Assessing Microbial Activity in the Laboratory and the Field: Lessons from the USGS Norman Landfill

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Sulfate Reducing Bacteria in the Terrestrial Subsurface: A Duel Edged Sword

Negative Activities

- H_2S Production
- Reservoir Souring
- Corrosion of Metals
- Methylmercury Formation
- Reduction in Hydrocarbon Quality

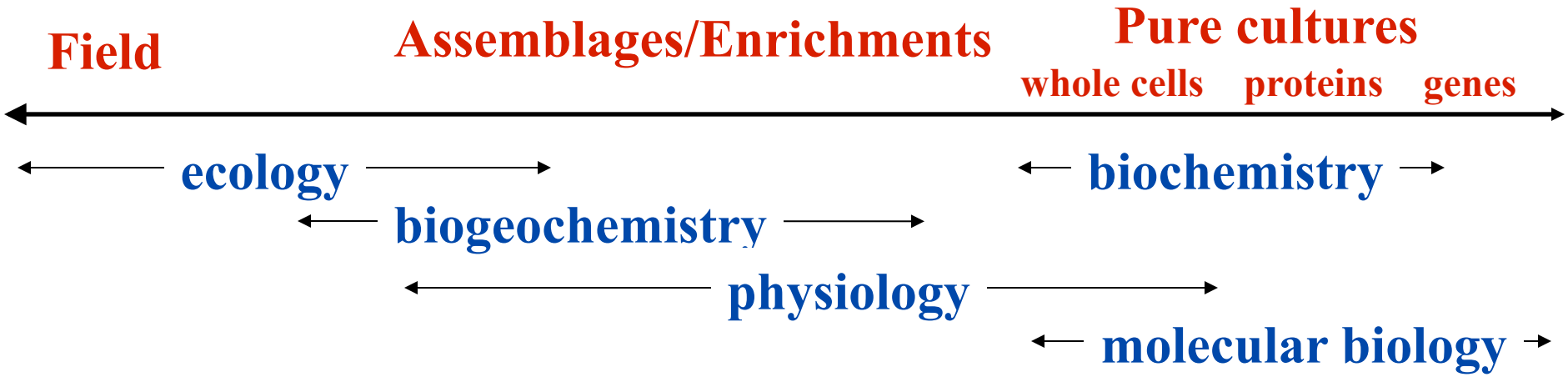
Positive Activities

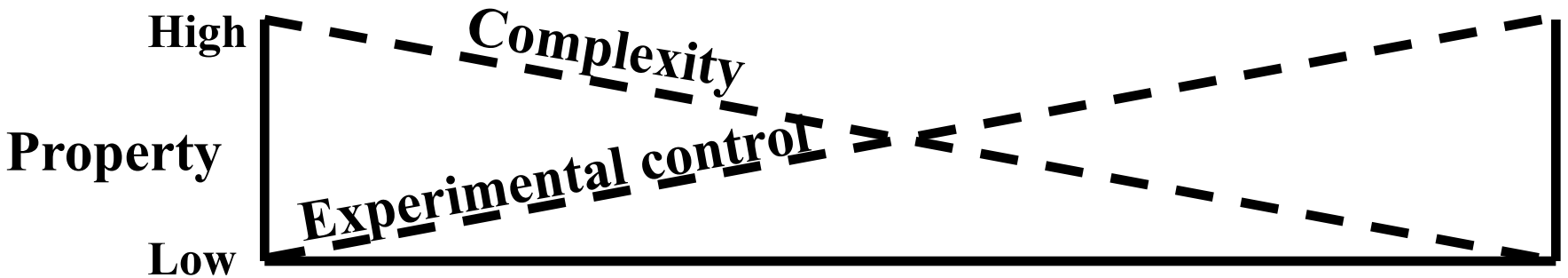
- Contaminant Bioremediation
  - Intrinsic
  - Engineered
- Immobilization of Metals and Radionuclides

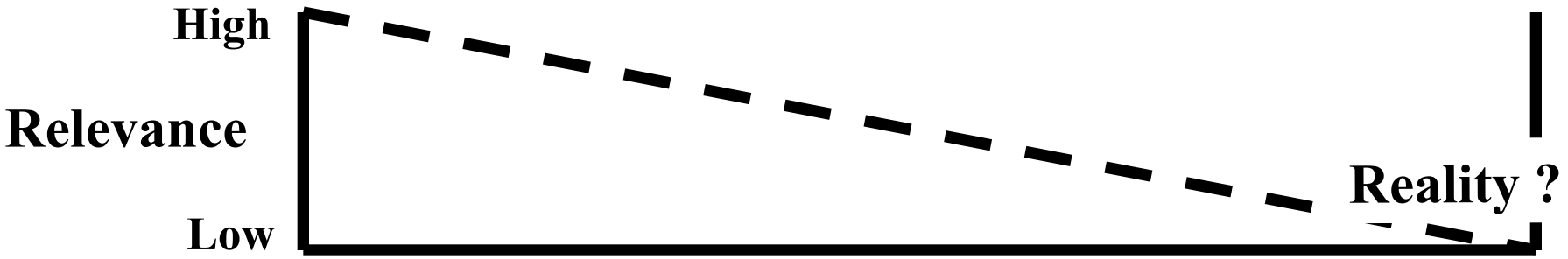
Approaches for the Control of Sulfate Reduction

- Broad-Spectrum Biocides
- Specific Inhibitors of Sulfate Reduction
- Use of Corrosion Resistant Alloys
- **Methods Based on Microbial Ecology**
  - A) Use of Nitrate/Nitrite
  - B) Factors Influencing Metabolic Activity

APPROACHES TO ASSESSING MICROBIAL ACTIVITIES







Adapted from Madsen, E. *Environ. Sci. Technol., 1998, 32 (4), 429-439*

So How To Determine *in situ* Microbial Activities??

Develop Lines of Evidence:

