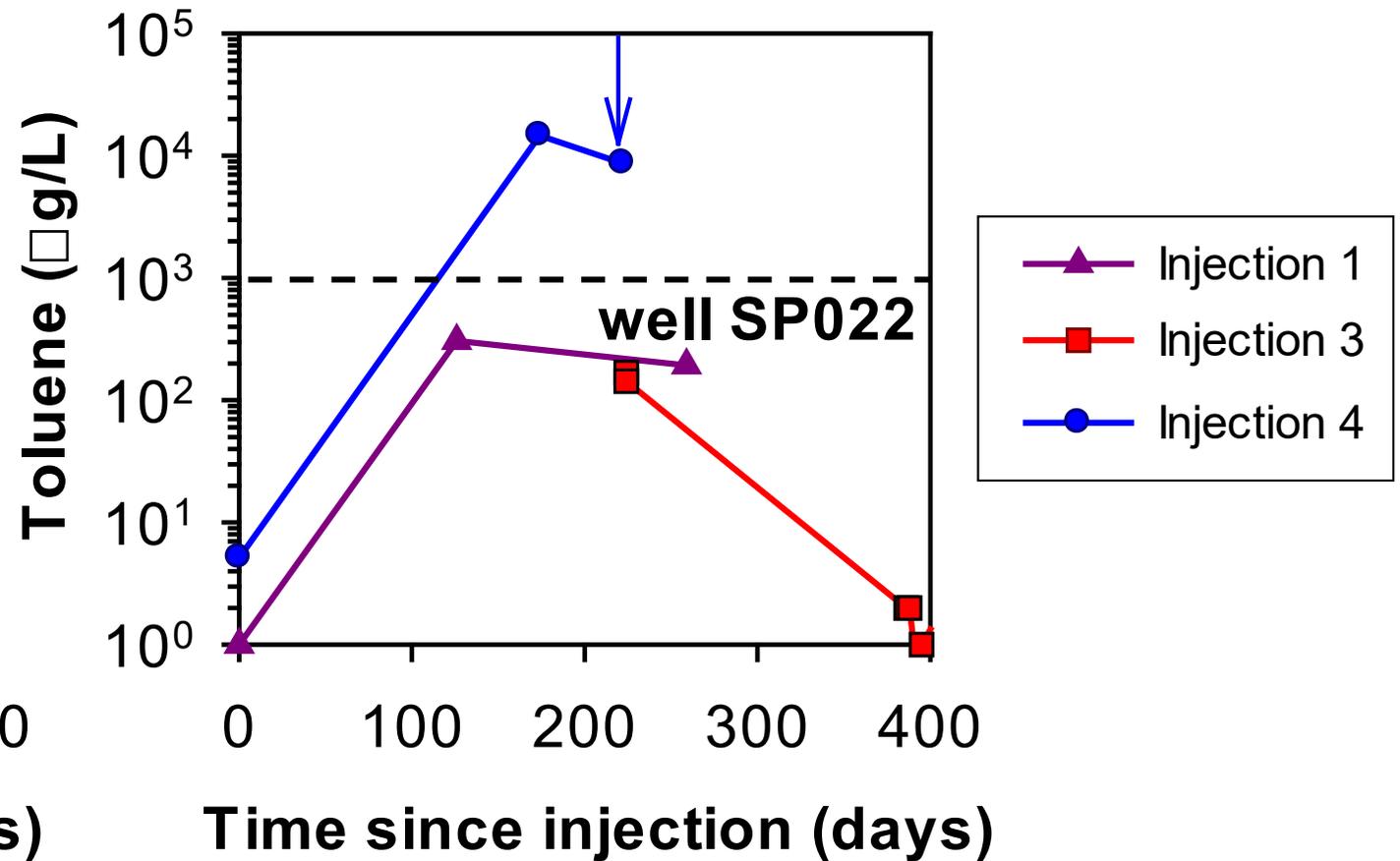
