Thermodynamic network model for predicting effects of substrate addition and other perturbations on subsurface microbial communities

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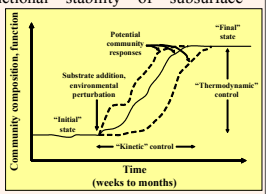
More information on this and related projects can be obtained at: *https://web.engr.oregonstate.edu/~istokj/grl-main.htm*

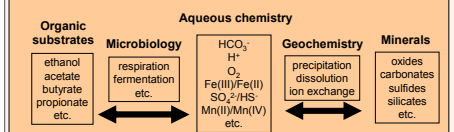
Introduction

The overall goal of this project is to develop and test a thermodynamic network model for predicting the effects of substrate additions and environmental perturbations on the composition and functional stability of subsurface microbial communities. The hypothesis is that a thermodynamic analysis of the energy-yielding reactions performed by broadly defined groups of microorganisms can be used to make quantitative and testable predictions of the change in microbial community composition that will occur when a substrate is added to the subsurface or when environmental conditions change.



Model Development

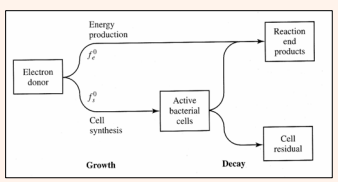
Coupling Microbial and Geochemical Processes



Assumptions

Define microbial groups based on ability to obtain free energy for growth from a particular reaction

Substrate partitioning and cellular yield



Growth Equations (after Rittman and McCarty, 2001)

$$R_i = f_{e,i} R_{a,i} + f_{s,i} R_{c,i} - R_{d,i} \quad (1)$$

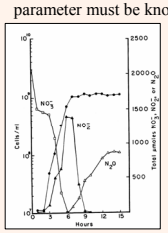
R_i = growth reaction for group i
$f_{e,i}$ = portion of electrons used for energy production
$f_{s,i}$ = portion of electrons used for cell synthesis
$R_{a,i}$ = electron acceptor half-reaction
$R_{c,i}$ = synthesis reaction
$R_{d,i}$ = electron donor half-reaction

Equation 1 is written once for every combination of donor and acceptor utilized by each metabolic group.

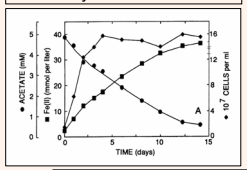
genes and lipid analysis.

Model Development cont.

A graphical user interface was developed to implement this approach. The user selects electron acceptor, electron donor, nitrogen source, and electron transfer efficiency. Application computes overall growth equation and thermodynamic constants. Energy transfer efficiency, ε parameter must be known for each growth equation.



Estimating electron transfer efficiency from the literature



Lactate
Cells grown = 6.0 x 10⁸
Nitrate consumed = 2 mM
ε = 0.69; f_s = 0.75; log K = -226
Chromobacterium violaceum
Bazylinski et al., AEM, 52(4):696-699

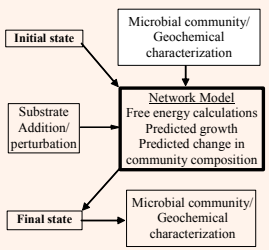
Acetate consumed = 36 mM
Cells grown = 1.5 x 10⁸
Fe(II) produced = 30 mM
ε = 0.43; f_s = 0.42; log K = -227
Desulfuromonas acetoxidans
Roden and Lovley, AEM, 59(3):734-742

Coupling Microbiology and Geochemistry

The Geochemist's Workbench® is a set of software tools for manipulating chemical reactions, calculating stability diagrams and the equilibrium states of natural waters, tracing reaction processes, and plotting the results of these calculations.

Growth equations and free energy values for defined microbial groups are added to the existing chemical thermodynamic data base.

Reactions paths are computed as a series of equilibrium states by minimizing overall system (microbiology and geochemistry) free energy.



Input
Water chemistry
Mineralogy
Boundary conditions (open vs closed system, etc.)
Numbers and types of microbial groups

Output
Water chemistry
Minerals formed
Cells grown for each group

Model Validation

We intend to amplify 16S rRNA genes and separate products using denaturing gradient gel electrophoresis. Sequencing of the rRNA genes will allow us to identify the major components of the microbial community as well as to monitor changes in composition of the community. Second, we will quantify functional groups using a combination of functional genes assessed quantitatively by Quantitative-PCR and the analysis of lipid composition.