multiple convergent independent

FIELD
distinguish abiotic/biotic

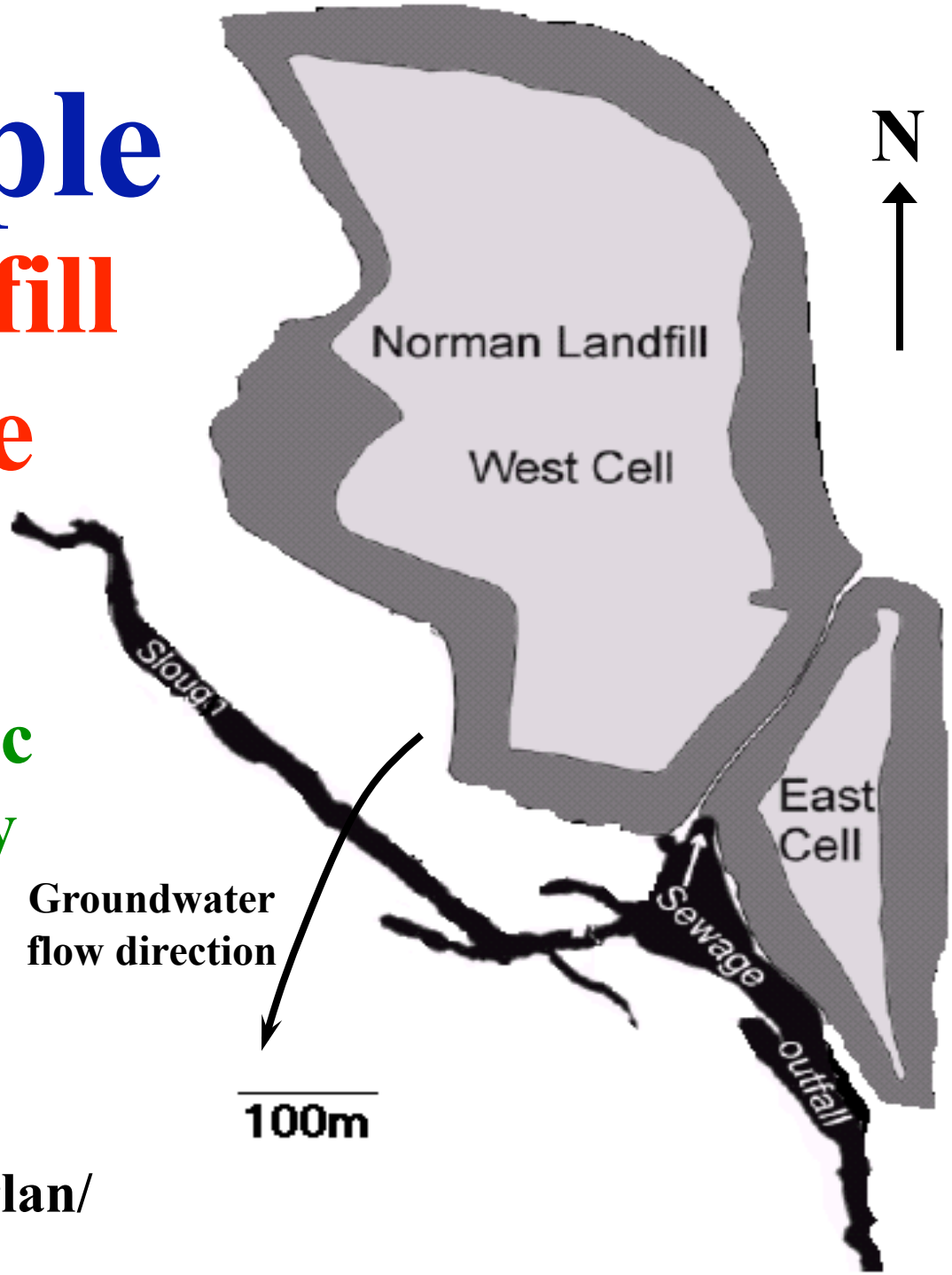
+

LABORATORY
ID controlling factors

Extrapolate Information to Other Locations

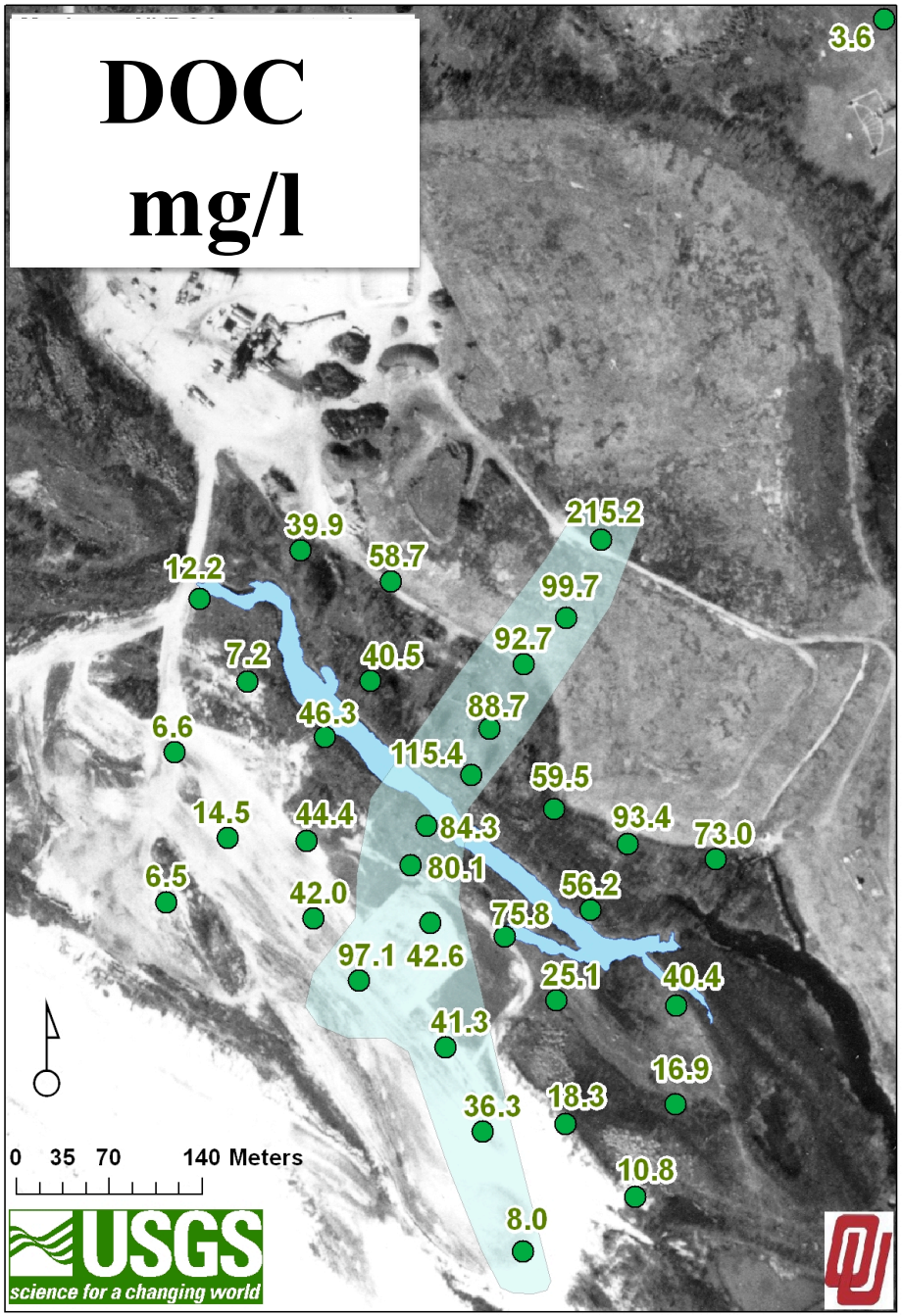
For Example

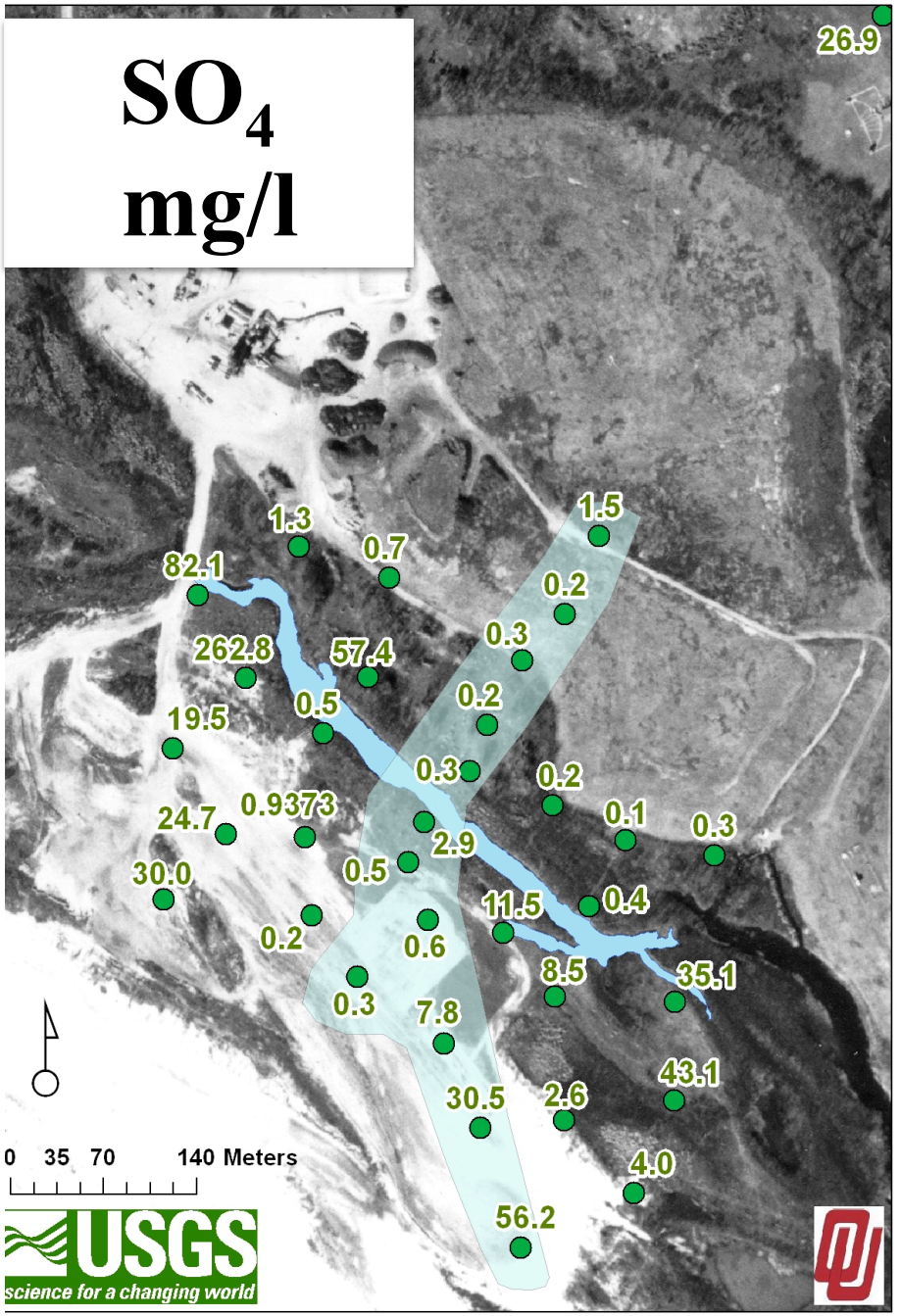
Norman Landfill Research Site



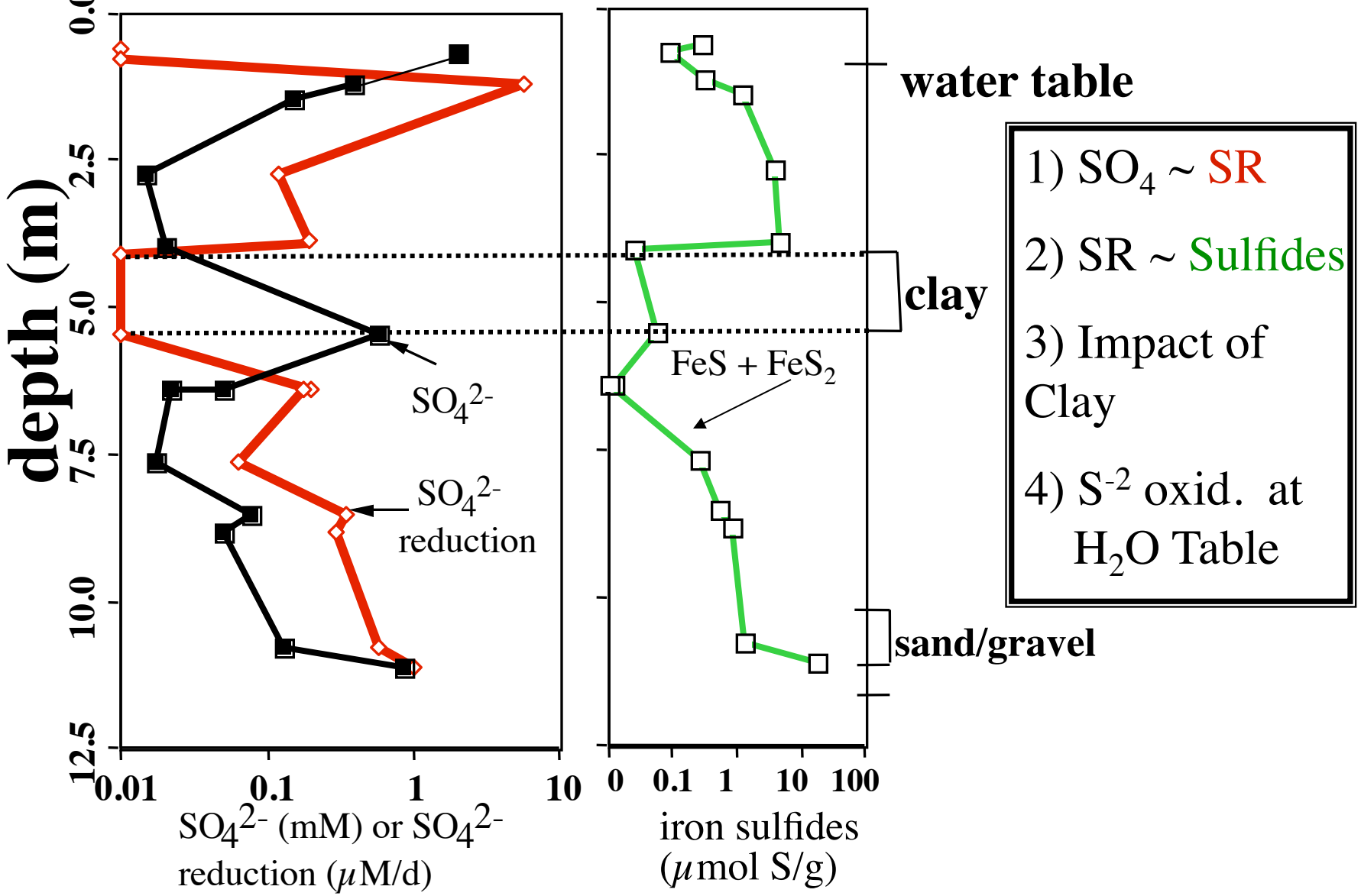
Part of the USGS Toxic Substances Hydrology Program

http://ok.water.usgs.gov/norlan/


DOC
mg/l
3.6
215.2
39.9
58.7
12.2
99.7
92.7
7.2
40.5
88.7
46.3
6.6
115.4
59.5
14.5
44.4
84.3
93.4
73.0
80.1
6.5
42.0
56.2
75.8
97.1
42.6
25.1
40.4
41.3
16.9
36.3
18.3
10.8
8.0
0 35 70 140 Meters
USGS
science for a changing world