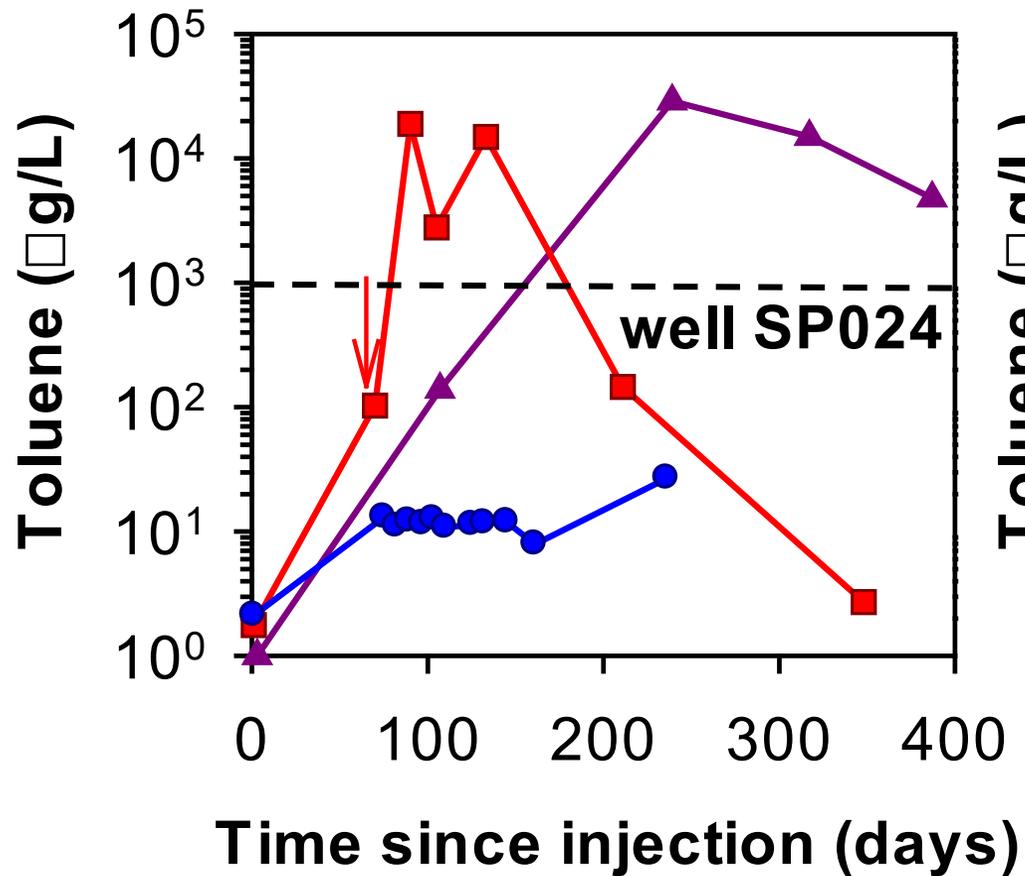


