Factors Controlling In Situ Uranium and Technetium Bioreduction at the NABIR Field Research Center

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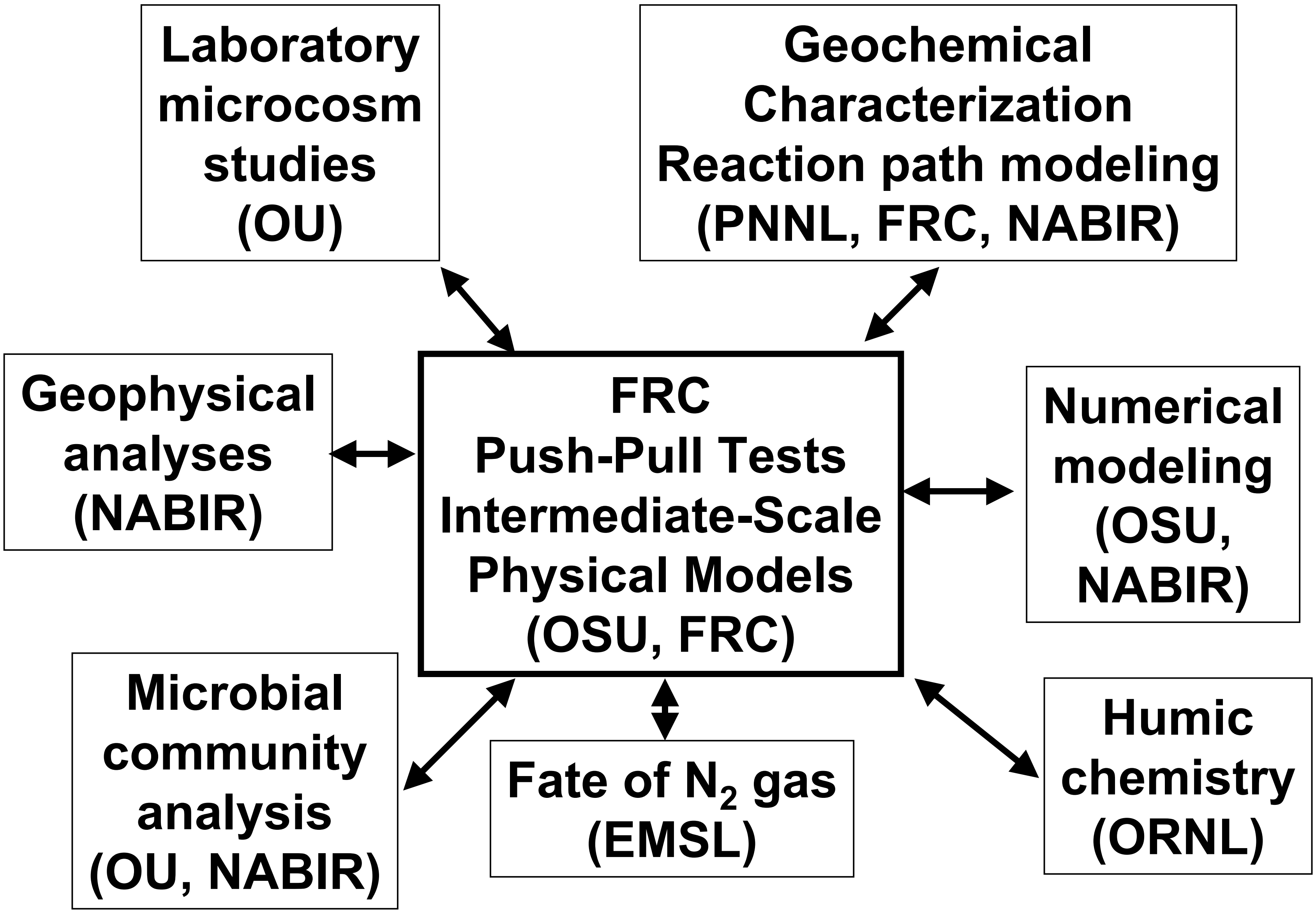
Research Hypotheses

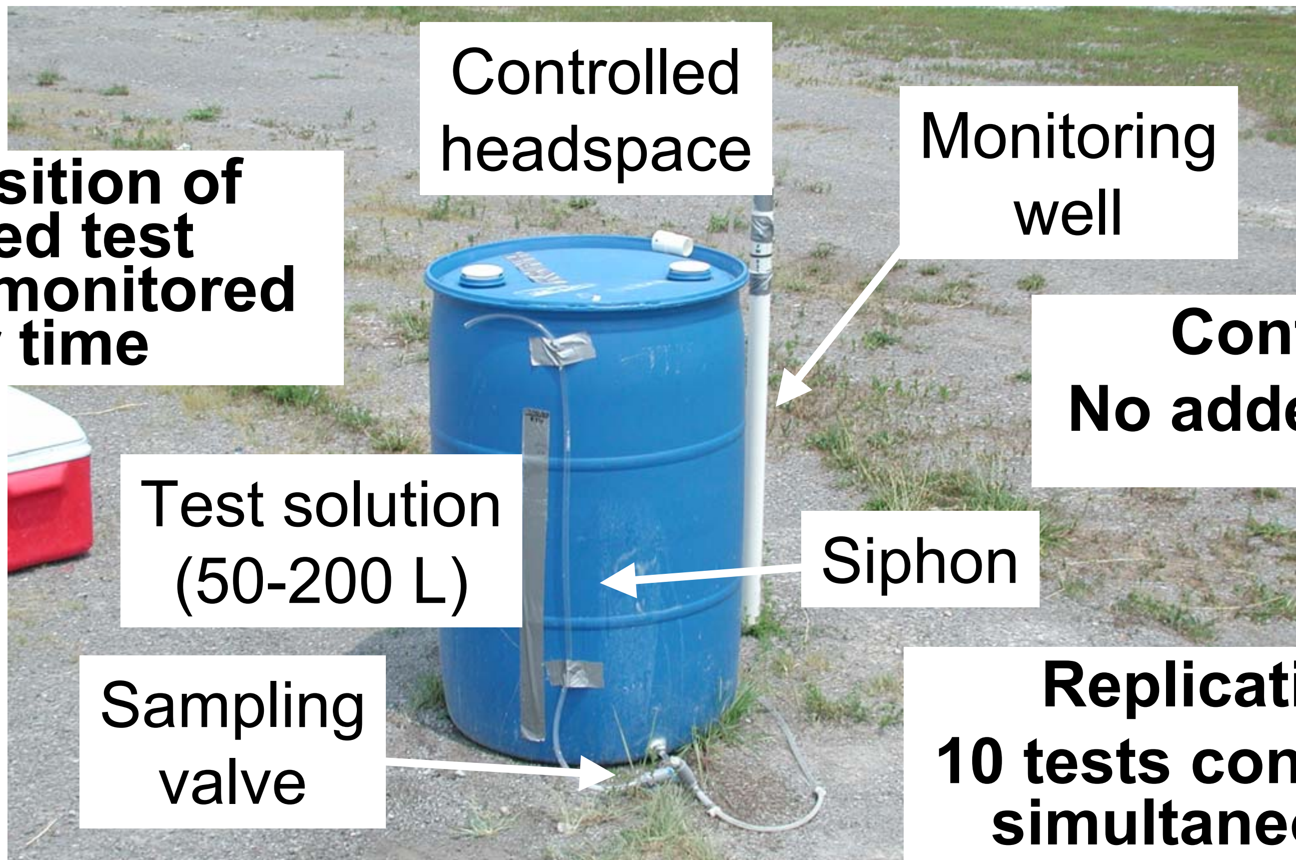
- **Indigenous microorganisms in the shallow aquifer at the FRC have the capability to reduce U(VI) and Tc(VII) but rates are limited by:**
  - **Scarce electron donor**
  - **Low pH and potentially toxic metals**
  - **High nitrate**
- **U(VI) and Tc(VII) reduction rates can be increased by:**
  - **Successive donor additions**
  - **Raising pH to precipitate toxic metals**
  - **Adding humics to complex toxic metals and serve as electron shuttles**

Project Organization



Processes Studied In Situ Using Push-Pull Tests

Site groundwater amended with tracers, +/-bicarbonate, +/- electron donor(s), +/- humics, +/- electron acceptors, +/- inhibitors and injected into existing monitoring wells



Composition of injected test solution monitored over time

Controls:
No added donor

Replication:
10 tests conducted simultaneously

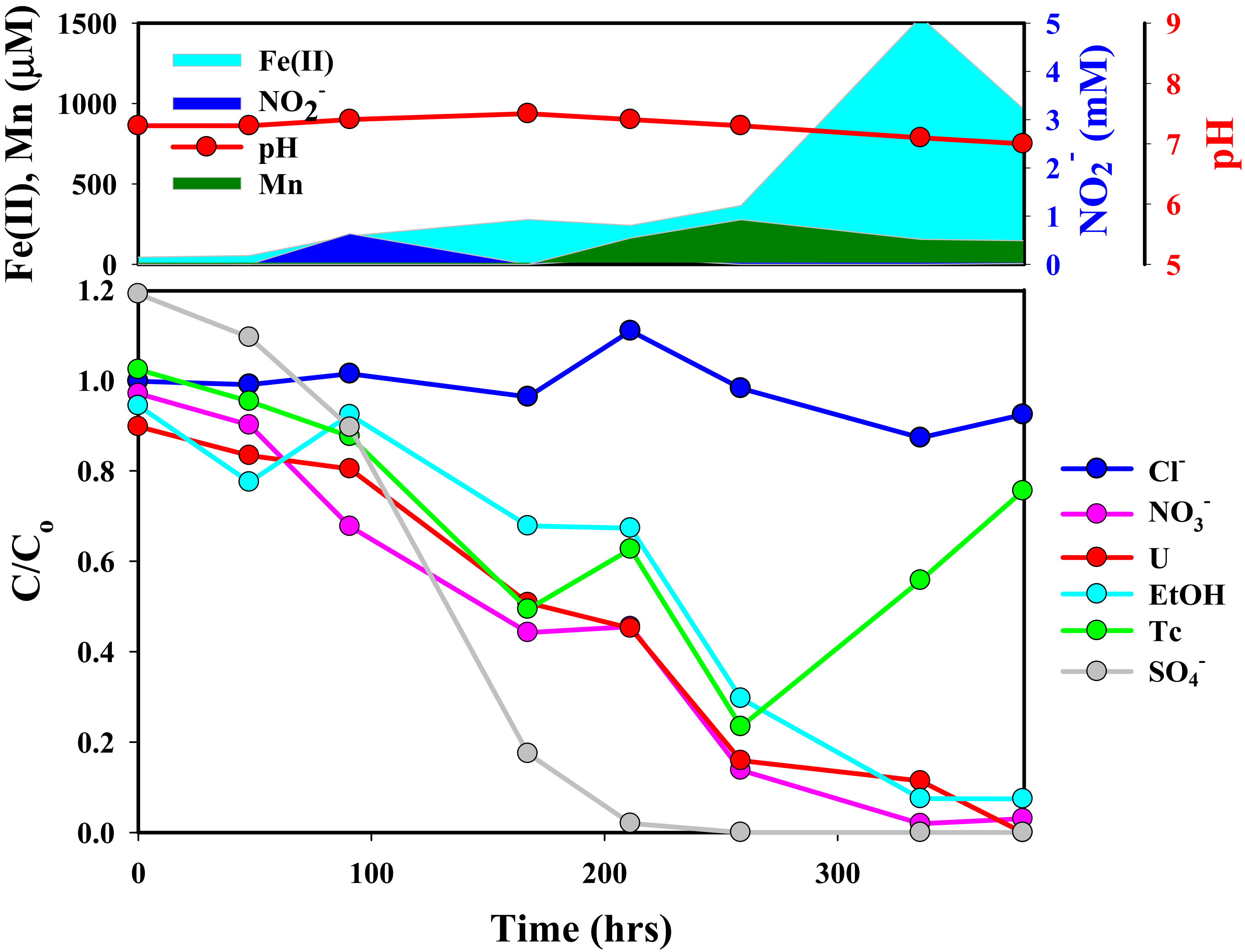
Groundwater Used to Prepare Test Solutions