SO_4
mg/l
26.9
1.5
1.3
0.7
82.1
0.2
0.3
262.8
57.4
0.2
0.5
19.5
0.3
0.2
24.7
0.9373
0.1
0.3
2.9
30.0
0.5
11.5
0.4
0.2
0.6
8.5
35.1
0.3
7.8
43.1
30.5
2.6
4.0
56.2
0 35 70 140 Meters
USGS
science for a changing world

SO_4, Sulfate Reduction, and Iron Sulfide Formation in the Aquifer

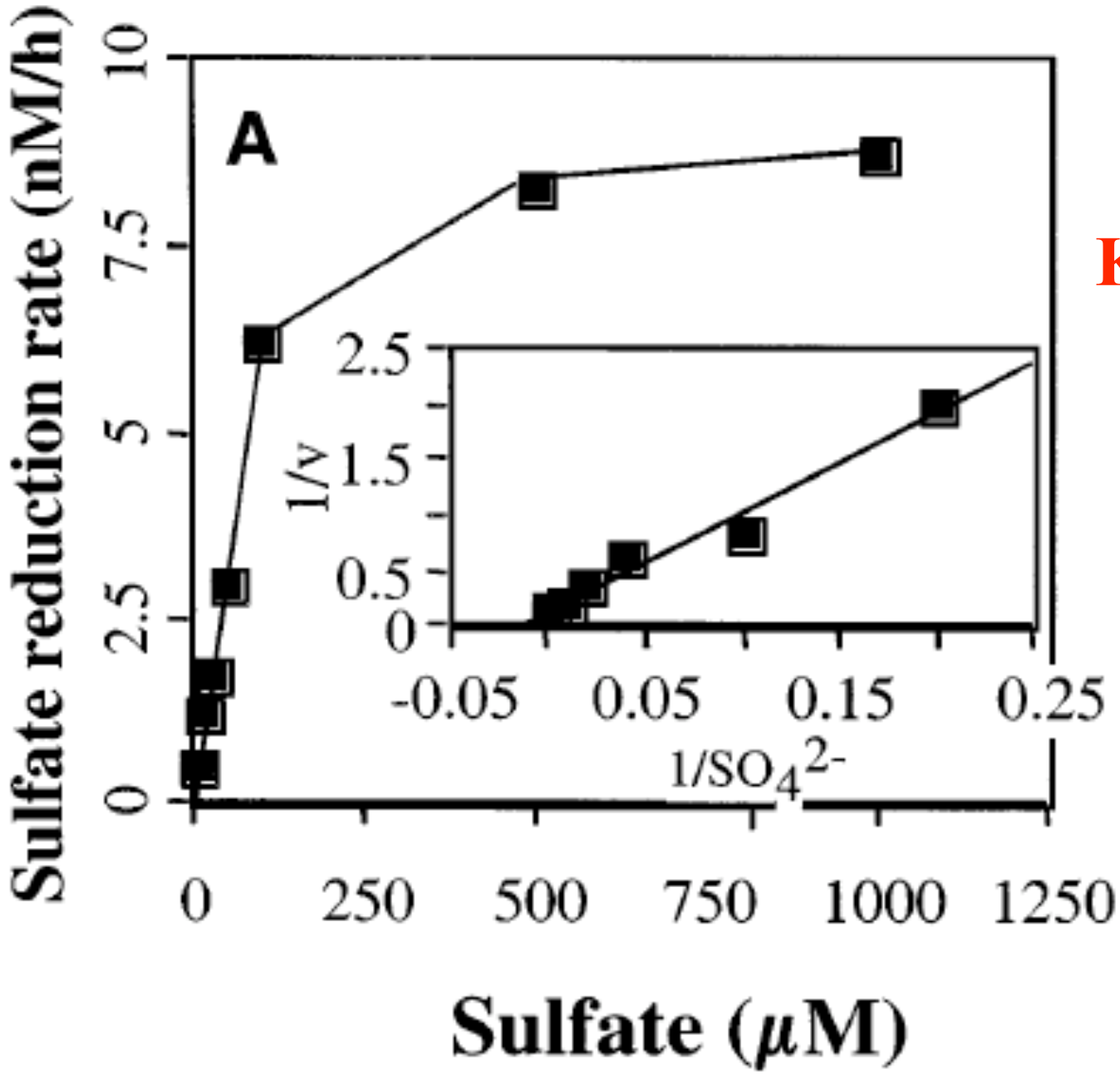


1) SO_4 ~ SR

2) SR ~ Sulfides

3) Impact of Clay

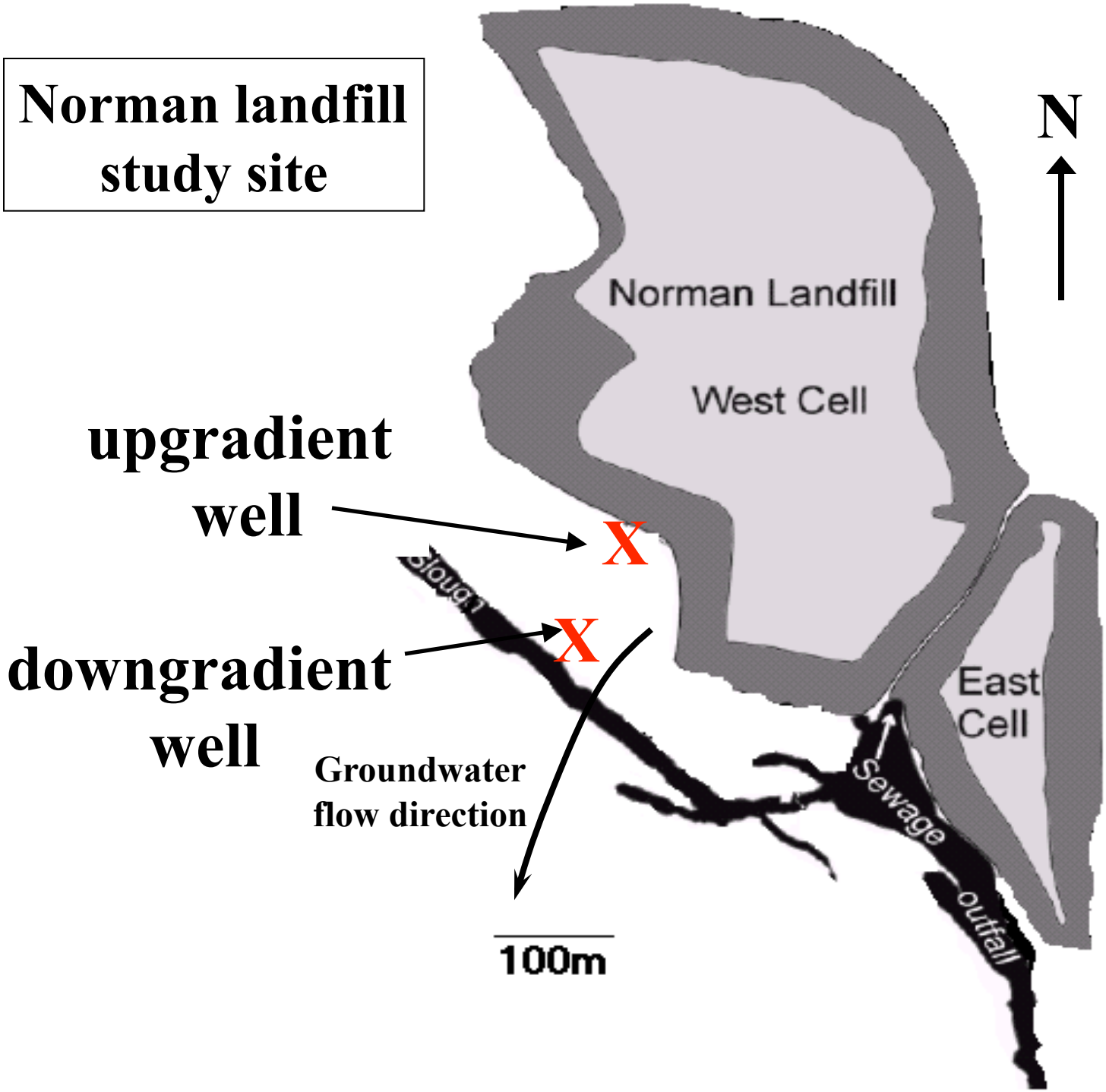
4) S^{-2} oxid. at H_2O Table



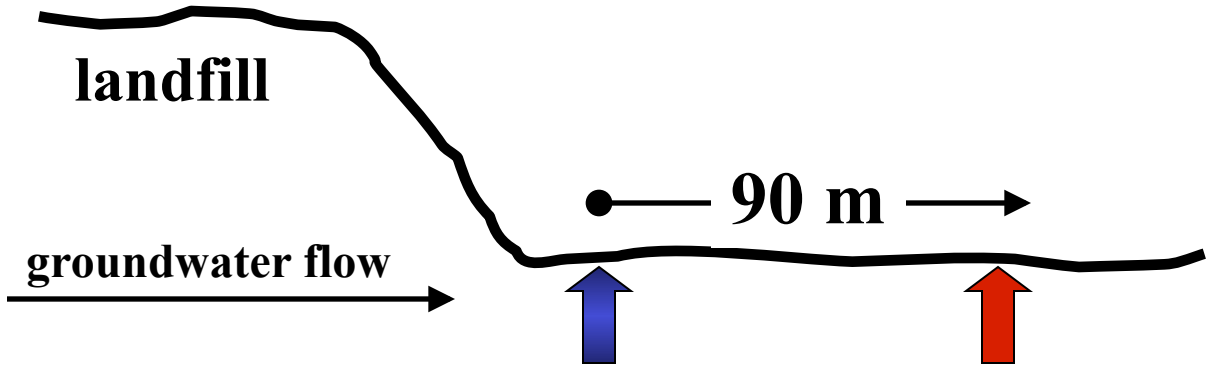
Apparent Km = 84μM

***In Situ* Sulfate ≤ 100 μM**

Norman landfill study site