Laboratory study: Part 1 (AG) (microcosms)

- Groundwater collected in sterile glass bottles
- Aliquots aseptically transferred to glass serum bottles sealed with butyl rubber stoppers and aluminum crimp caps
- Anaerobic
- Ambient laboratory temp

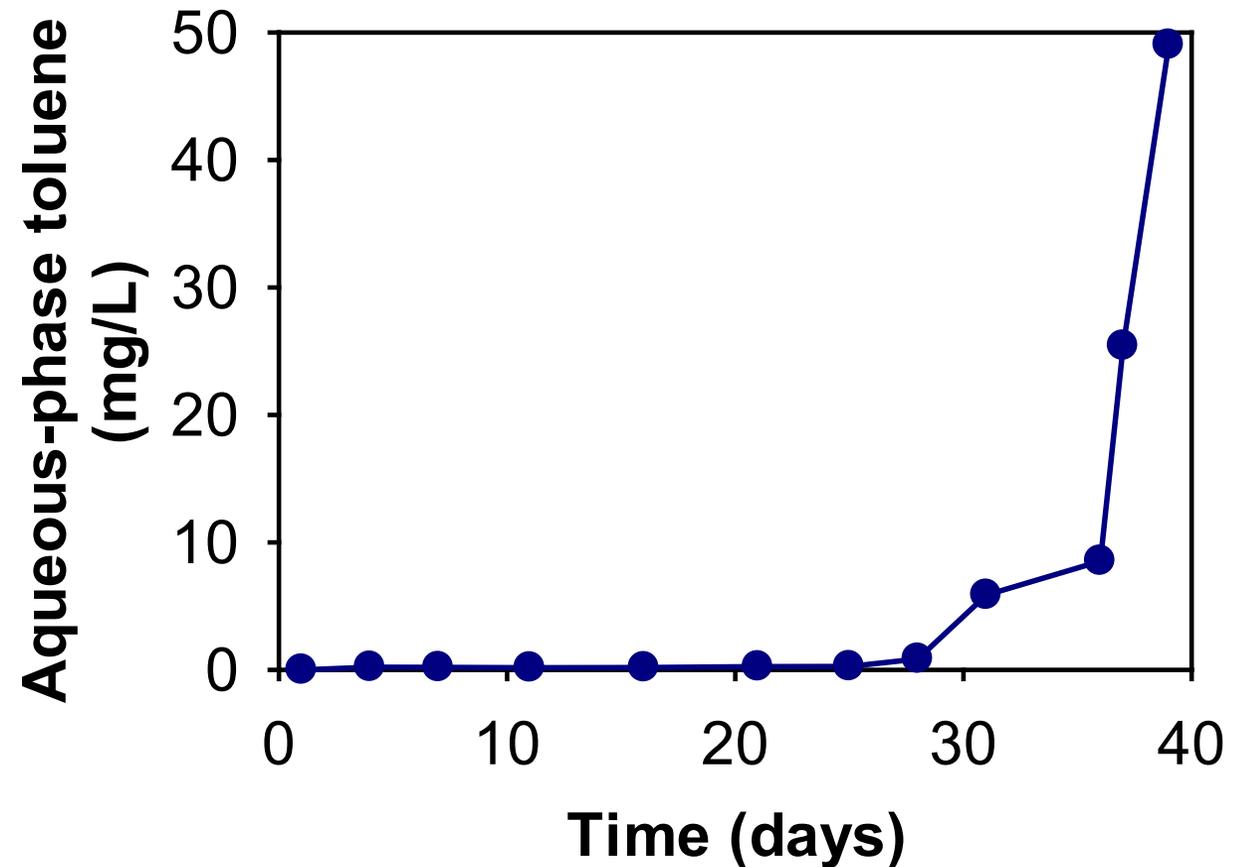


Field Site: More substrate injections, more toluene observations