	GW835 (μM)	FW021 (μM)
pH	6.4	3.3
Tc (pM)	410	18000
U	5	2
NO_3^-	1200	140000
Na	1100	23000
Ca	3500	19000
Al	0	12000
Mg	1100	8300
Cl^-	650	7900
Mn	50	2500
K	120	980
SO_4^{2-}	830	430

Push-Pull Test Overview

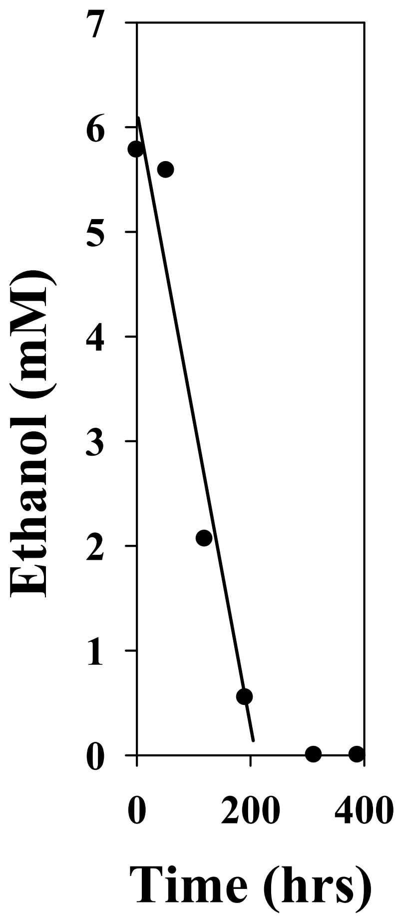
- Moderate pH (5.2 - 6.6) Area 1 (59 tests)
  - Low vs high nitrate; + tracer; + HCO_3^-; +/- donor; +/- acetylene; +/- humics
- Low pH (3.5 – 4.5) Area 1 (24 tests)
  - Low vs high nitrate; + tracer; + HCO_3^-; +/- donor; +/- acetylene; +/- humics
- Moderate pH (5.5 – 6.8) Area 2 (40 tests)
  - Low vs high nitrate; + tracer; + HCO_3^-; +/- donor; +/- sulfate; +/- humics