Groundwater Chemistry at the Norman Landfill Sites

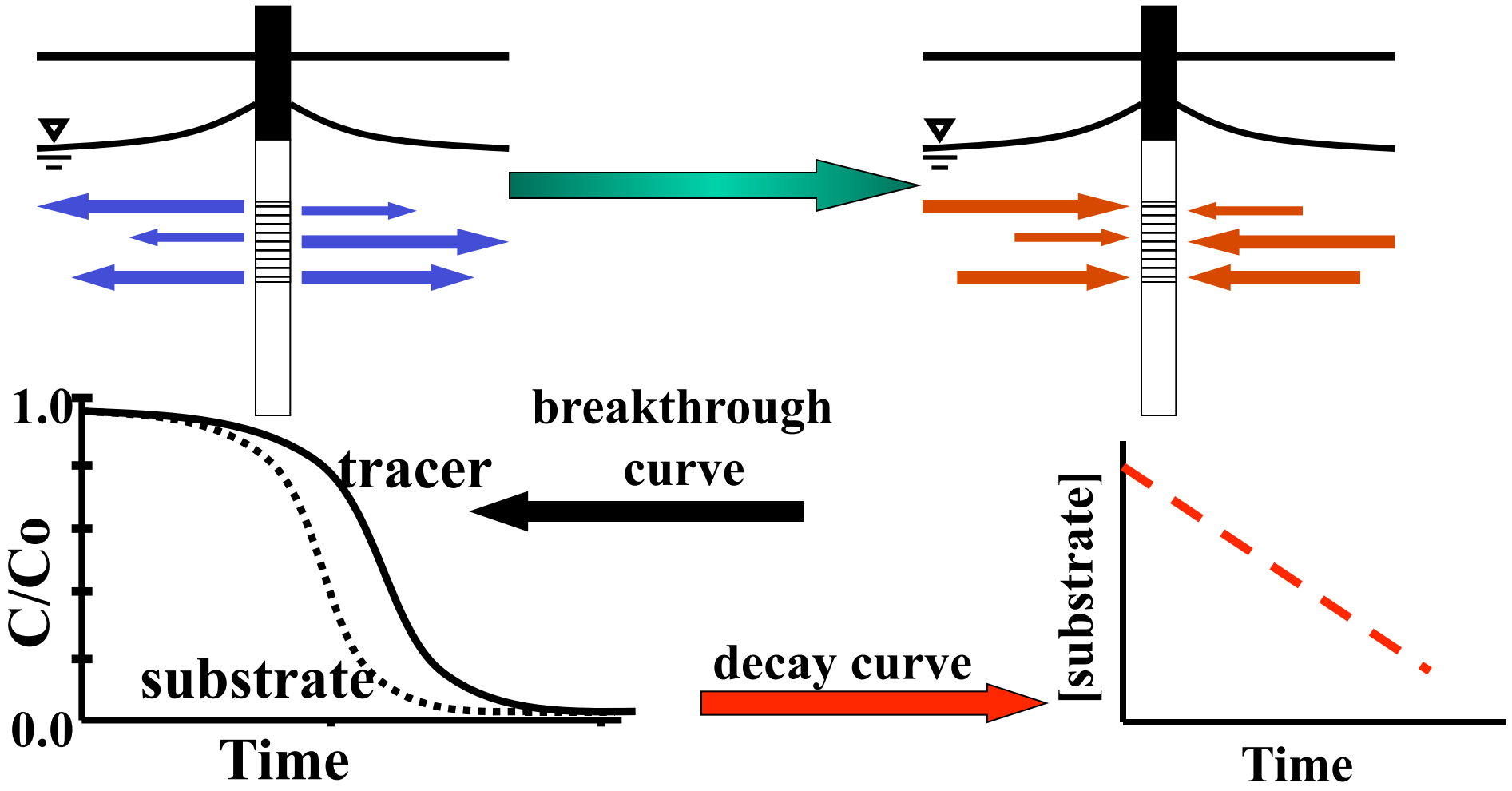


constituent	up-gradient	down-gradient	back-ground
dissolved org. C (mM)	~8	3.3	0.2
sp. conductance ($\mu S\ cm^{-1}$)	4990	5940	1570
sulfate (mM)	0.04	7.1	1.2
chloride (mM)	9.7	13.6	5.1
hydrogen (nM)	1.6	ND	ND
oxygen ($mg\ L^{-1}$)	<0.3	<0.3	ND

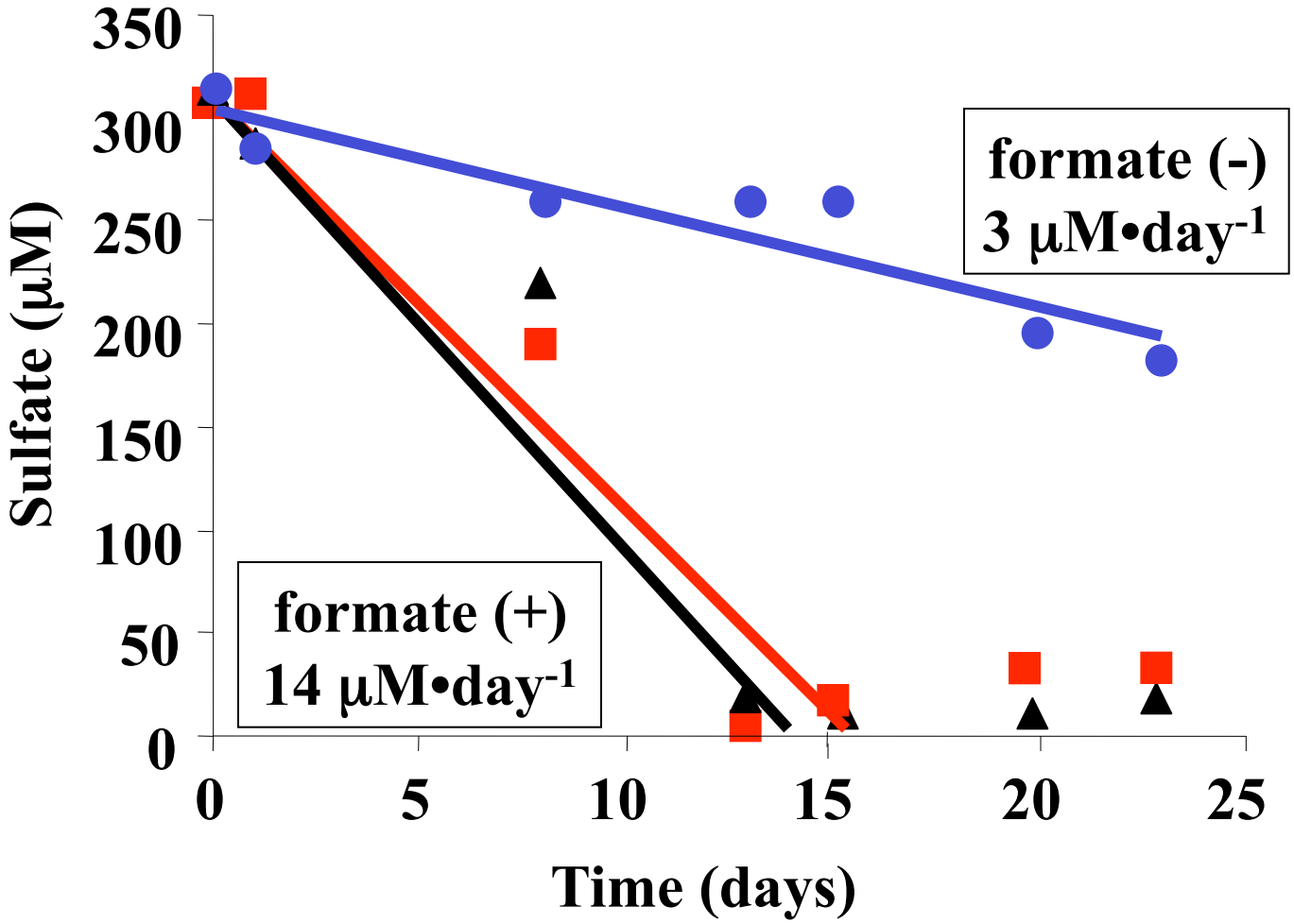
Push-pull test procedure

sparge groundwater
add reactant/NaBr & inject

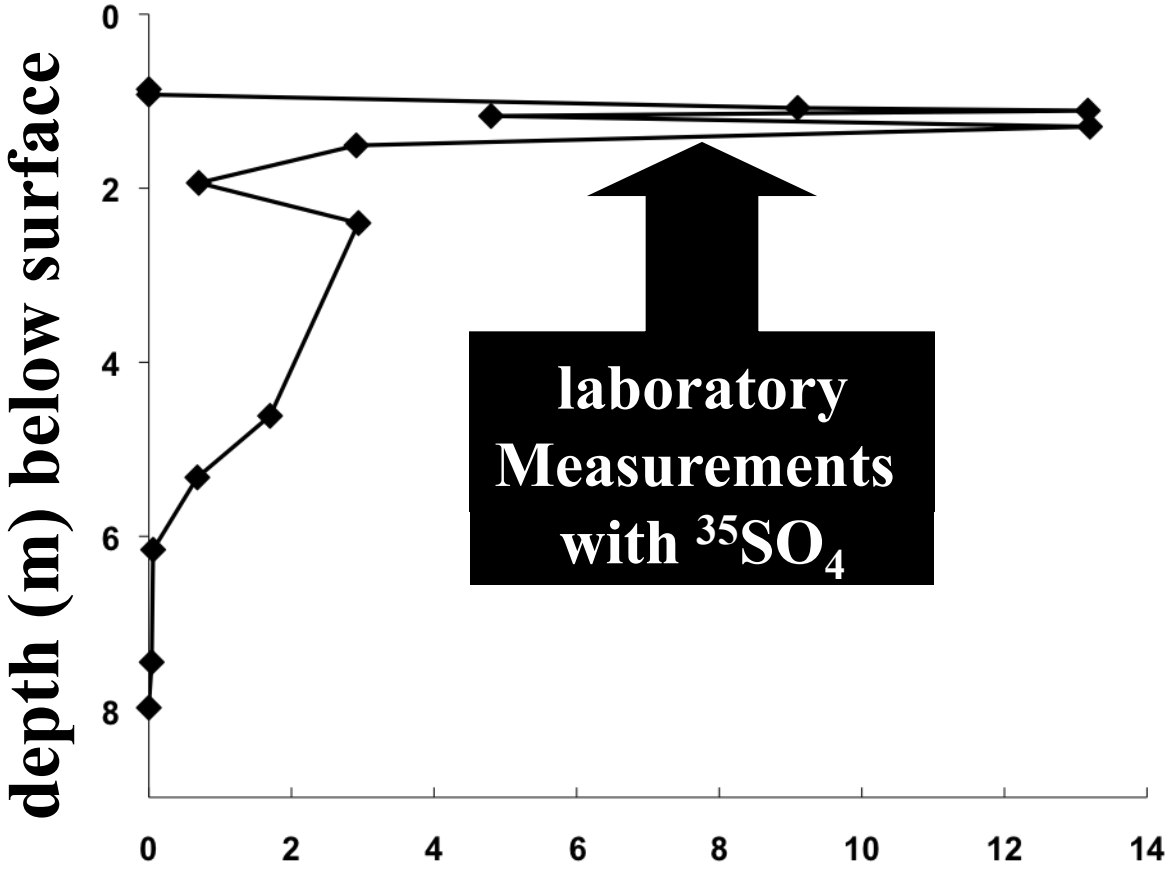
extract solution and sample
for Br^- and reactant vs. time