Example 1

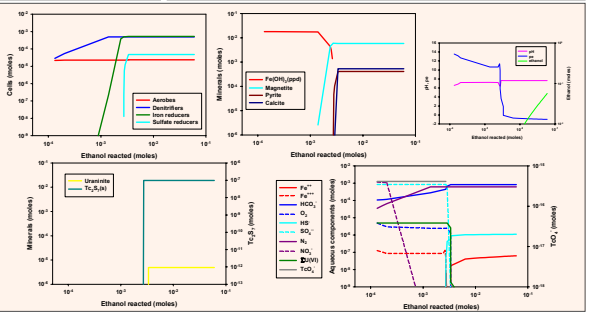
Reducing Area 2 groundwater/ sediment system with ethanol. Cell growth and minerals formed.

Geochemistry
pH 6.43

O_2	68.0	μM
NO_3^-	1.21	mM
SO_4^{2-}	0.83	mM
Fe(III) in sediment as Fe(OH)3(ppd)		
HCO_3^-	90.0	μM
Ca^{2+}	3.52	mM
Mg^{2+}	1.11	mM
K^+	0.12	mM
Al^{3+}	0.06	μM
Na^+	1.08	mM
Cl^-	0.65	mM
U	4.90	μM
Tc	411	pM

Microbiology
- NH_4^+ as nitrogen source
- Ethanol as electron donor
- Four functional groups
  - "Aerobes"
  - "Denitrifers"
  - "Iron reducers"
  - "Sulfate reducers"

	moles	grams	volume (cm3)
Iron Reducers	5.2 x10^{-4} (48%)	0.059	0.059
Denitrifiers	5.0 x10^{-4} (45%)	0.056	0.056
Sulfate Reducers	4.8 x10^{-5} (4%)	0.005	0.005
Aerobes	2.4 x10^{-5} (2%)	0.003	0.003
Magnetite	5.9 x 10^{-3}	1.36	0.261
Calcite	5.3 x 10^{-4}	0.053	0.02
Pyrite	4.1 x 10^{-4}	0.05	0.01
Uraninite	4.9 x 10^{-6}	0.001	0.0001
NiS$_2$S4	3.8 x 10^{-7}	0.0001	0.00002
Tc2S7	9.7 x 10^{-8}	0.00004	-



Example 2

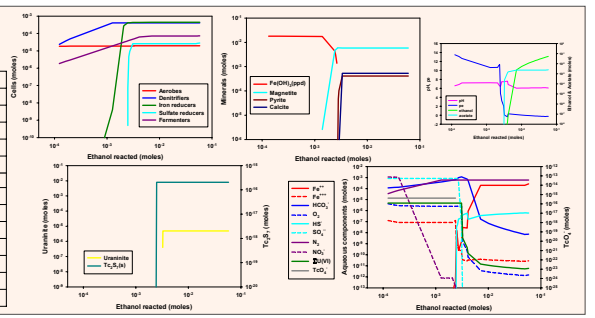
Cell growth and minerals formed.

Geochemistry
pH 6.43

O_2	68.0	μM
NO_3^-	1.21	mM
SO_4^{2-}	0.83	mM
Fe(III) in sediment as Fe(OH)3(ppd)		
HCO_3^-	90.0	μM
Ca^{2+}	3.52	mM
Mg^{2+}	1.11	mM
K^+	0.12	mM
Al^{3+}	0.06	μM
Na^+	1.08	mM
Cl^-	0.65	mM
U	4.90	μM
Tc	411	pM

Microbiology
- NH_4^+ as nitrogen source
- Ethanol as electron donor
- Nine functional groups
  - "Aerobes" (ethanol or acetate)
  - "Denitrifers" (etc.)
  - "Iron reducers"
  - "Sulfate reducers"
  - "Fermenters"

	Example 2 (moles)	Example 1 (moles)
Iron Reducers	4.5 x10^{-4} (46%)	5.2 x10^{-4} (48%)
Denitrifiers	4.1 x10^{-4} (42%)	5.0 x10^{-4} (45%)
Sulfate Reducers	2.7 x10^{-5} (3%)	4.8 x10^{-5} (4%)
Aerobes	2.0 x10^{-5} (2%)	2.4 x10^{-5} (2%)
Fermenters	7.4 x 10^{-5} (7%)	-
Magnetite	5.9 x 10^{-3}	1.36
Calcite	5.2 x 10^{-4}	0.053
Pyrite	4.1 x 10^{-4}	0.05
Uraninite	4.9 x 10^{-6}	0.001
NiS$_2$S4	3.8 x 10^{-7}	0.0001
Tc2S7	9.7 x 10^{-8}	0.00004
Pyrrhotite	6.2 x 10^{-4}	-
Gibbsite	3.7 x 10^{-6}	-