Example Results: 1 mM Nitrate

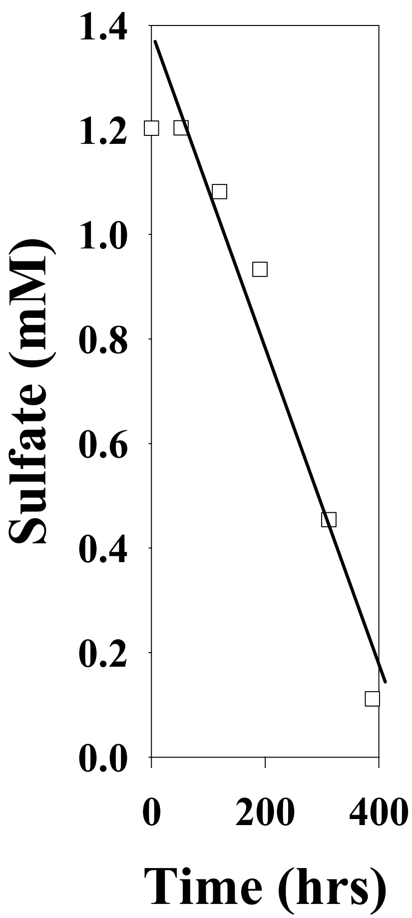


Area 2
GW835
+ Br^-
+ HCO_3^-
+ EtOH

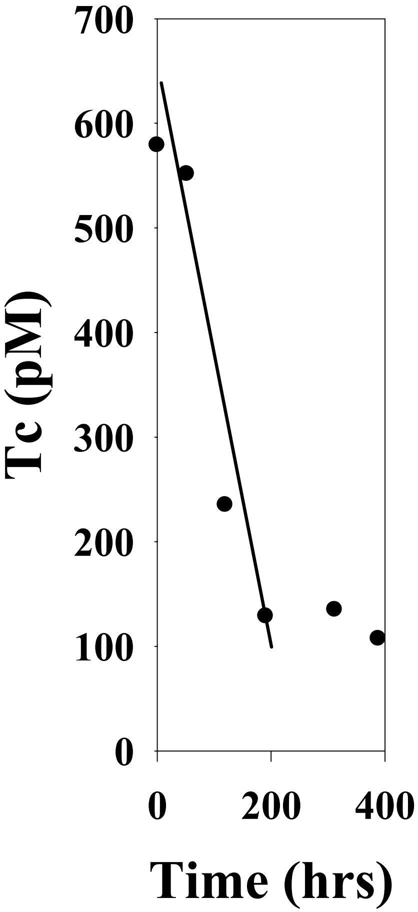
Rate Calculations: 1 mM Nitrate



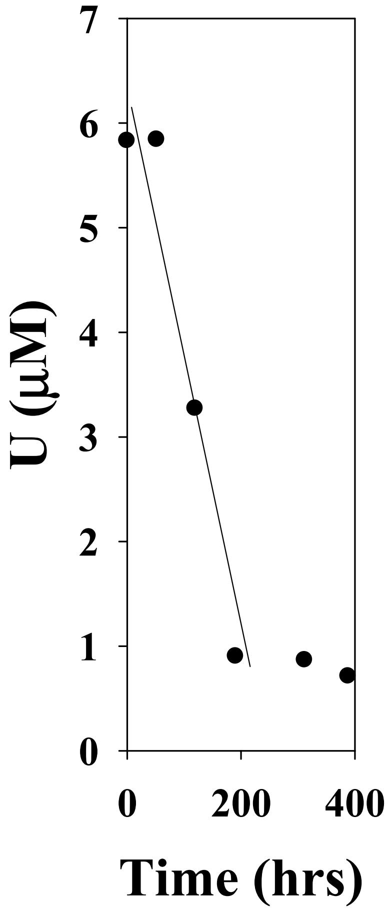
30 μM/hr



3 μM/hr

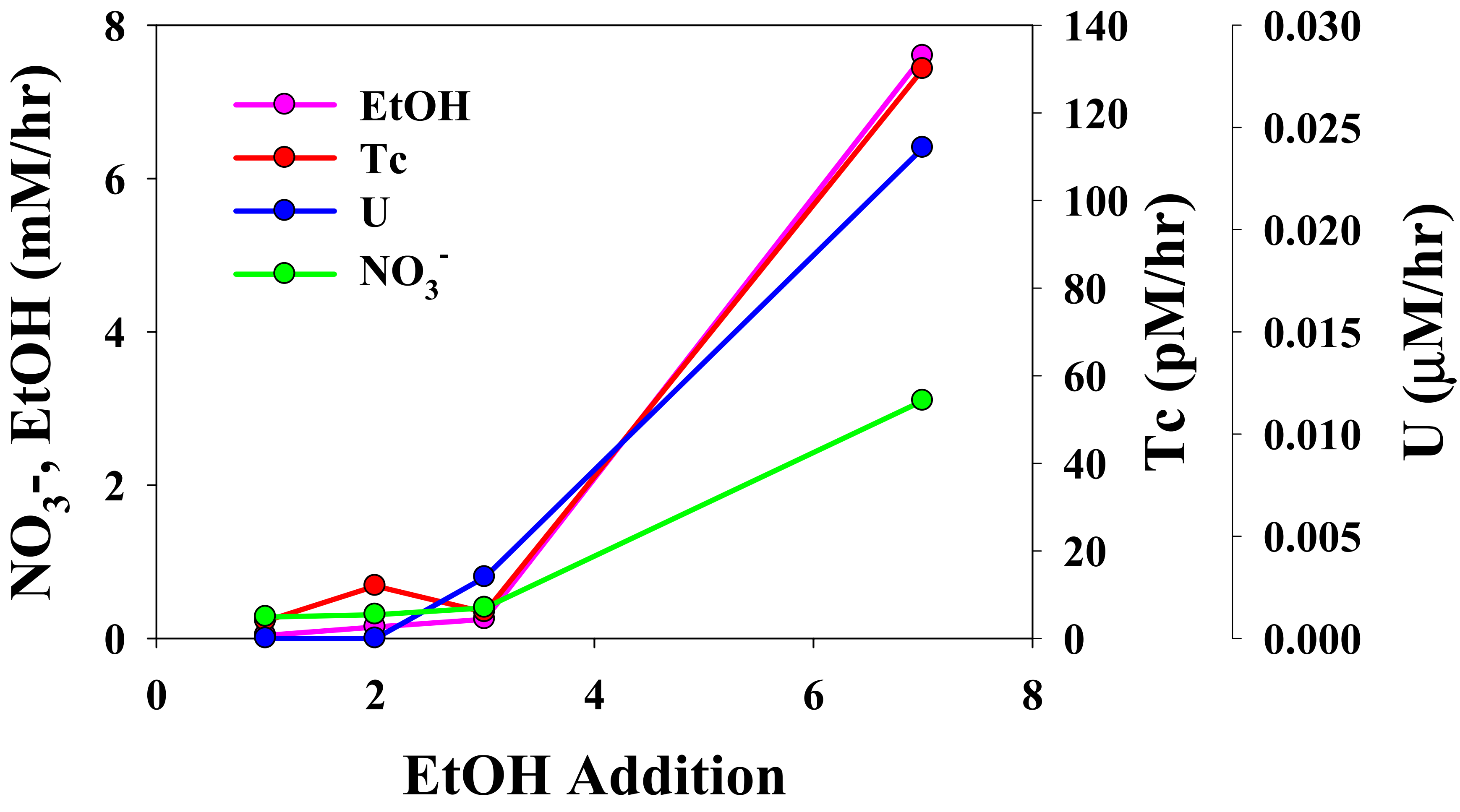


2.6 pM/hr

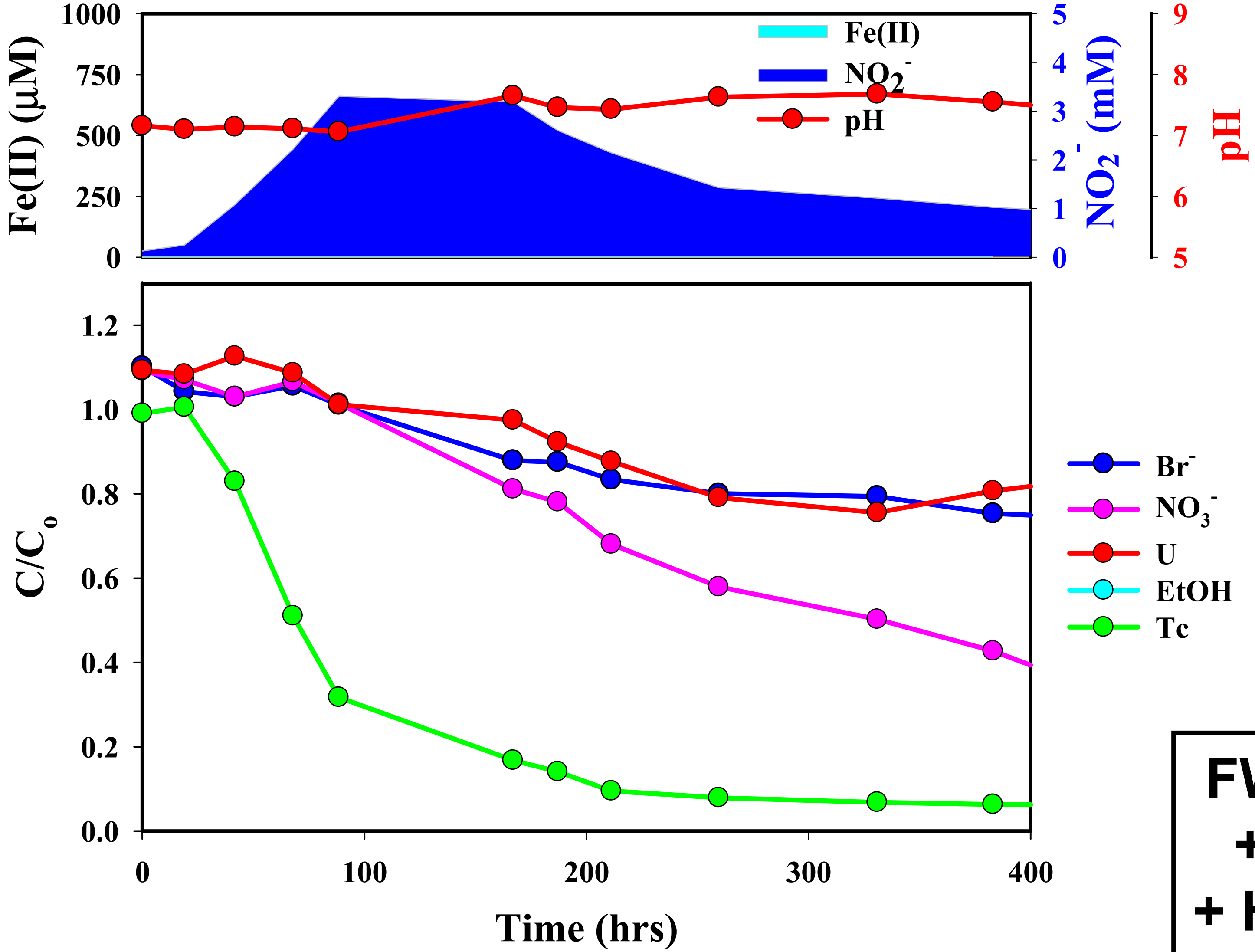


0.03 μM/hr

Successive Donor Additions Stimulates Microbial Activity

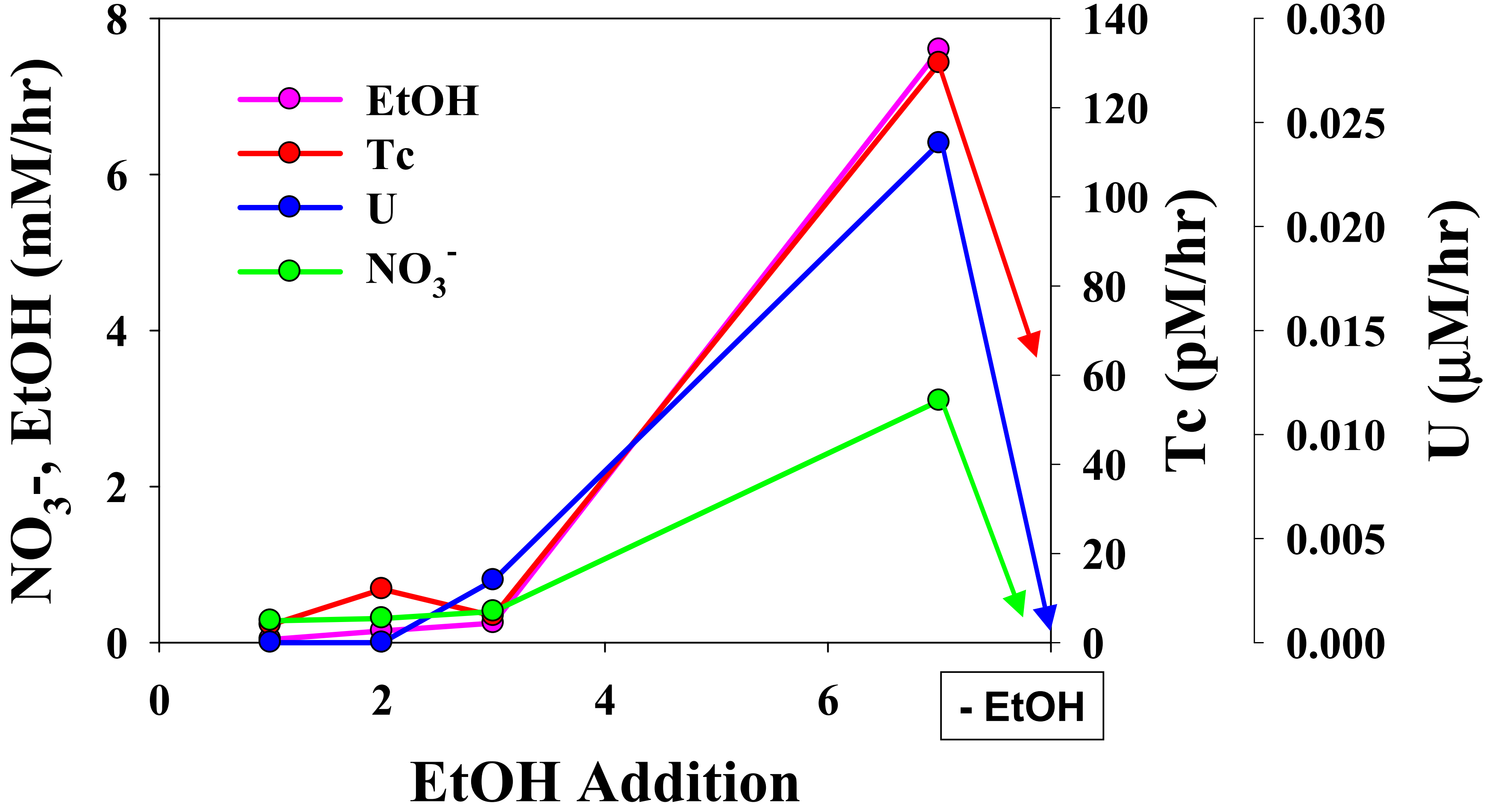


Decreased Activity With No Added Donor (After Biostimulation)

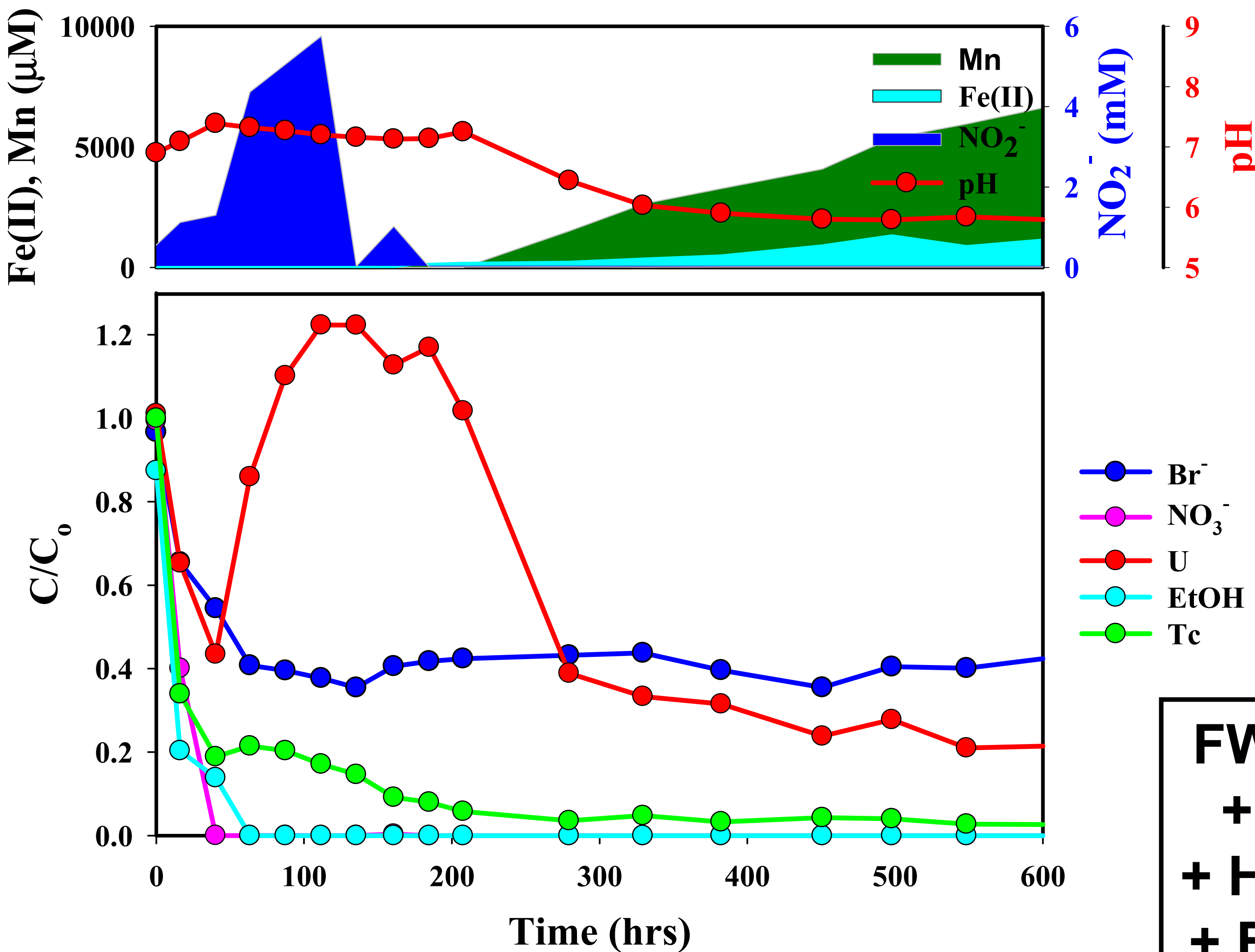


FW021
+ Br$^-$
+ HCO_3^-

Rate Decrease With No Added Donor (After Biostimulation)