Field Sulfate Consumption Rates From Push-Pull Tests at the Upgradient Site at the Norman Landfill



Comparison of Sulfate Reduction Rates Measured in Intact Cores and *in situ* Tests



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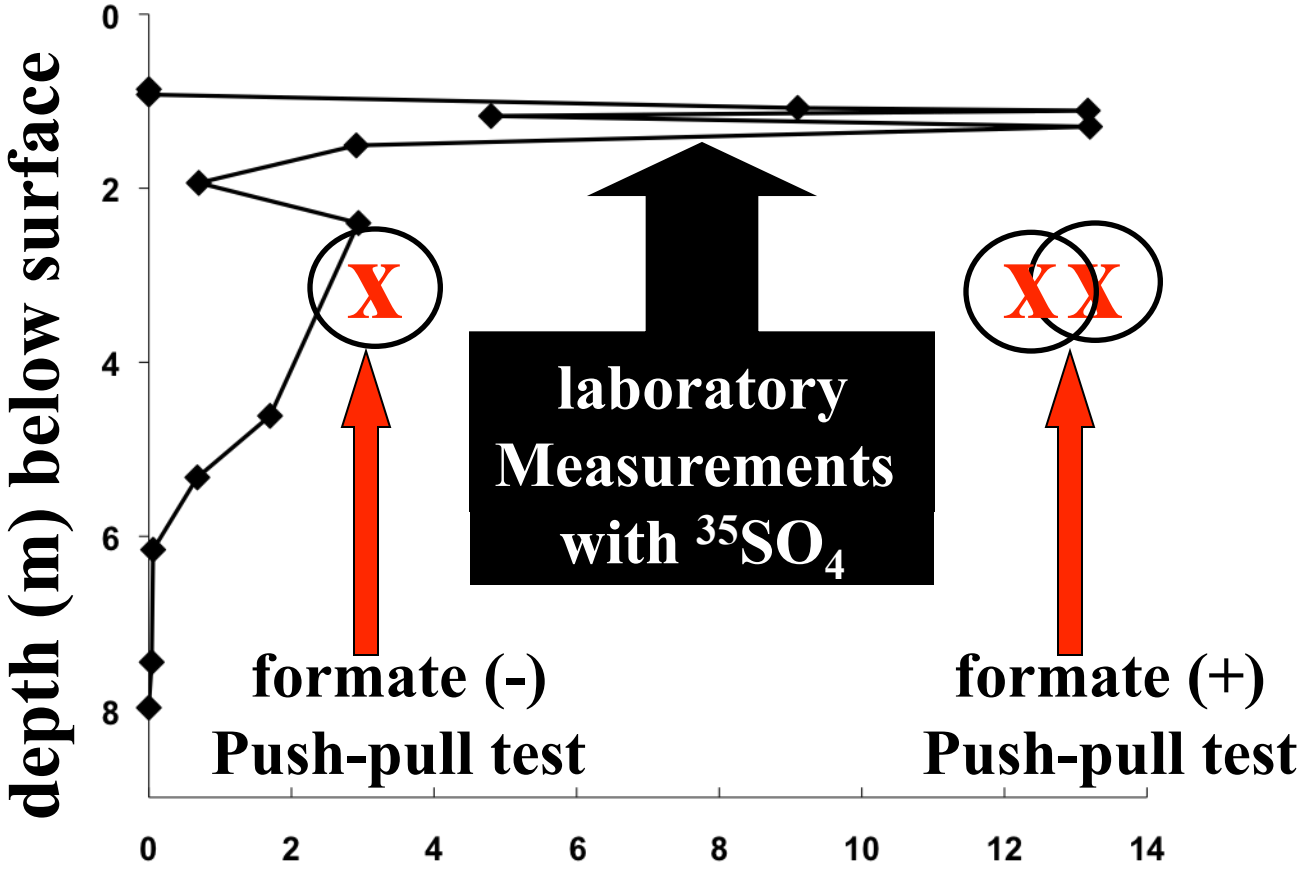
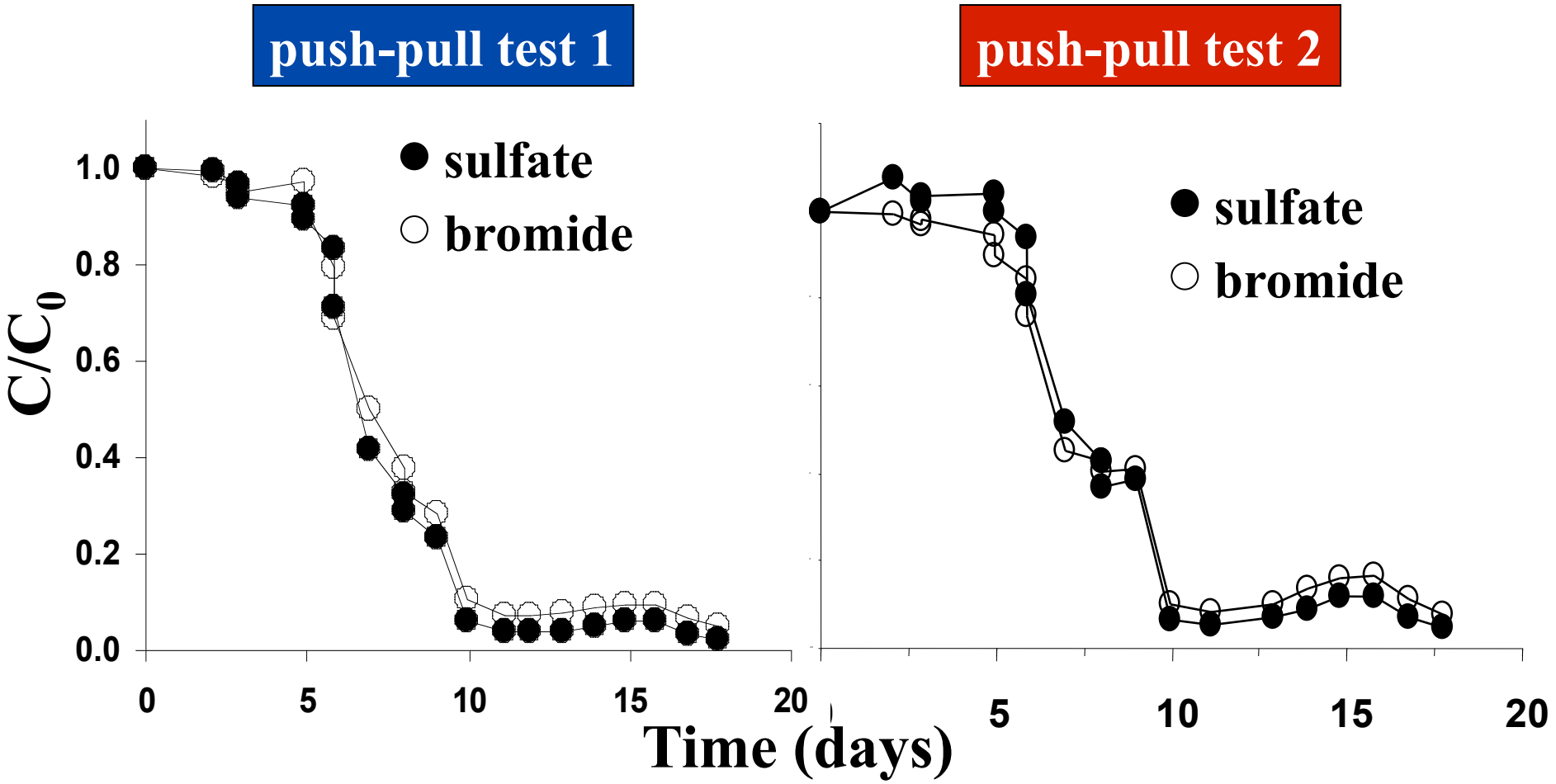
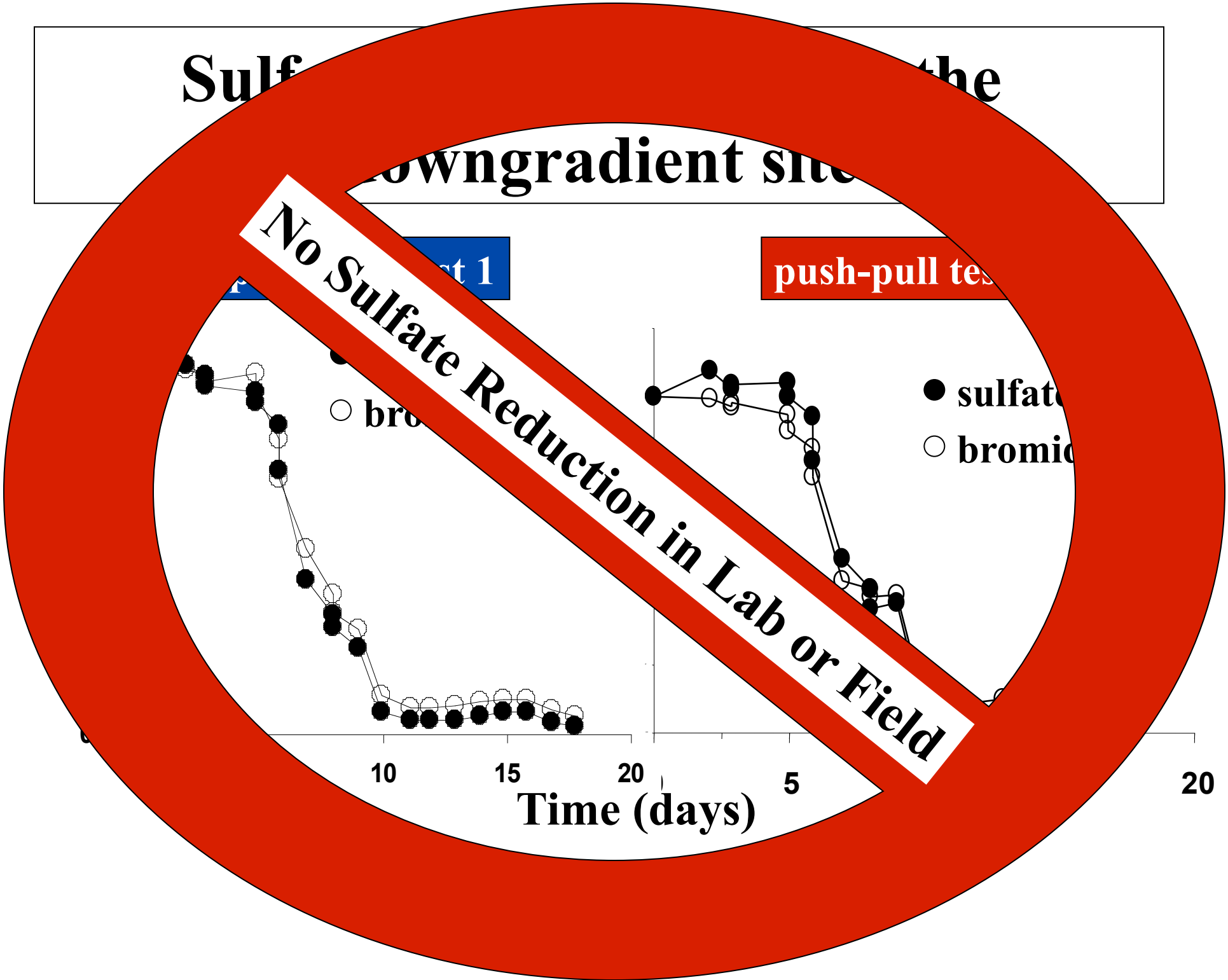