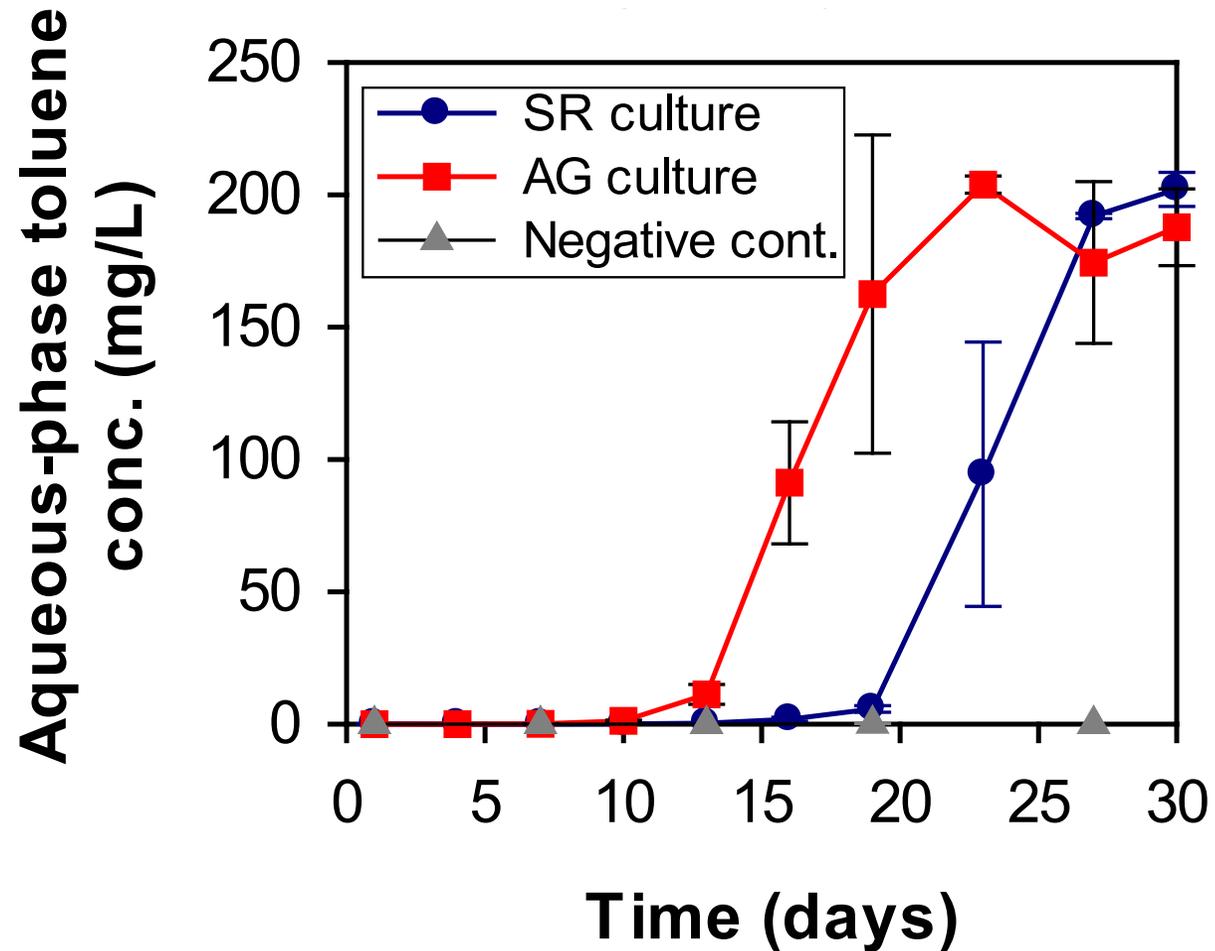


Laboratory study: Part 2 (SR) (enrichment cultures)

- Groundwater collected in sterile glass bottles
- Inoculation into anoxic phenylacetic acid-containing growth medium with glucose
- Anaerobic gas headspace
- Incubation at ambient laboratory temperature