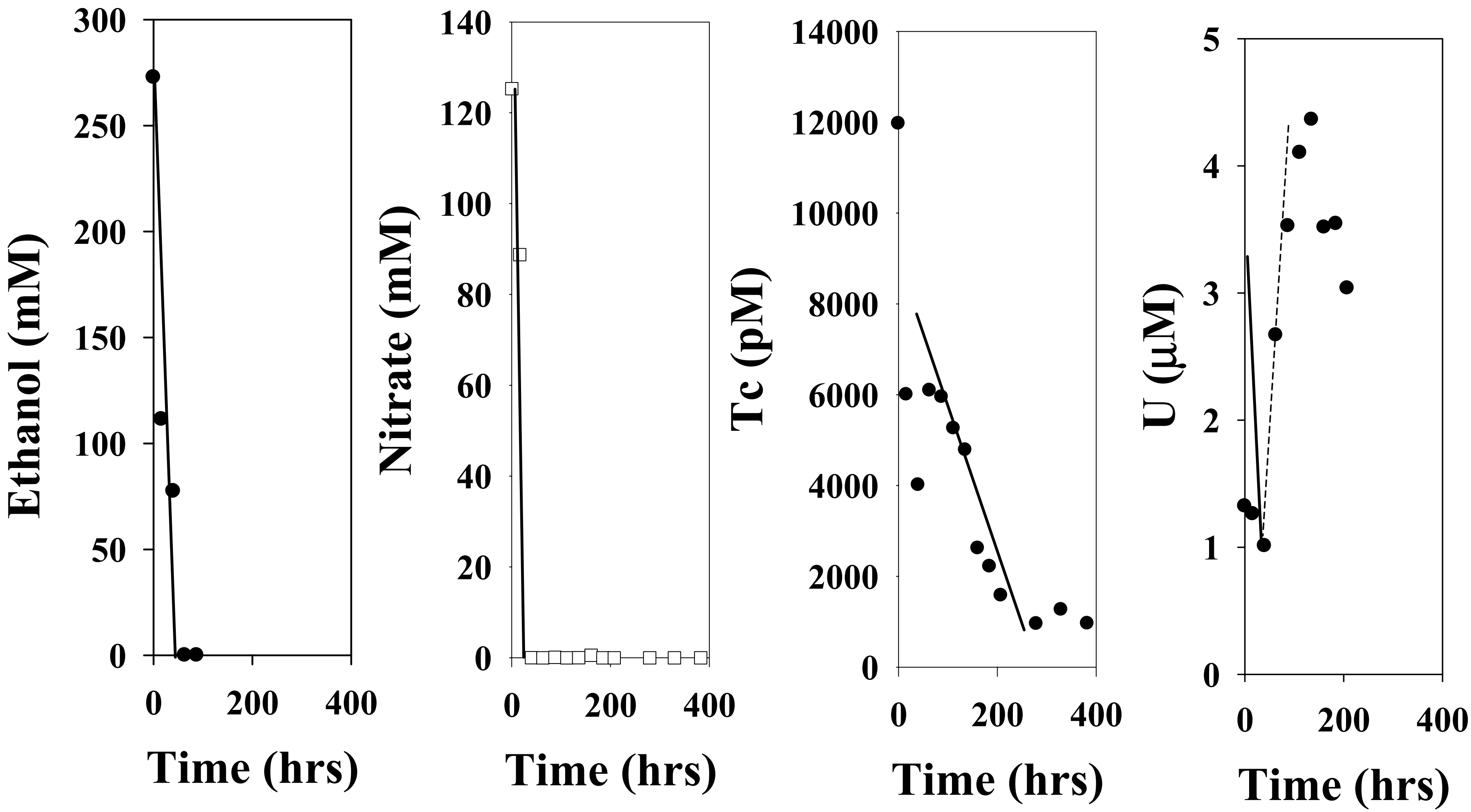


Example Results: 140 mM Nitrate



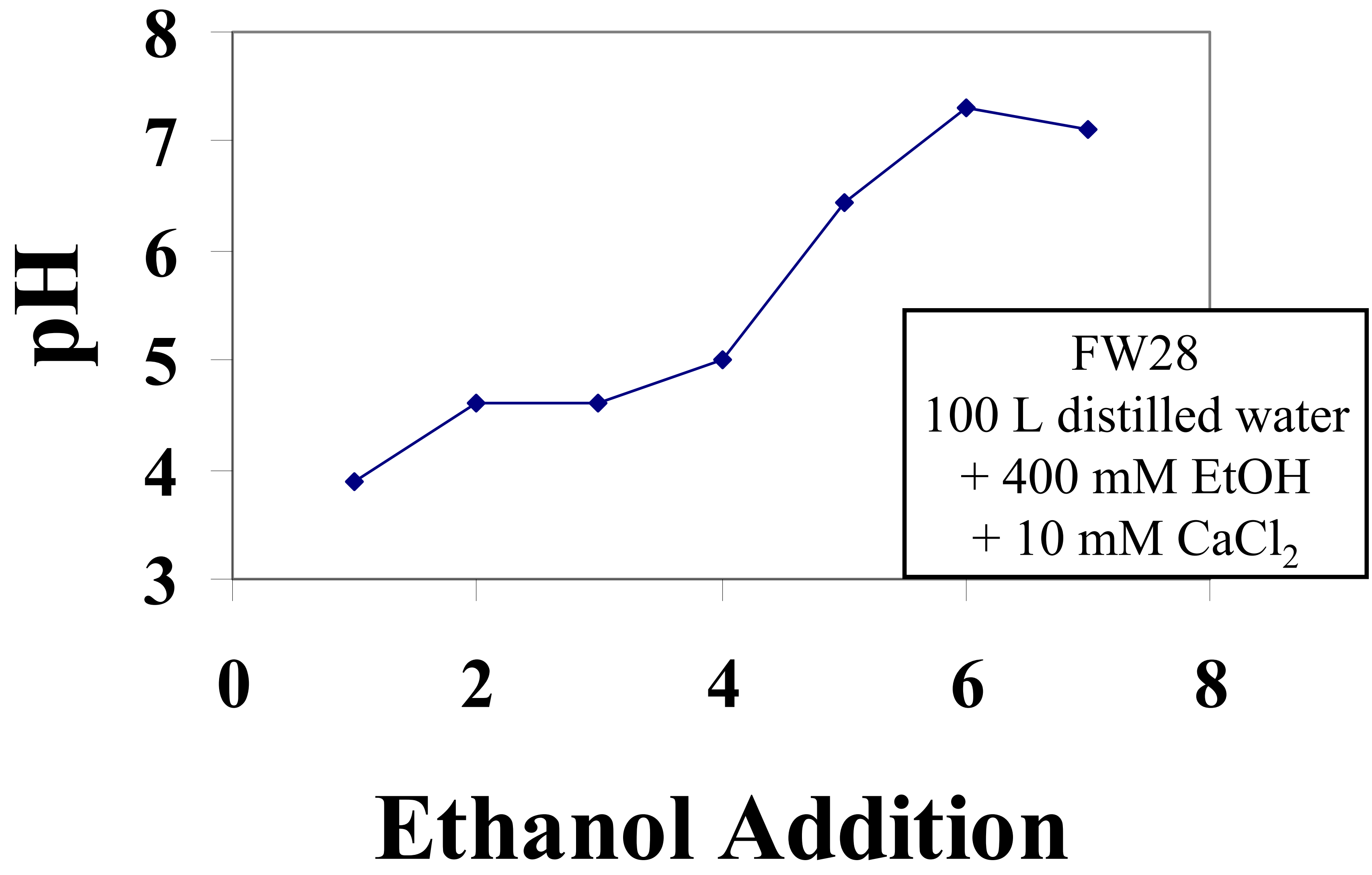
FW021
+ Br^-
+ HCO_3^-
+ EtOH

Rate Calculations: 140 mM Nitrate

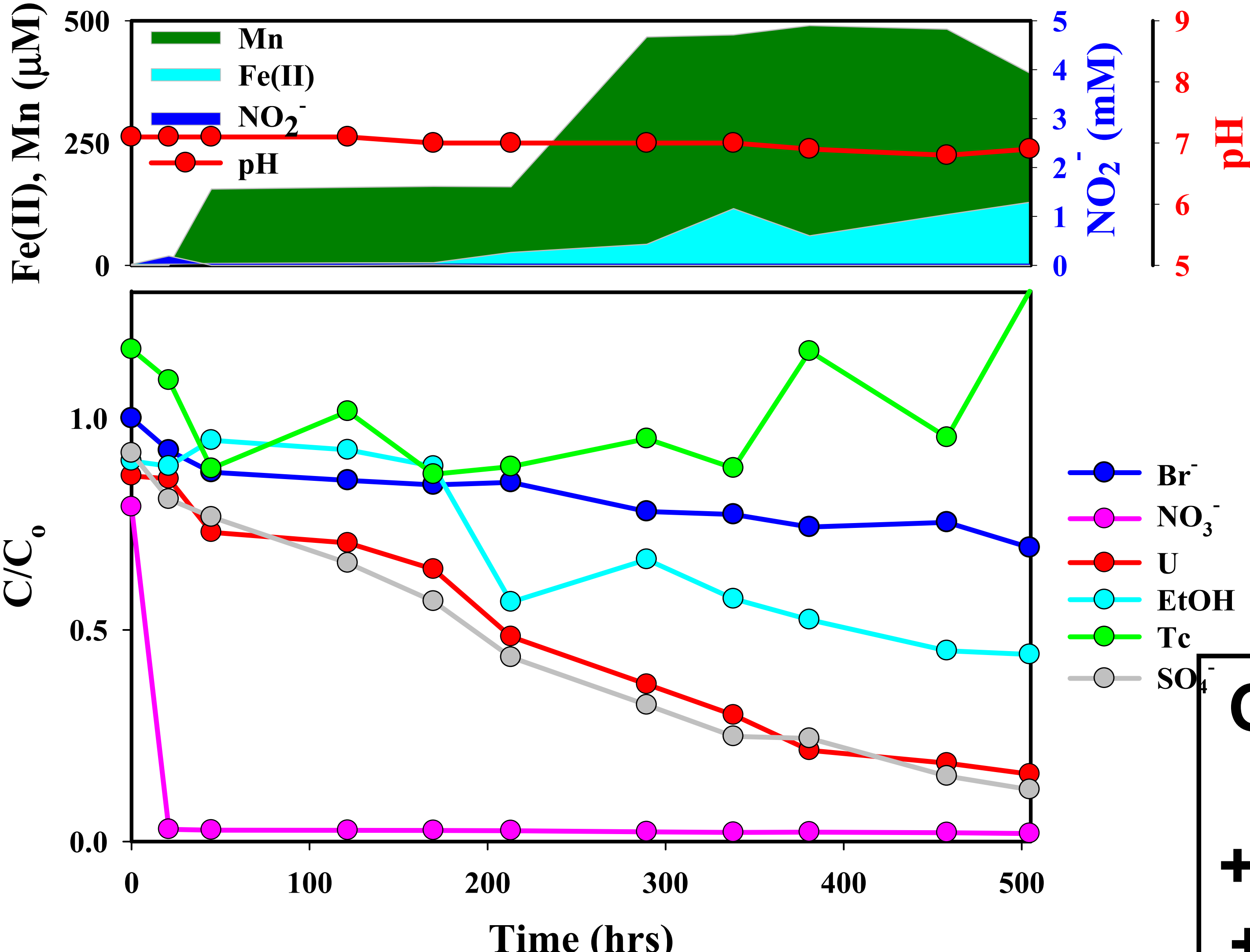


5.5 mM/hr 13 mM/hr 30 pM/hr +/-0.3 μM/hr

Effect of Biostimulation on pH

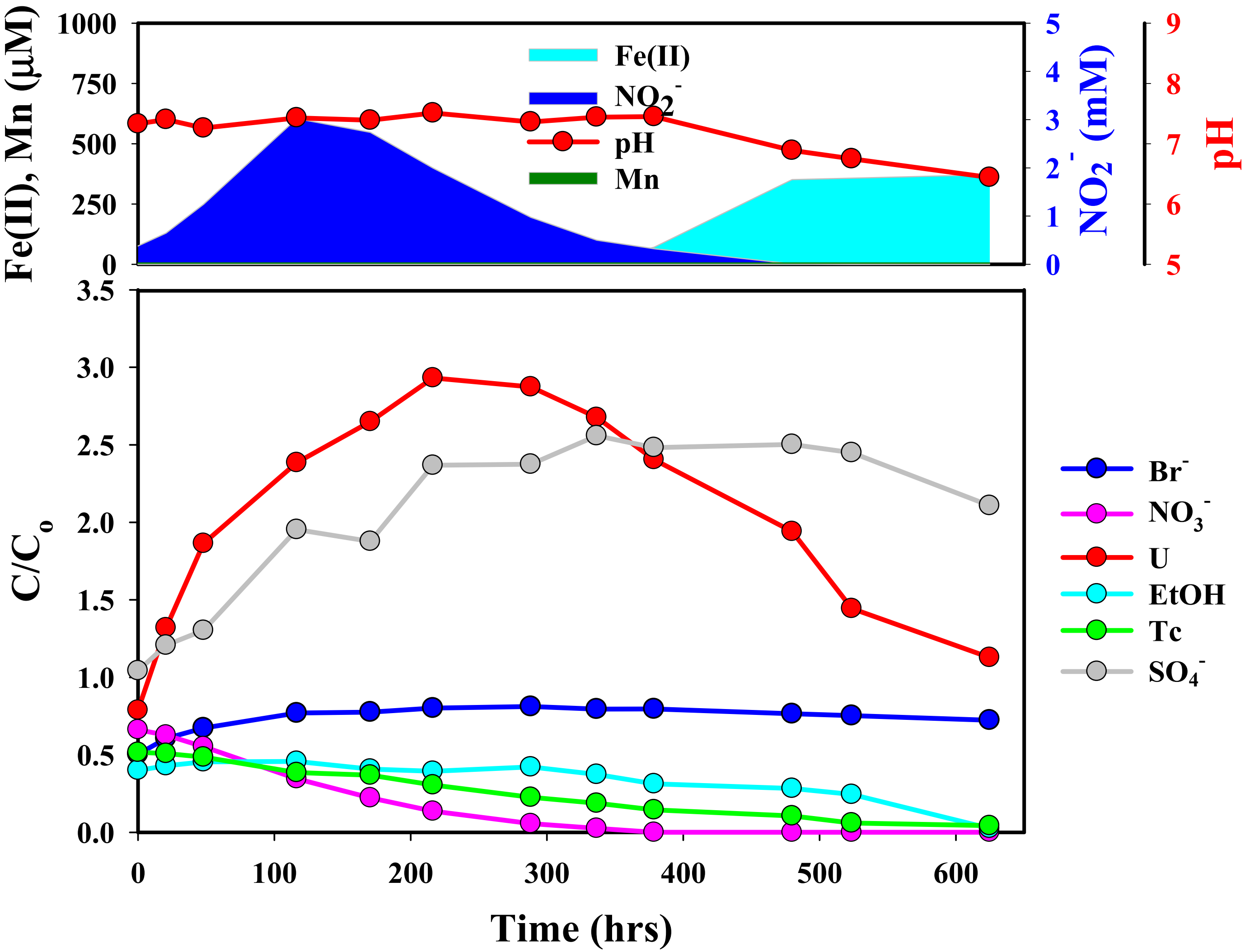