Sulfate push-pull tests at the downgradient site



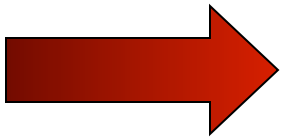

Sulf
the
owngradient site
t 1
push-pull tes
sulfat
bromic
bro
No Sulfate Reduction in Lab or Field
10
15
20
5
20
Time (days)

Possible hypotheses for the lack of sulfate reduction activity at the downgradient site

- ✓ lack of sulfate reducing microorganisms
- ✓ presence of an inhibitory compound
- ✓ lack of suitable electron donors

To address these issues:

examine microbial sulfate reduction under more controlled conditions



intact cores
&
aquifer samples

^{35}S-sulfate reduction assay in intact cores

- section core ~ (20 x 5 x 0.5 cm)
- apply ^{35}S-sulfate to core face
- incubate anaerobically

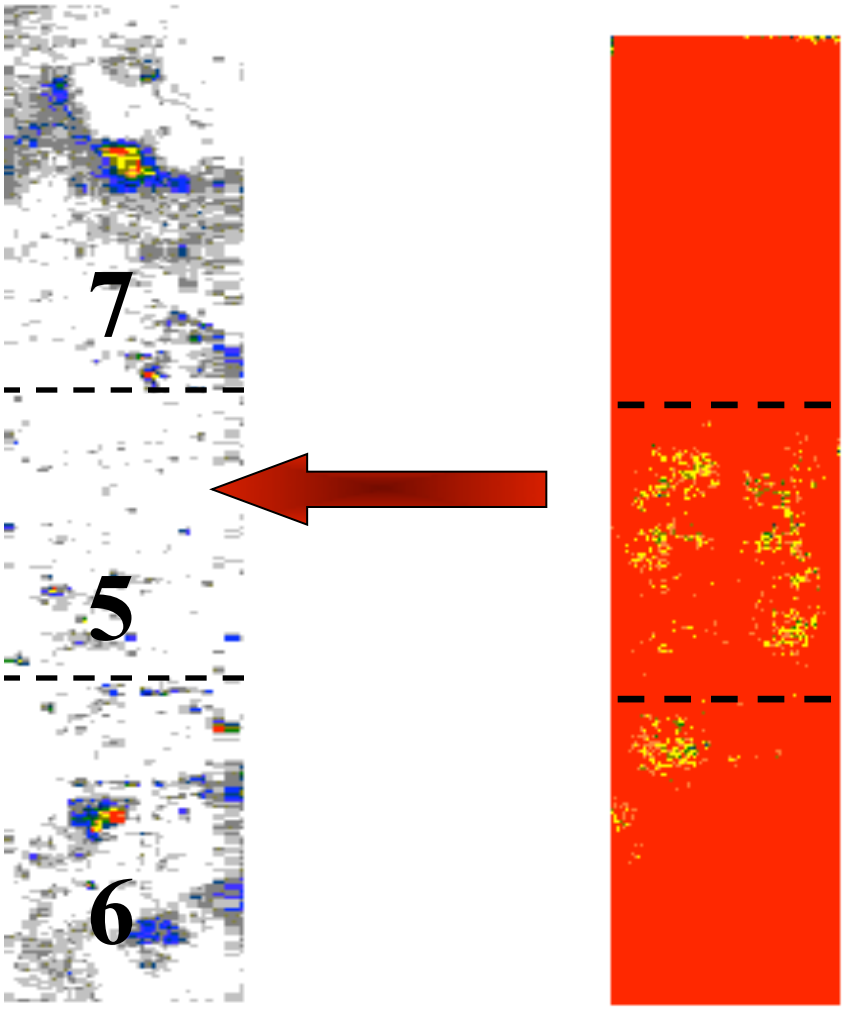
$^{35}SO_4^{=}$ (soluble) —SRB→ $H_2{}^{35}S^{-}$ (precipitated)

- wash unreacted $^{35}SO_4^{=}$ & image $^{35}S^{=}$

Sulfate reduction activity in a core segment incubated with ^{35}S-sulfate, lactate and *Desulfovibrio* preparations

Before treatment

(cpm/cm^2)



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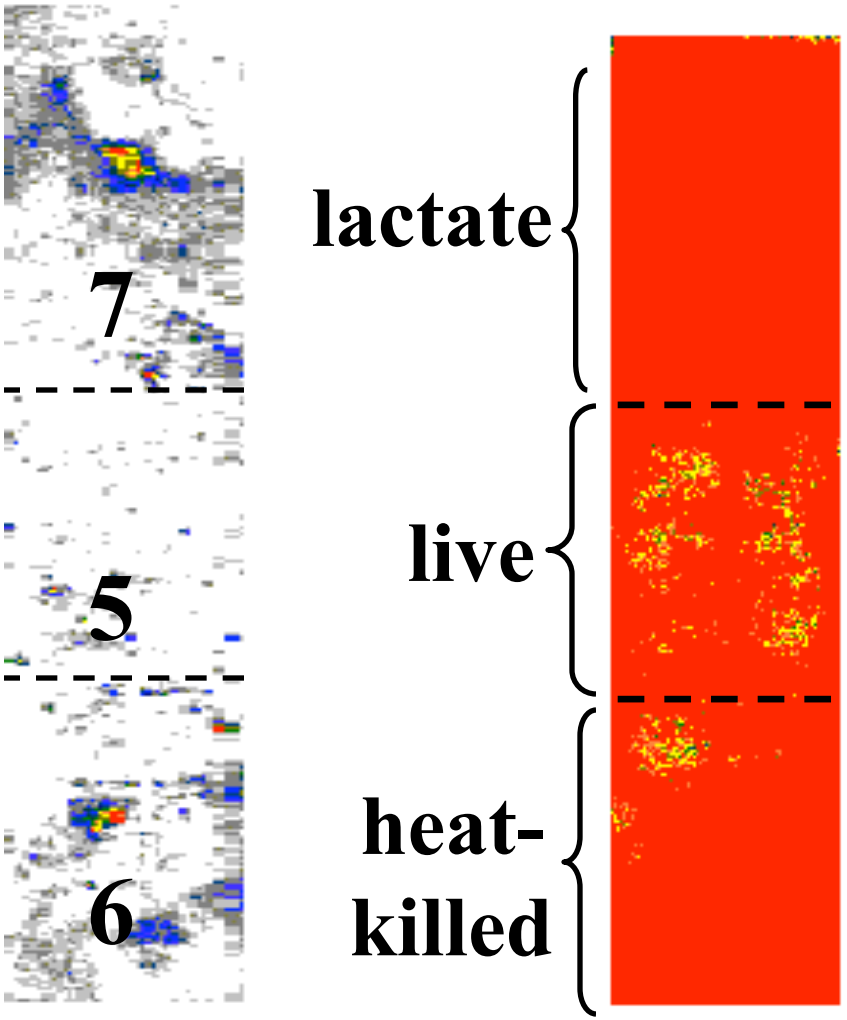