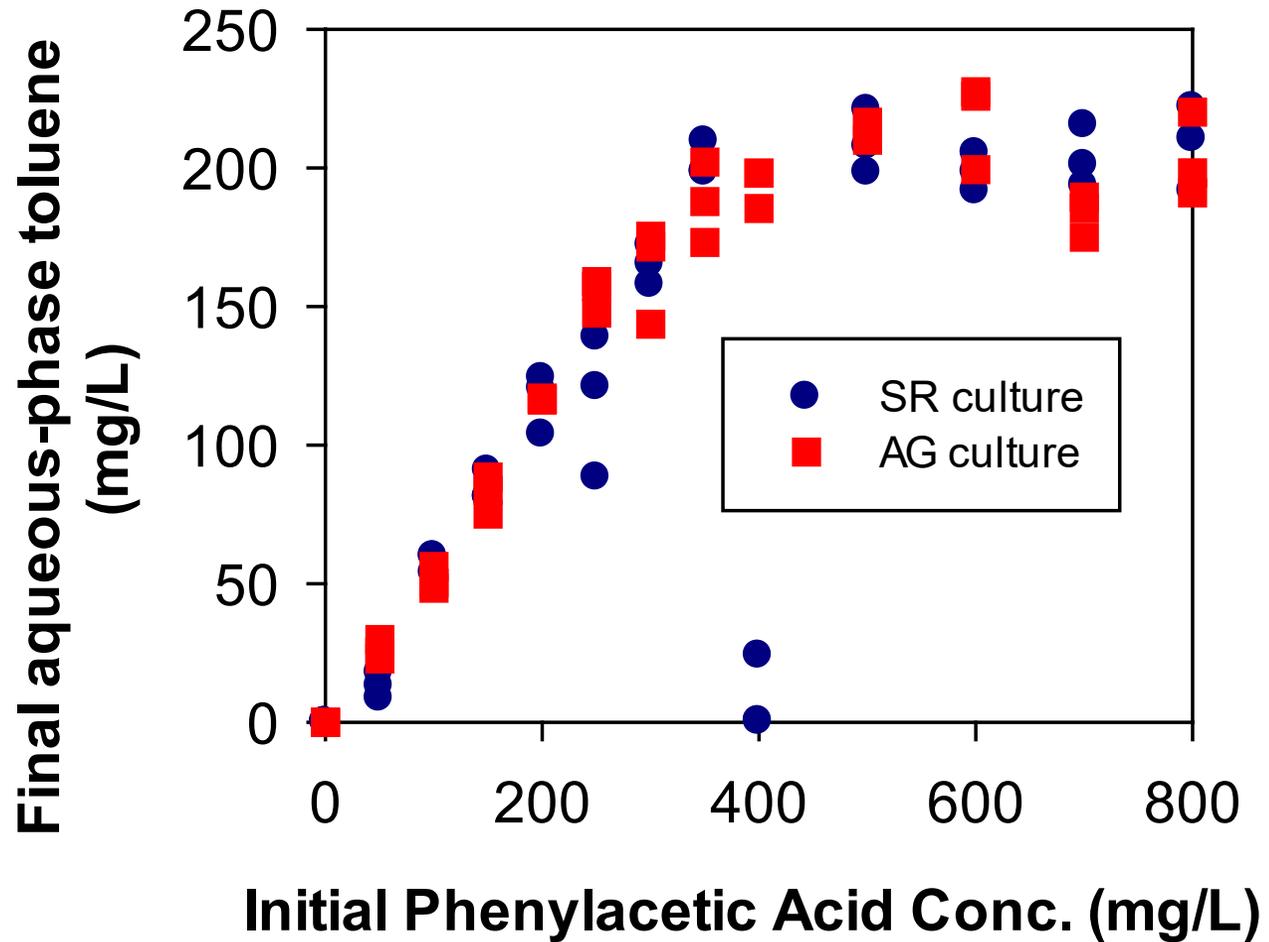


Functionally stable enrichment cultures

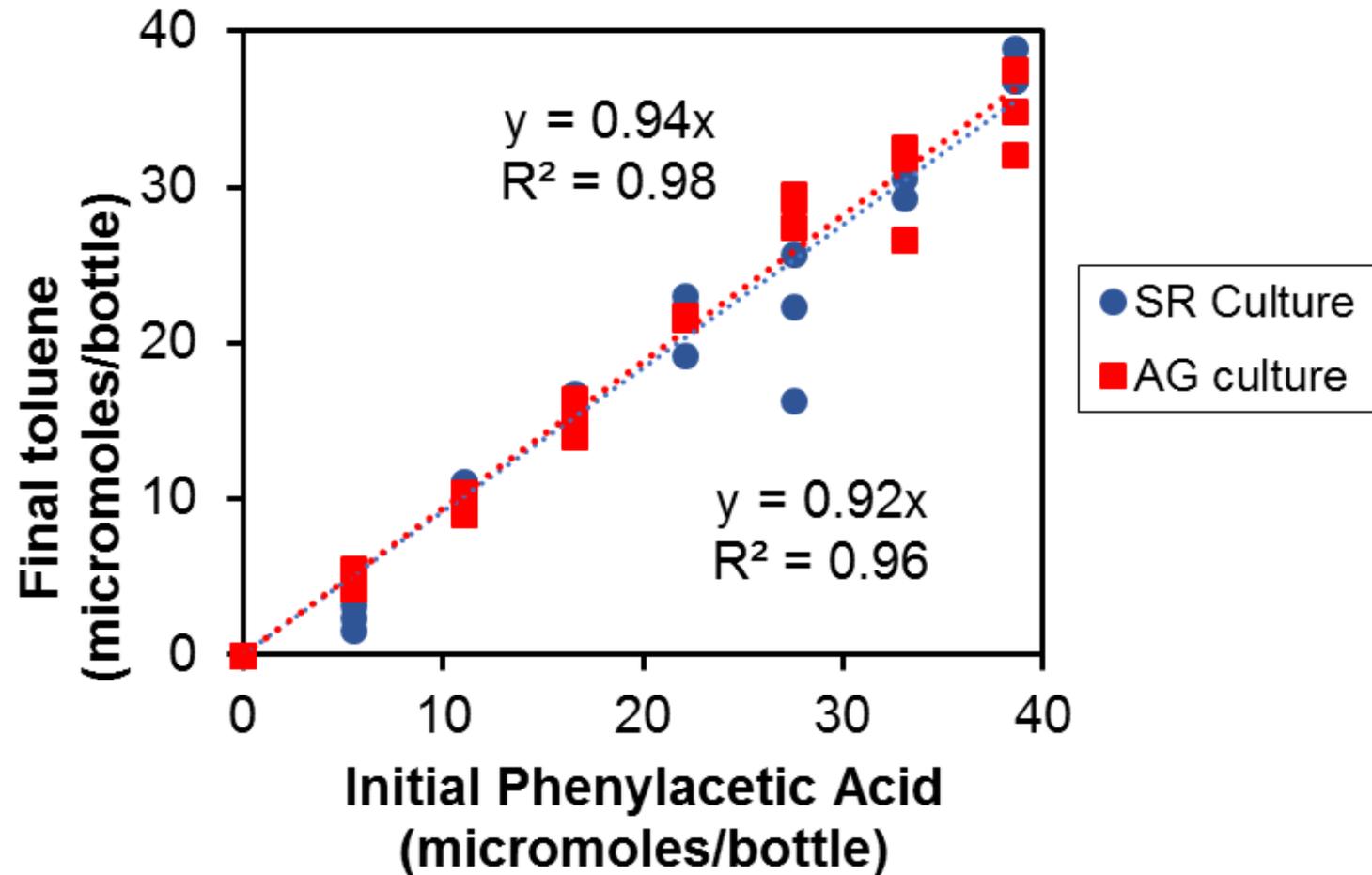


Propagation in defined medium with glucose (1 g/L) and phenylacetic acid (350 mg/L)

Effect of varying phenylacetic acid

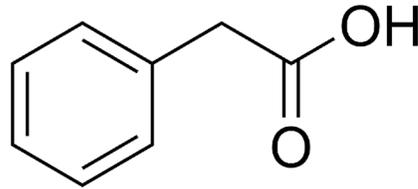


Toluene accumulation highly correlated w/ phenylacetic acid supplied (when ≤ 350 mg/L)