3.8 Initial pH, 1 mM Nitrate After Biostimulation



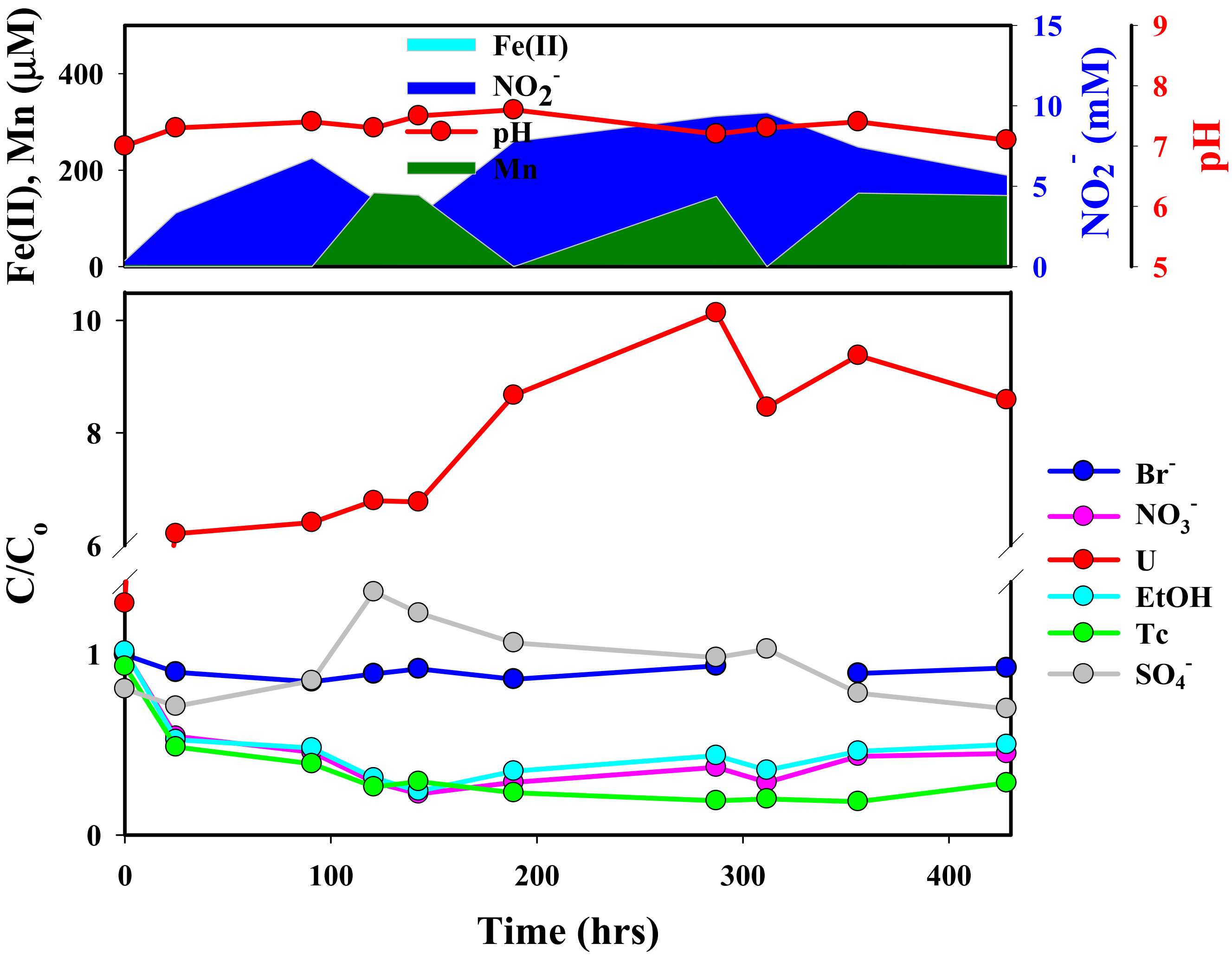
GW835
+ Br^-
+ HCO_3^-
+ EtOH

3.8 Initial pH, 140 mM Nitrate After Biostimulation



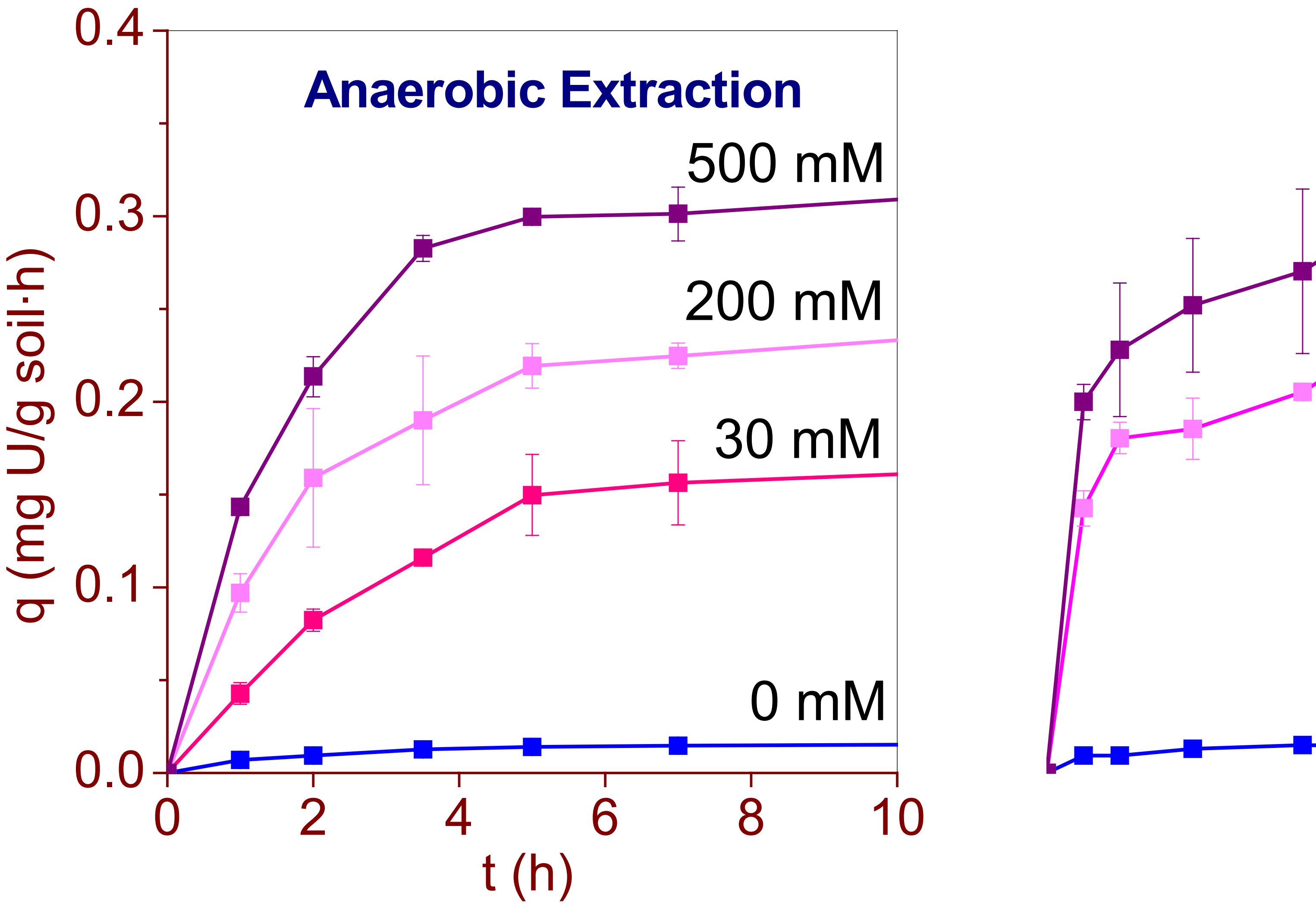
FW021
+ Br^-
+ HCO_3^-
+ EtOH

6.8 Initial pH, Area 2
100 mM Nitrate

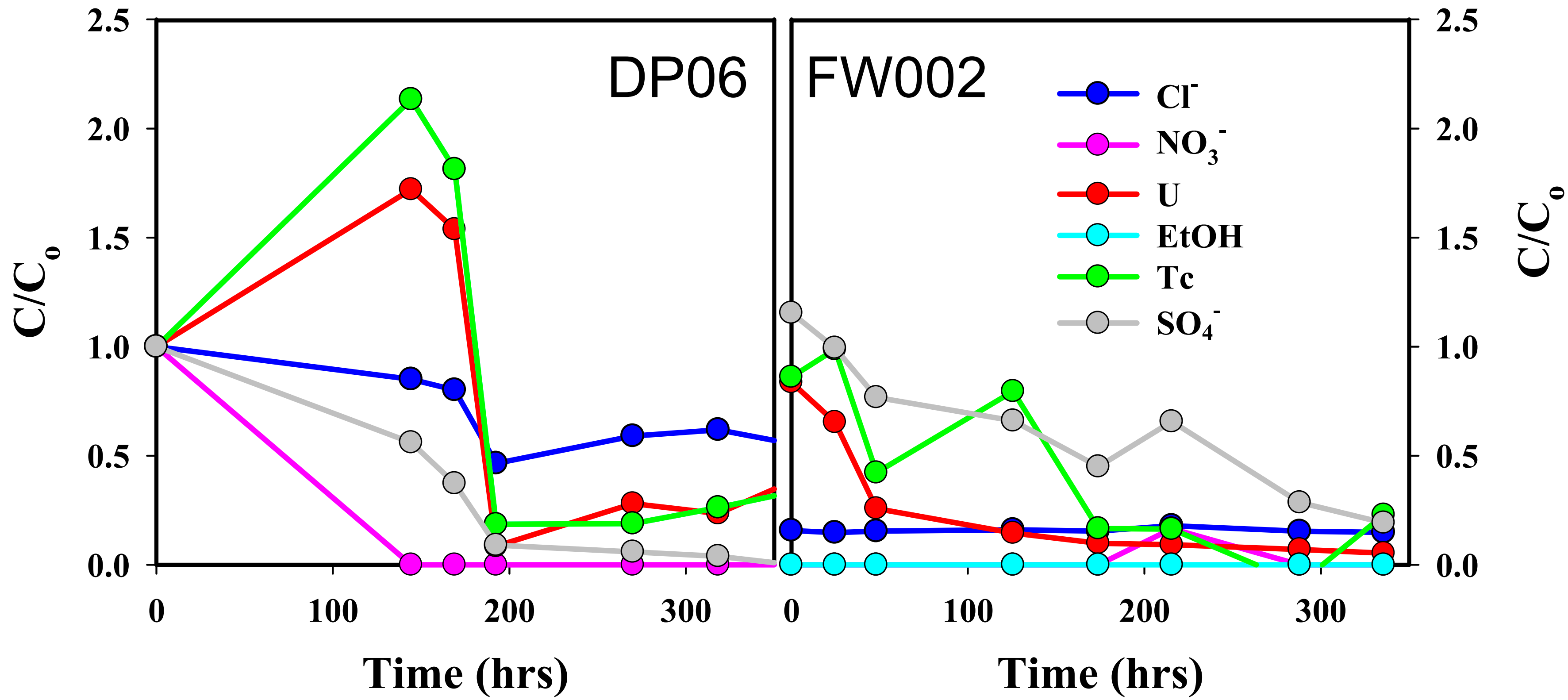