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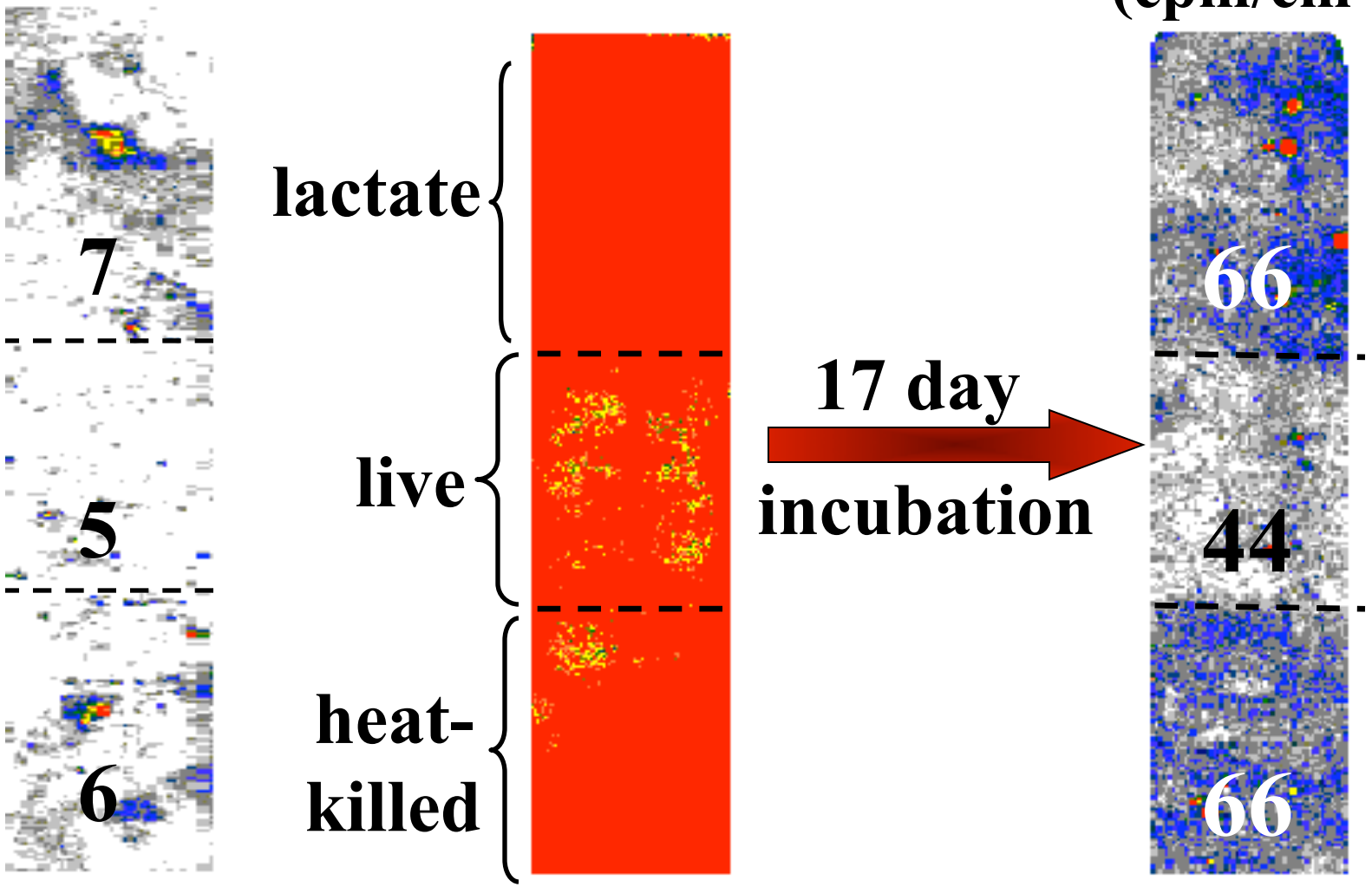
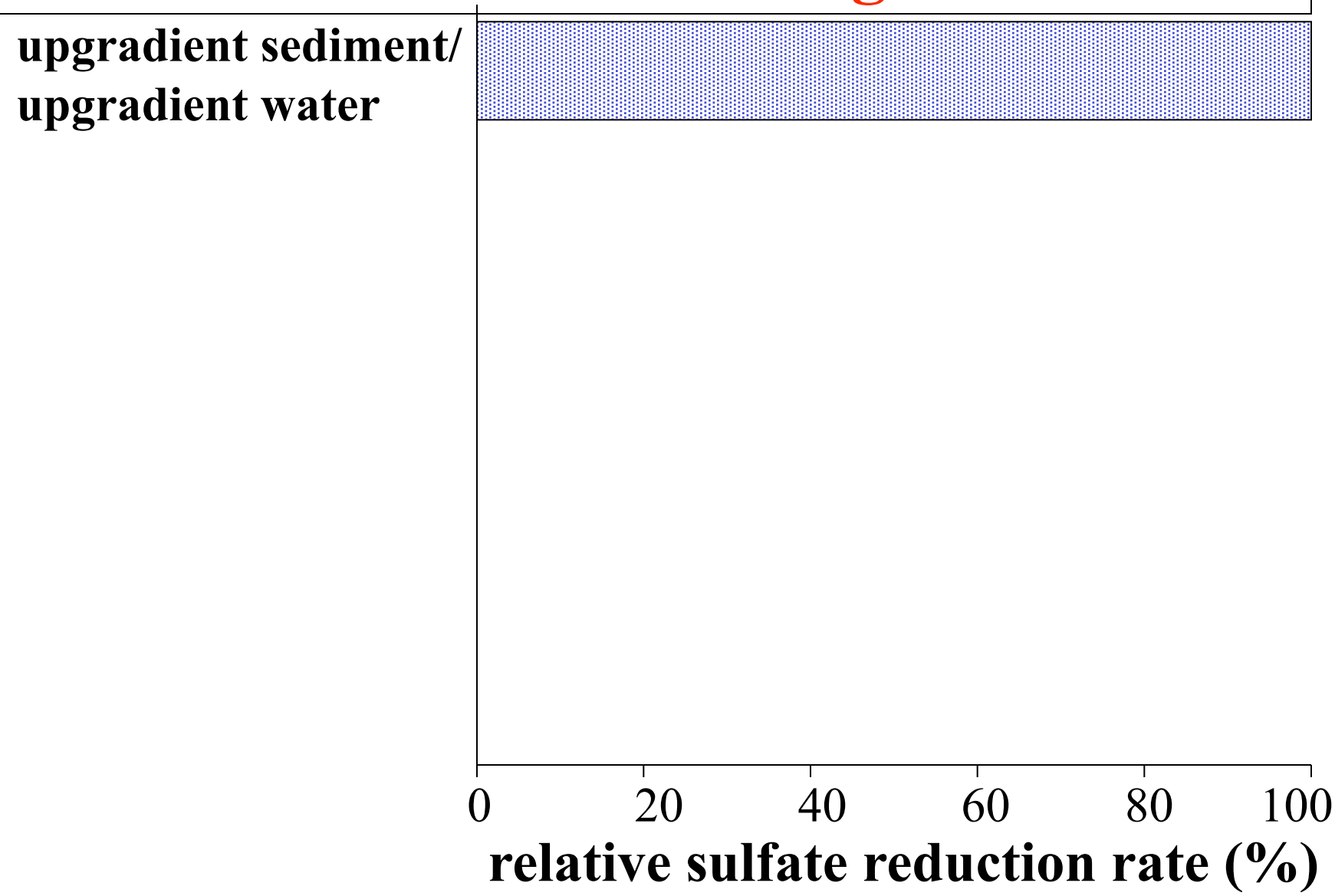


Sulfate reduction in aquifer slurries using sediment inocula and sterile groundwater



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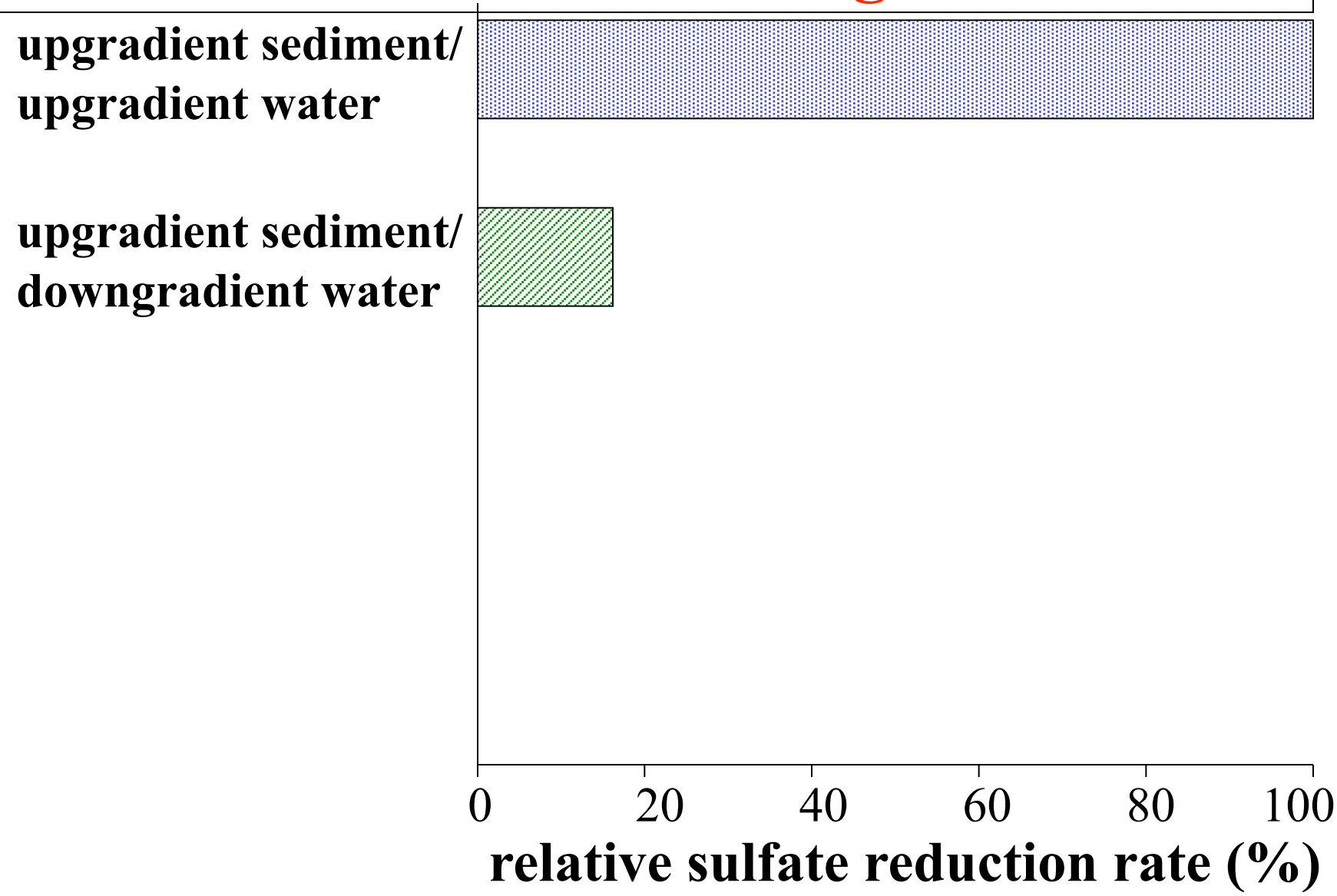