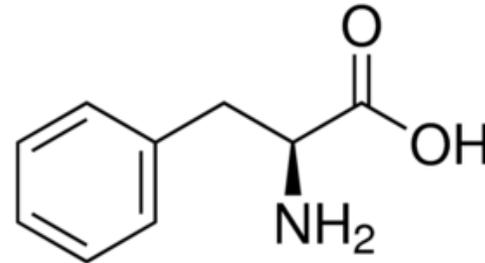


Multiple precursors

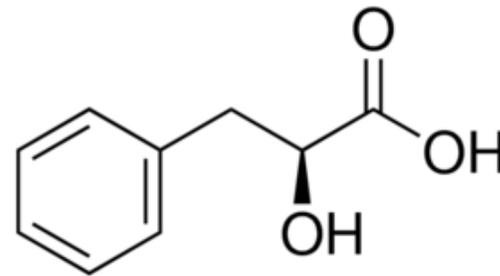
Phenylacetic acid



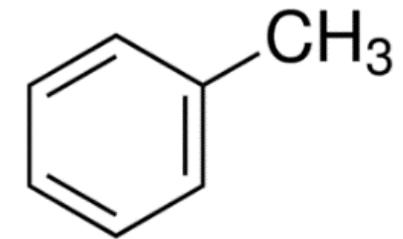
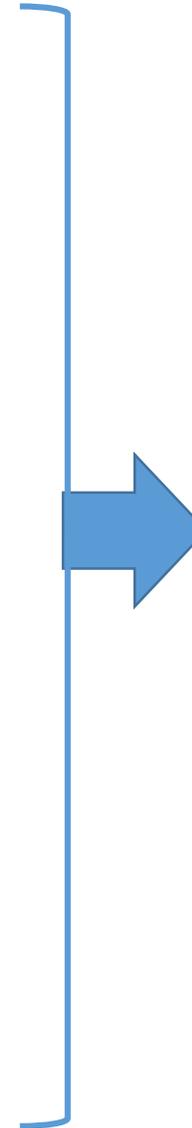
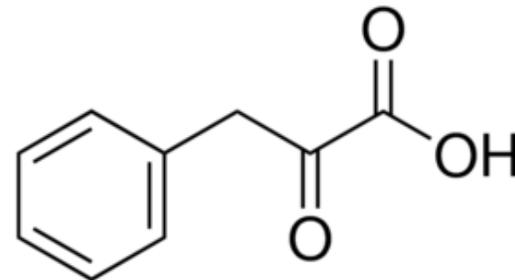
Phenylalanine



Phenyllactic acid



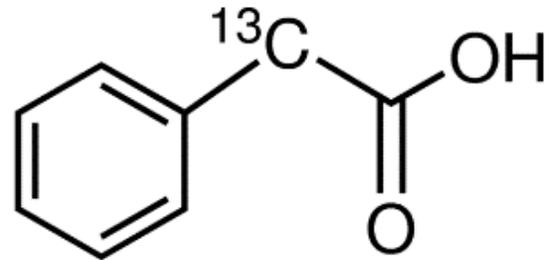
Phenylpyruvic acid



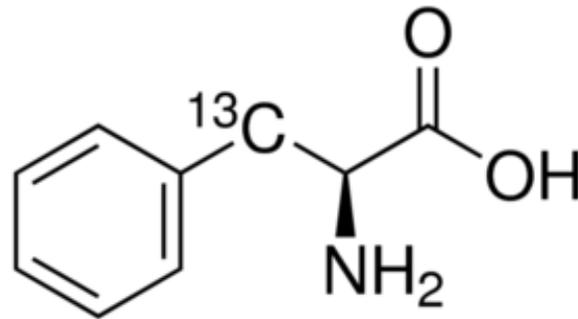
Toluene

¹³C precursor studies confirm biotransformation

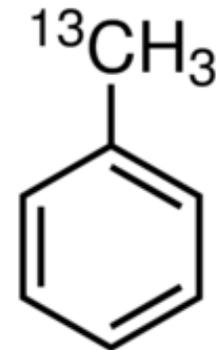
Phenylacetic acid-2-¹³C



L-Phenylalanine-3-¹³C



Toluene-α-¹³C



Aromatic precursor concentrations in molasses

- Agricultural feed grade molasses from two bulk distributors was assayed
- Potential precursors in molasses itself could not account for the tens of mg/L toluene observed at the field site
- Endogenous decay or fermentation products?

Compound	A (mg/g)	B (mg/g)
Phenylalanine (total)	0.23	0.20
Phenylacetic acid	0.012	0.035
L-(-)-3-Phenyllactic acid	<0.01	<0.01
Phenylpyruvic acid	0.35	0.27

Conclusions / Implications

- Toluene concentrations as high as 56 mg/L were observed following field-scale biostimulation
- Microbial populations indigenous to the site were shown to produce toluene from a variety of precursor molecules
- Further research is needed to identify the causative microorganisms and site conditions leading to *in-situ* toluene production
- Monitoring aromatic hydrocarbons during chlorinated solvent bioremediation is warranted

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