GW835
+ Br^-
+ NO_3^-
+ HCO_3^-
+ EtOH

Effect of $NaHCO_3$ Concentration on U(VI) Extraction

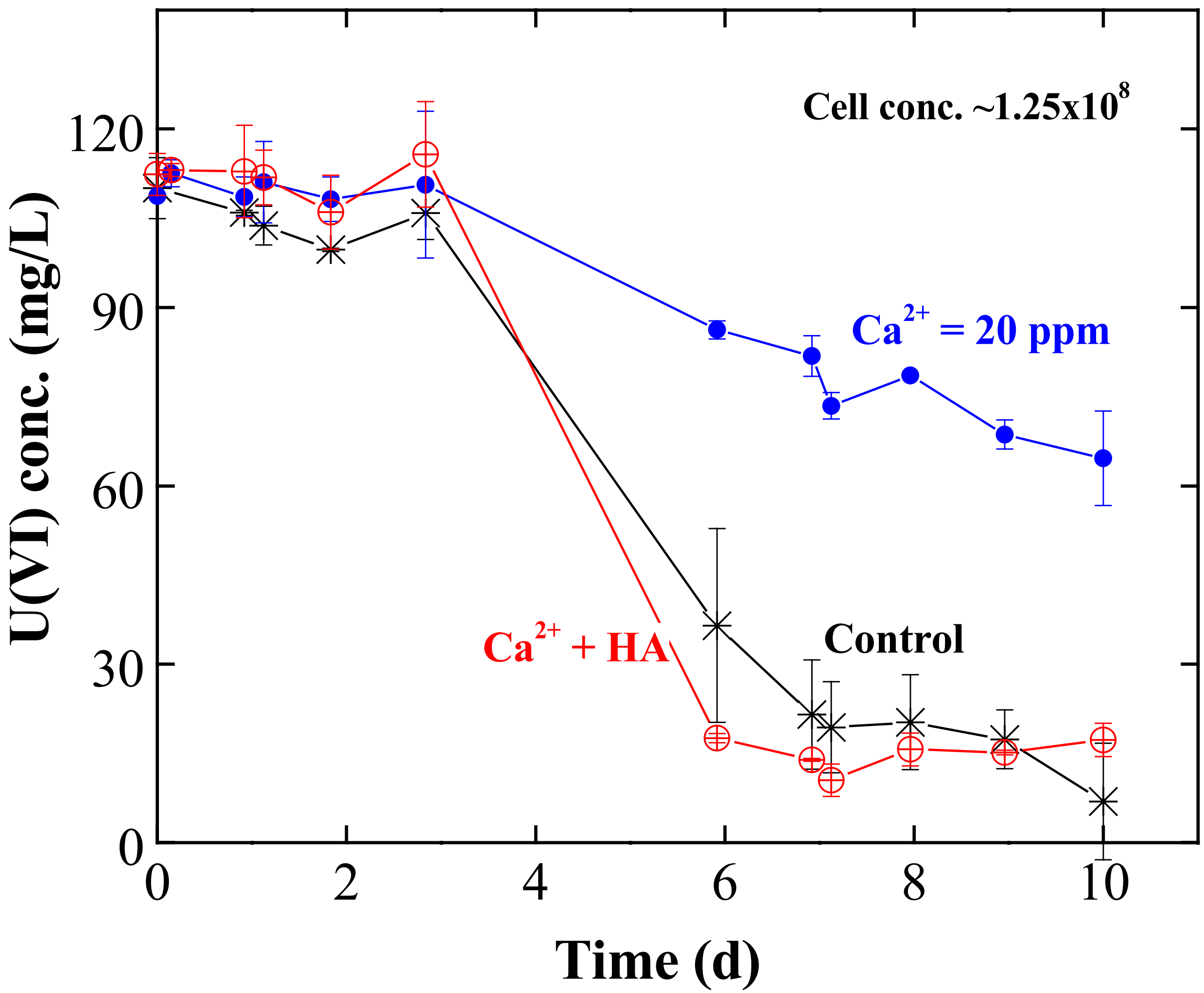


6.8 Initial pH, Area 2
100 mM HCO_3^-

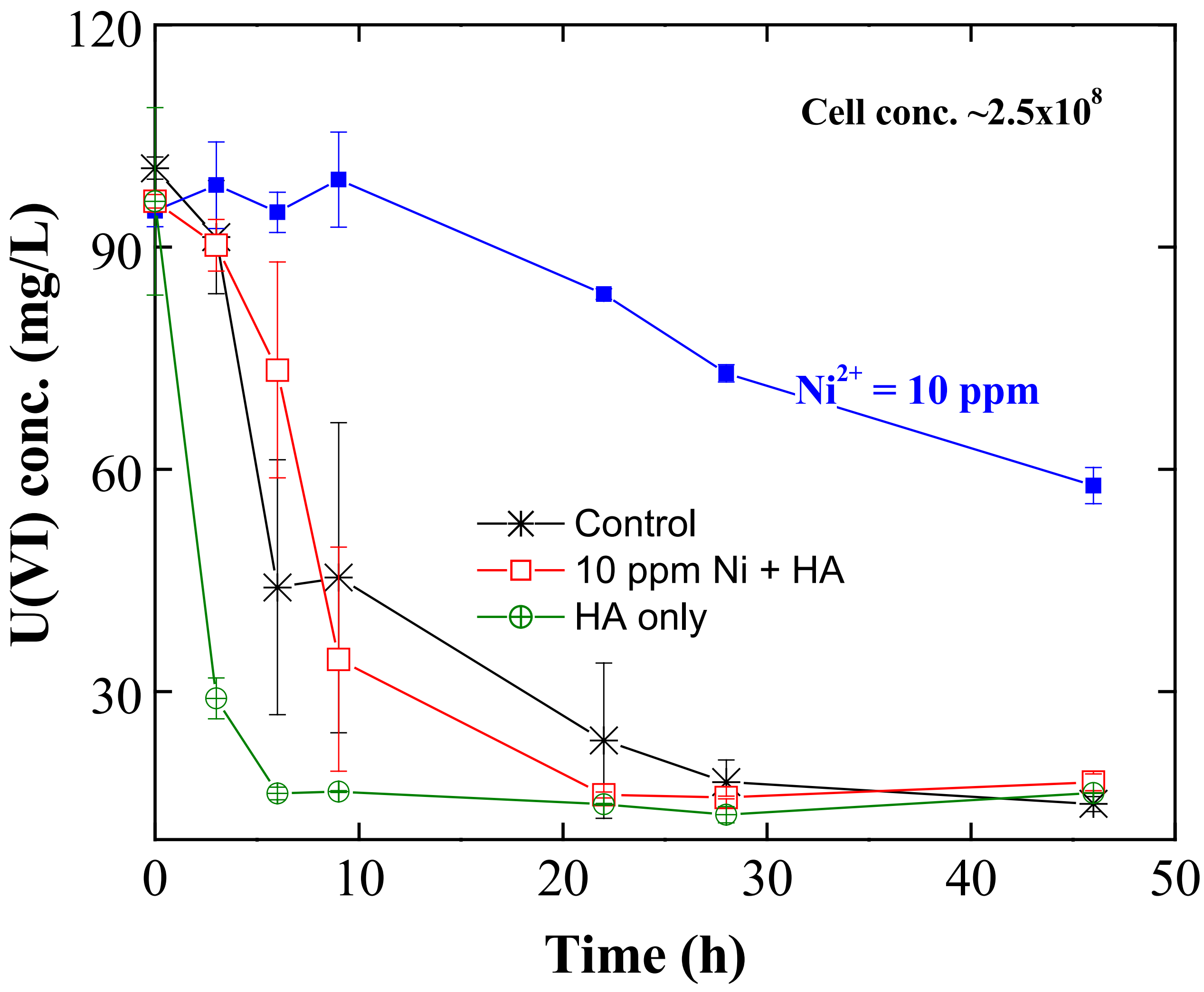


GW835 + Br^- + HCO_3^-

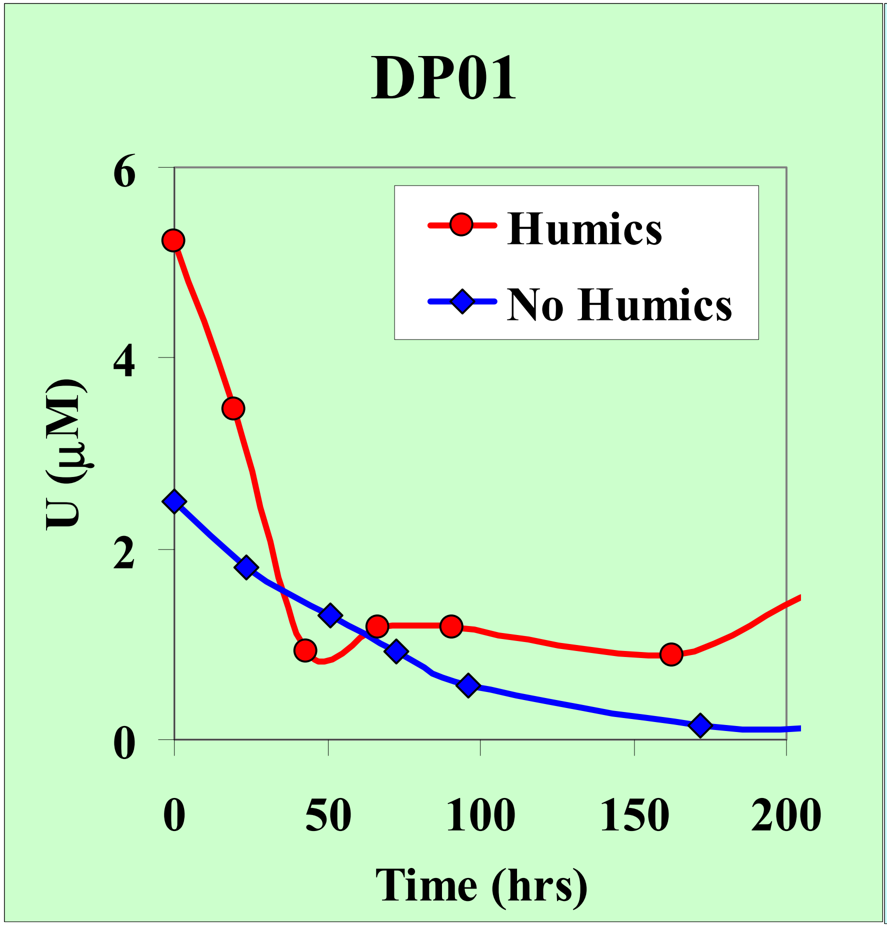
Effects of Ca^{2+} and HA on U(VI) Reduction