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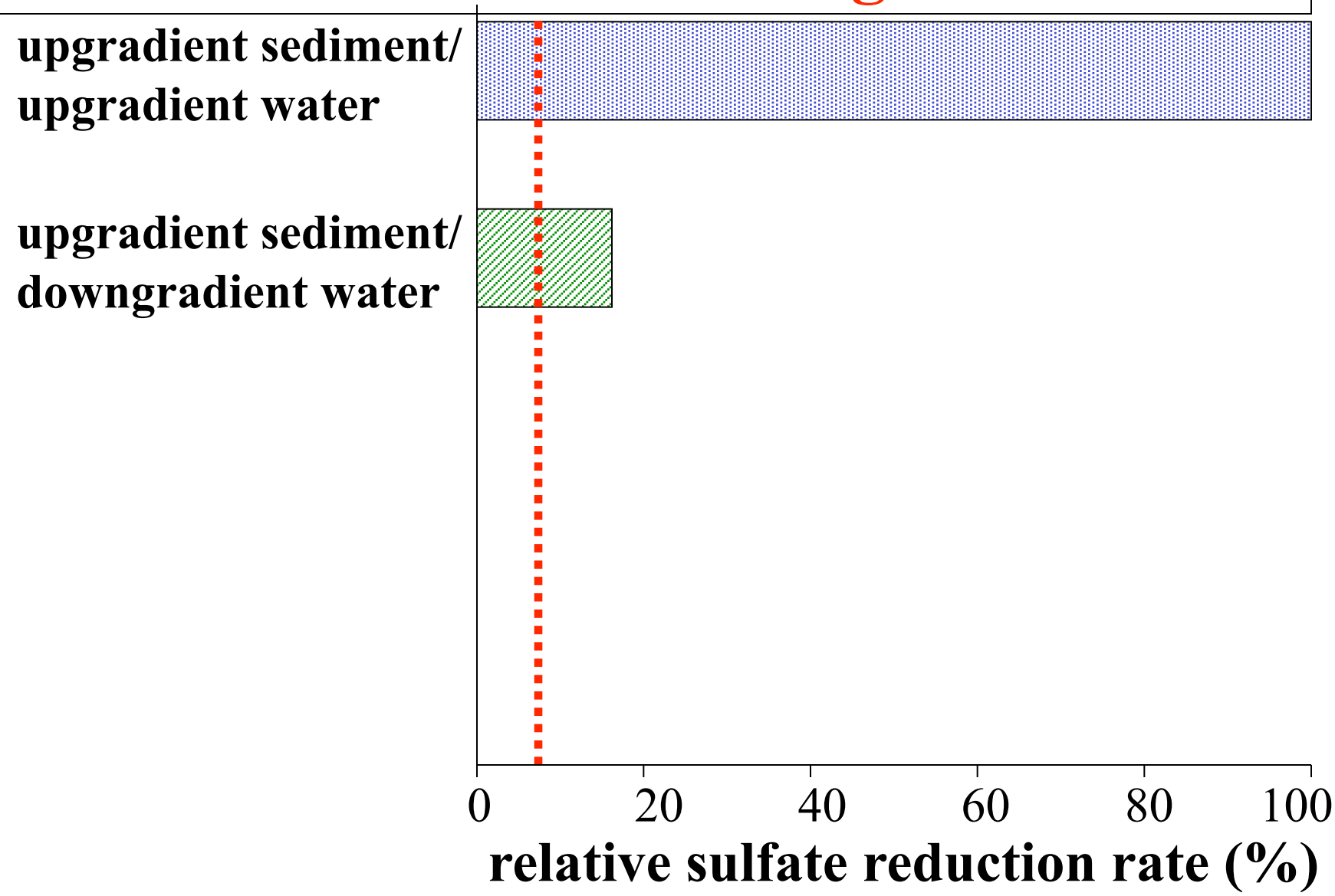

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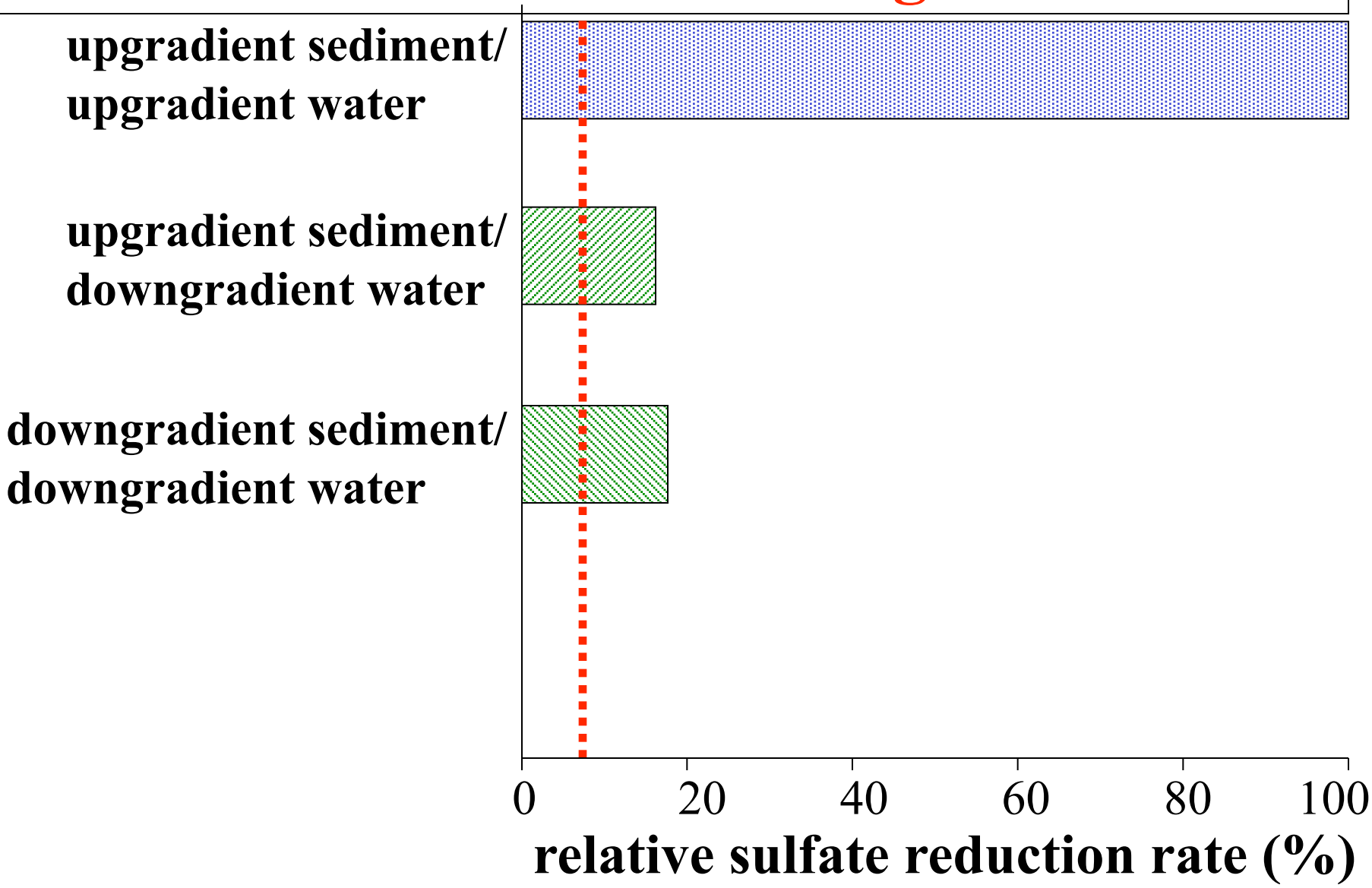

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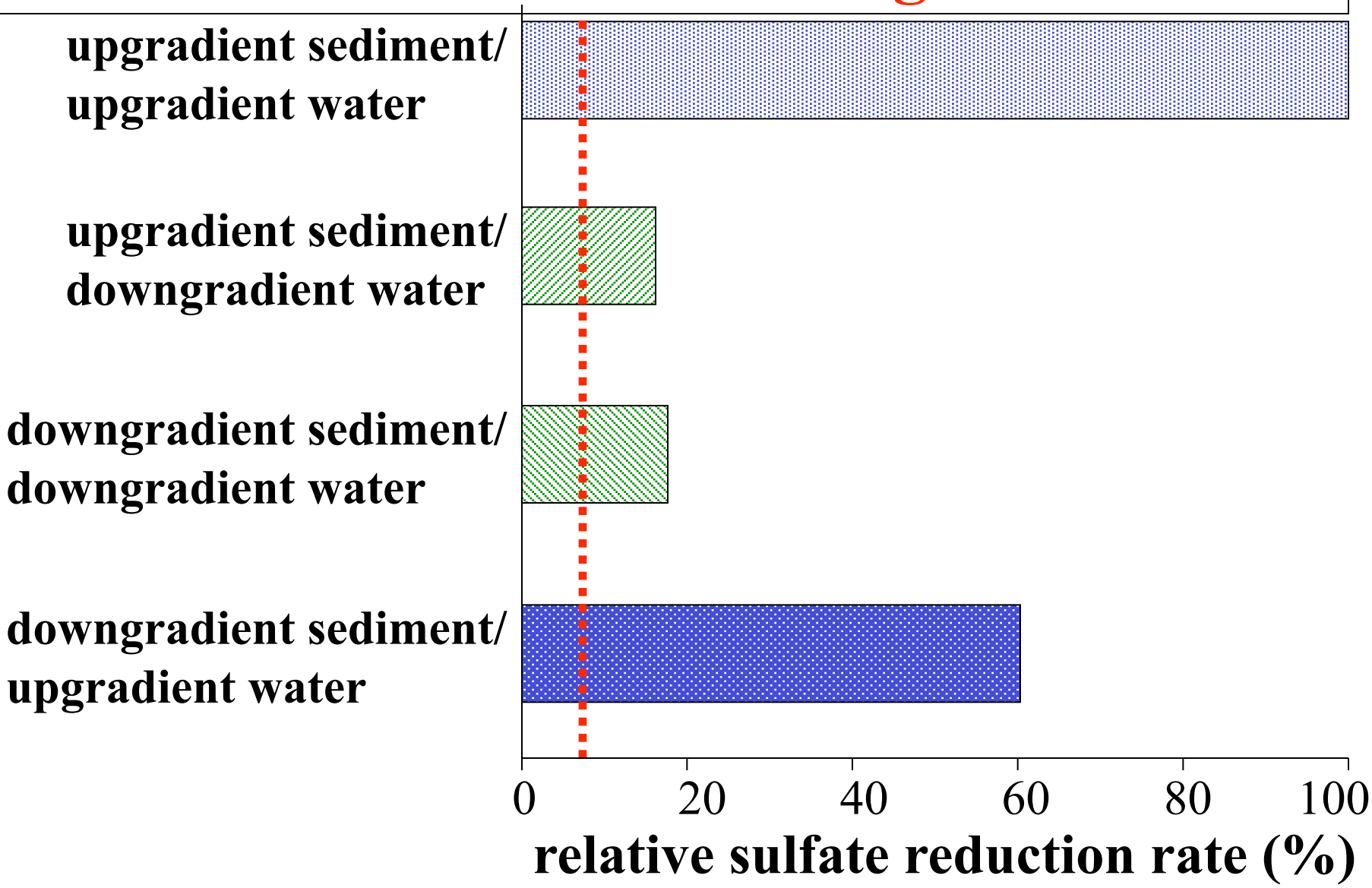

What Can We Conclude

- **sulfate reduction at the distal site was not limited by:**
  - ✓ **sulfate concentration**
  - ✓ **electron donor quantity**
  - ✓ **lack of metabolic potential**
  - ✓ **inhibitory substance**
- **was limited by electron donor QUALITY**
- **microbial inoculants can be a source of electron donors in bioaugmentation studies**