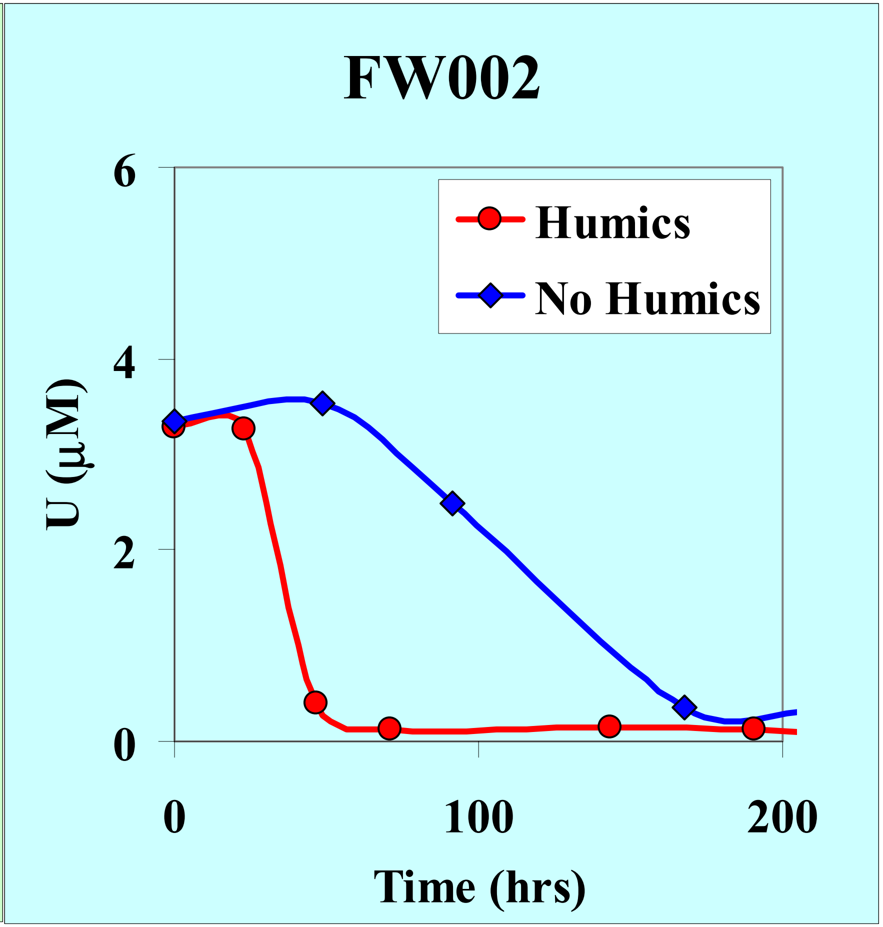


Effects of Ni^{2+} on U(VI) Reduction



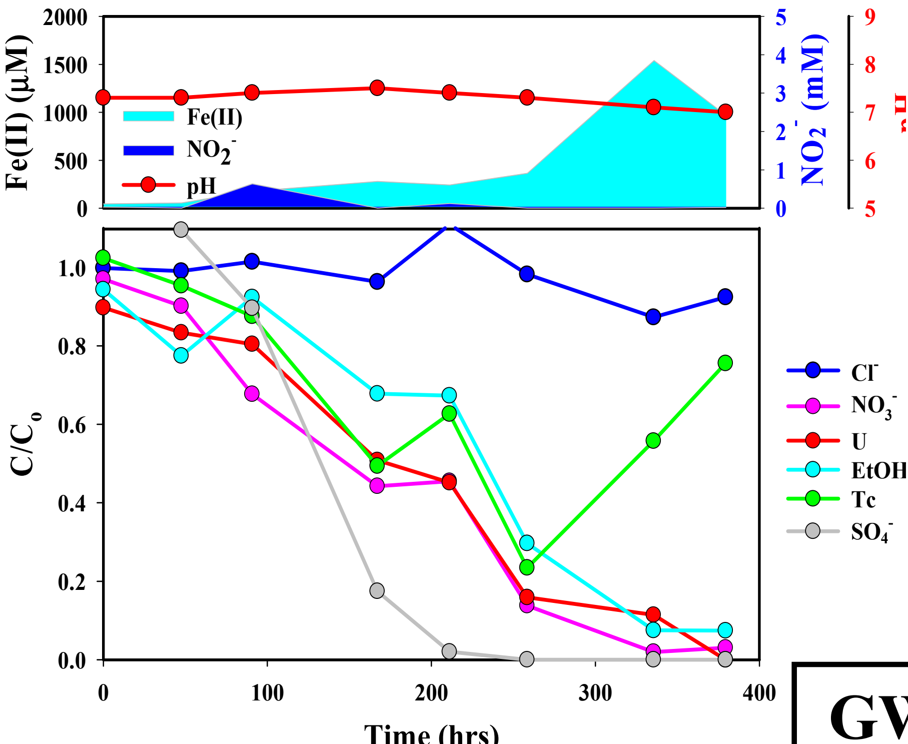
Effect of Added Humics on U(VI) Reduction (In Progress)



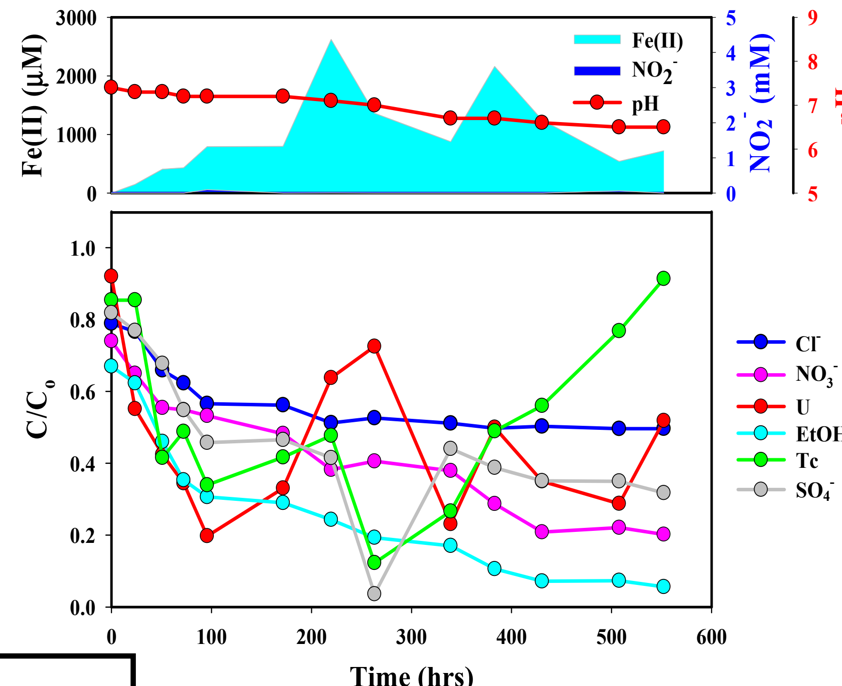


Sulfide Production Mitigates U(IV) Remobilization (In progress)

+ 20 mM sulfate + 20 mM nitrate



-Added sulfate + 20 mM nitrate



GW835
+ HCO_3^-
+ EtOH

Summary of In Situ Testing

Donor (ethanol, glucose, or acetate) additions increased pH and stimulated microbial activity in a wide range environments in shallow subsurface at FRC:

Initial Conditions

pH	NO_3^- (mM)	SO_4^{2-} (mM)	U(VI) (μM)	Tc(VII) (pM)
3.3-3.9	100-140	0-1	5-12	10000-15000
5.2-5.6	90-100	0-1	5-12	10000-15000
5.6-7.2	0-6	1-2	1-7	200-1000

Summary of In Situ Testing

- **Rates of denitrification, sulfate reduction, U(VI) and Tc(VII) in all environments tests were comparable following biostimulation**
- **High initial nitrate inhibits U(VI) reduction**
- **Added bicarbonate remobilizes U and Tc**
- **Added humics increased U(VI) reduction rates**

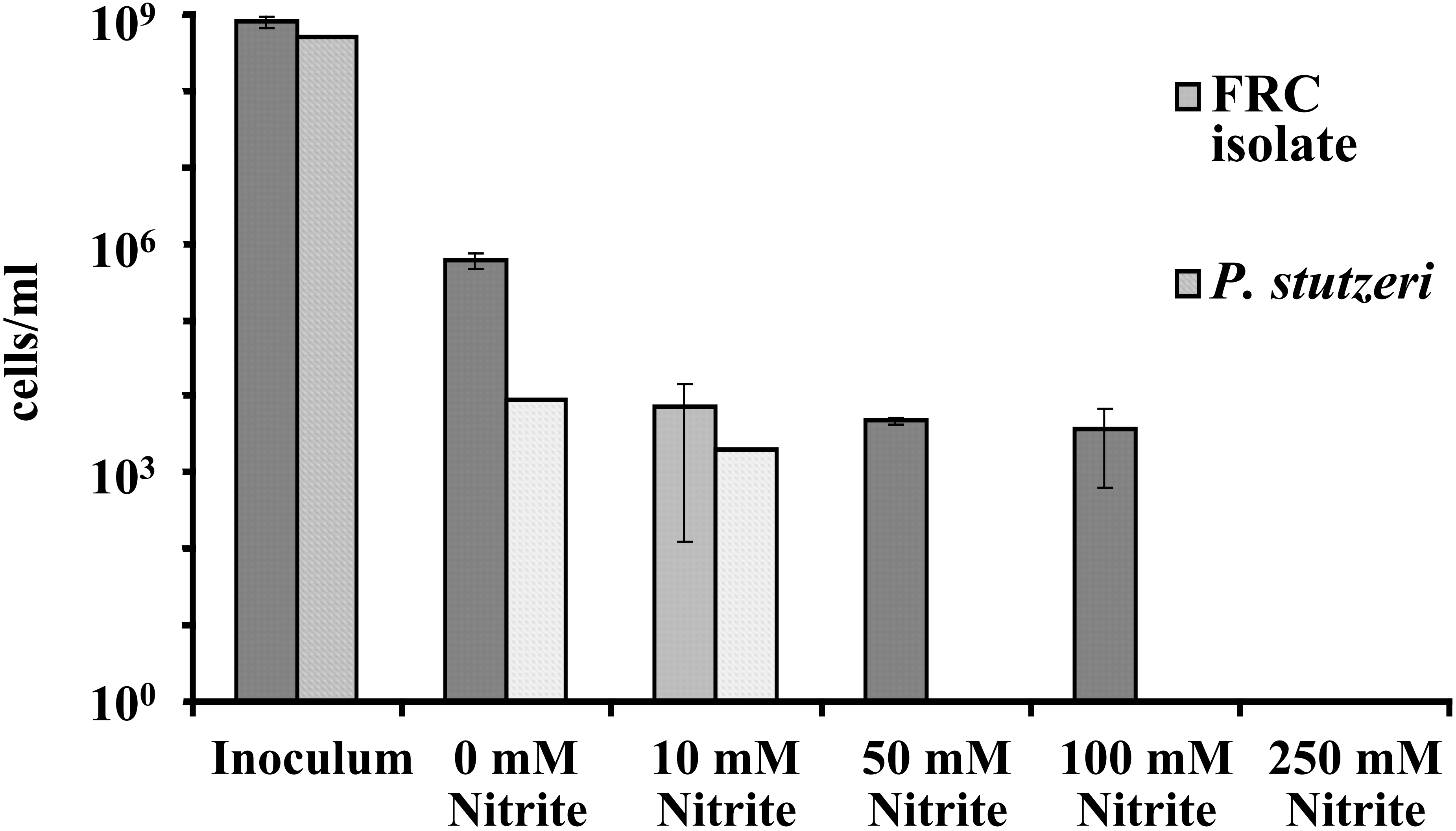
In Situ Activity Measurements

Initial pH	EtOH (mM/hr)	NO_3^- (mM/hr)	SO_4^{2-} (mM/hr)	U(VI) (μM/hr)	U(IV) (μM/hr)	Tc(VII) (pM/hr)
3.3 – 3.9	0.3 – 1.0	0.1 – 0.4	0 – 0.01	$10^{-4} – 10^{-3}$	$10^{-3} – 10^{-2}$	4 – 30
5.2 – 5.6	0.3 – 4.0	0.3 – 4.0	0 – 0.01	$10^{-4} – 10^{-3}$	$10^{-3} – 10^{-2}$	10 – 150
5.6 – 7.2	0.1 – 2.0	0.1 – 2.0	0 – 0.03	$10^{-4} – 10^{-3}$	$10^{-3} – 10^{-2}$	4 - 10

Denitrifying Isolates (A. Spain)

Isolate ID	Phylogenetic affiliation	Optimal pH	Min. pH with growth	Nitrite accum. at low pH?	Nitrite reductase	Nitrite reductase
GN 32#1	*Agrobacterium tumefaciens*	6.5	5.5	no	Nap only	nirK
GN 32#2	*Agrobacterium tumefaciens*	6.5	4.5	no	Nap only	N/A
GN 32#3	*Agrobacterium tumefaciens*	6.5	5.5	no	Nap only	nirK
GN 33#1	*Pseudomonas* sp.	8.0	6.0	yes	Nap and Nar	nirK
AN 33#1	*Klebsiella pneumoniae*	8.0	5.5	yes	Nap and Nar	N/A

Effect of Nitrite on Survival in Laboratory Incubations (J. Senko)



Results from NABIR Collaborators

Conclusion that donor additions stimulated the growth and activity of metal-reducing organisms (e.g. *Geobacter*) supported by findings of NABIR collaborators:

- **PLFA, DMA, DGGE of 16s rRNA (groundwater, microbial samplers, sediments: A. Peacock, D. White, J. Chang)**
- **16s rRNA, Q-PCR (sediments): N. North, S. Dollhopf, L. Petrie, D. Balkwill, J. Kostka)**
- **Mossbauer spectroscopy (sediments), J. Stucki)**

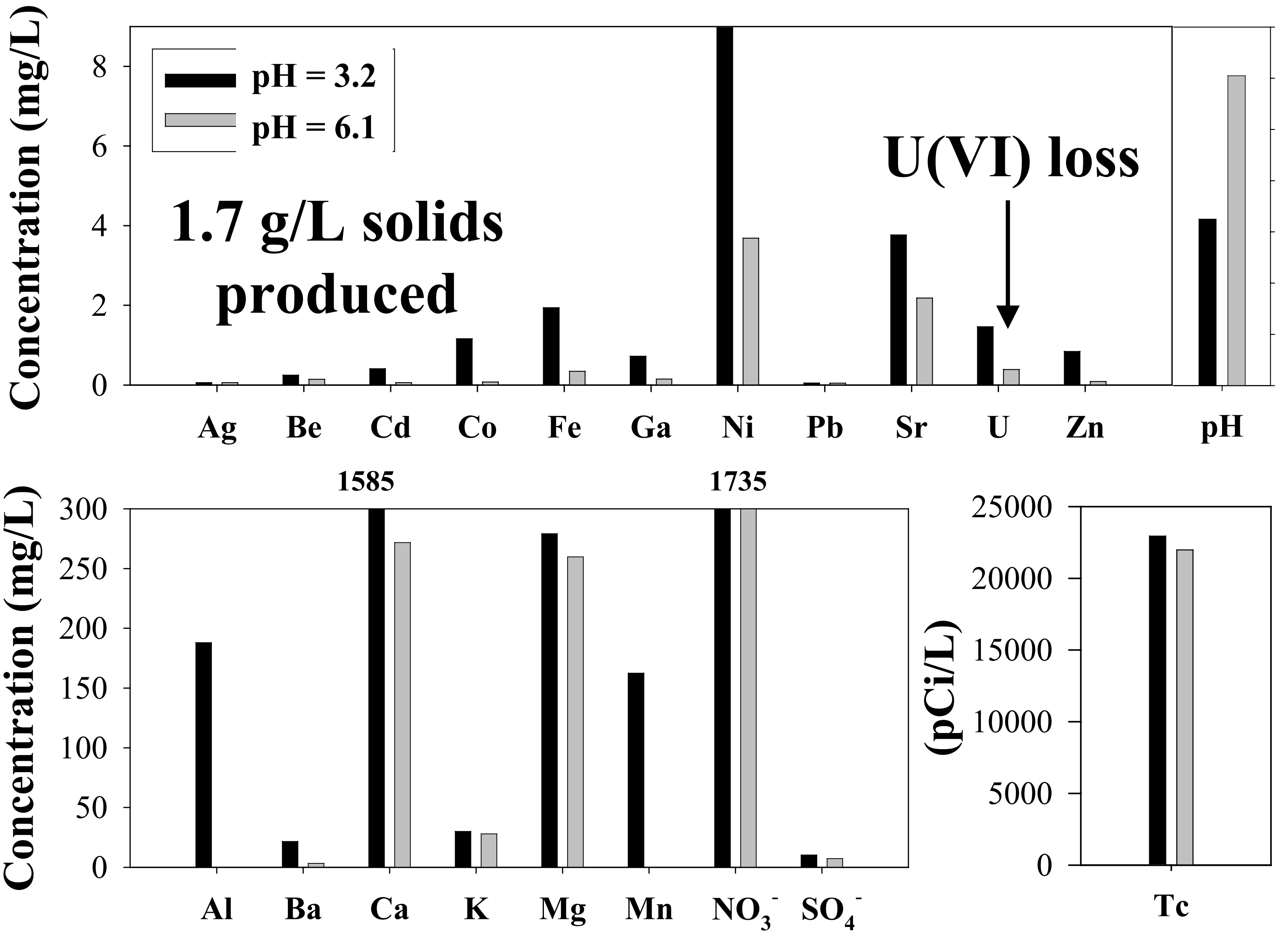
Some Additional Comments

- **Desired metabolic capability is widespread in shallow subsurface at FRC**
- **Nitrate removal necessary for U(VI) reduction**
- **pH increases resulting from donor addition will produce precipitates containing U(VI) from low pH groundwater**
- **Clogging of aquifer by precipitates, biomass, and (perhaps) N_2 gas is possible in the long-term**

Effect of Biostimulation on Aquifer Hydraulic Conductivity (partial data set)

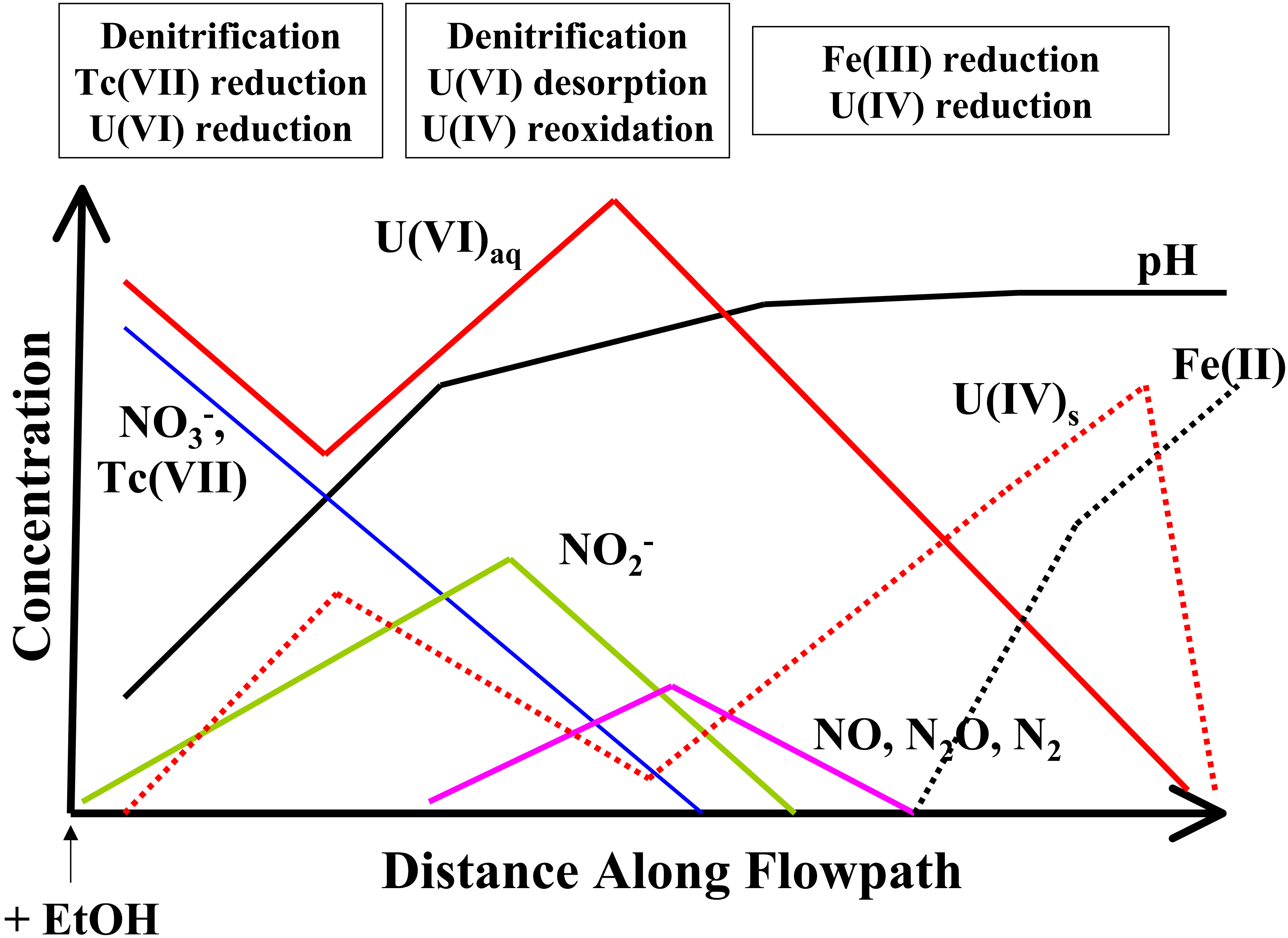
Well	Initial	Final
DP15D	16.5	16.7
DP01	17.5	16.7
DP06	2.0	2.2
FW002	2.8	2.9
FW003	2.8	2.9
FW34	250	0.4
FW28	106	3.2
FW29	190	8.3
FW30	800	14.3

Precipitate Formation



Collaboration with EMSL Flow and Transport Lab (M. Oostrom, T. Wietsma)

- FRC Background Sediment and Maynardsville Limestone
- Denitrifying activity stimulated with ethanol
- Gas and liquid saturations monitored to track fate of N_2 gas


Denitrification
Tc(VII) reduction
U(VI) reduction
Denitrification
U(VI) desorption
U(IV) reoxidation
Fe(III) reduction
U(IV) reduction
$U(VI)_{aq}$
pH
Fe(II)
$U(IV)_s$
NO_3^-,
Tc(VII)
NO_2^-
NO, N_2O, N_2
Concentration
Distance Along Flowpath
+ EtOH

Intermediate-Scale Physical Models

Currently 4 models:
GW835 and FW021
+ EtOH and – EtOH controls