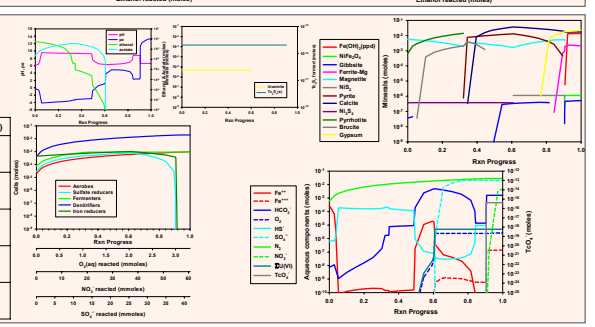
Example 3

Reoxidation of reduced system. Predicted change in community composition (NH_4^+ not limiting).

React reduced system with 50 volumes of original groundwater (NH_4^+ not limiting)

Initial Reduced Conditions (mol)		Reacting masses:		
0.0026	Acetate	O_2	106	mg
0.0551	Ethanol	NO_3^-	60.5	mM
0.0011	Na+	SO_4^{2-}	41.5	mM
2.1e-16	$Tc_2S_3(s)$	Ca^{2+}	176	mM
4.9e-6	Uraninite	Cl^-	32	mM
0.0057	Magnetite	HCO_3^-	4.5	mM
8.7e-5	Pyrite	Na^+	54	mM
0.0007	Pyrrhotite	Mg^{2+}	55	mM
1.9e-5	"Aerobes"	Al^{3+}	5e-4	mM
7.4e-5	"Fermenters"	K^+	6.2	mM
4.5e-4	"Iron reducers"			
2.9e-5	"Sulfate reducers"			
4.1e-4	"Denitrifiers"			

	Example 2 (moles)	Example 3 (moles)
Iron Reducers	4.5 x10^{-4} (45%)	~ 0
Denitrifiers	4.1 x10^{-4} (42%)	0.019 (91%)
Sulfate Reducers	2.7 x10^{-5} (3%)	~ 0
Aerobes	2.0 x10^{-5} (2%)	9.7 x10^{-4} (5%)
Fermenters	7.4 x10^{-5} (7%)	9.0 x10^{-4} (4%)



Example 4

Reoxidation of reduced system. Predicted change in community composition (NH4+ limiting).

React reduced system with 50 volumes of original groundwater: no NH_4^+ supplied

Initial Conditions (mol)		Reacting mass:		
0.0026	Acetate	O_2	106	mg
0.0551	Ethanol	NO_3^-	60.5	mM
0.0011	Na+	SO_4^{2-}	41.5	mM
2.1e-16	$Tc_2S_3(s)$	Ca^{2+}	176	mM
4.9e-6	Uraninite	Cl^-	32	mM
0.0057	Magnetite	HCO_3^-	4.5	mM
8.7e-5	Pyrite	Na^+	54	mM
0.0007	Pyrrhotite	Mg^{2+}	55	mM
1.9e-5	"Aerobes"	Al^{3+}	5e-4	mM
7.4e-5	"Fermenters"	K^+	6.2	mM
4.5e-4	"Iron reducers"			
2.9e-5	"Sulfate reducers"			
4.1e-4	"Denitrifiers"			

	Example 2 (moles)	Example 3 (moles)	Example 4 (moles)
Iron Reducers	4.5 x10^{-4} (45%)	~ 0	~ 0
Denitrifiers	4.1 x10^{-4} (42%)	0.019 (91%)	~ 0
Sulfate Reducers	2.7 x10^{-5} (3%)	~ 0	5.4 x10^{-4} (55%)
Aerobes	2.0 x10^{-5} (2%)	9.7 x10^{-4} (5%)	0
Fermenters	7.4 x10^{-5} (7%)	9.0 x10^{-4} (4%)	4.4 x10^{-4} (45%)
	(+ NH_4^+)	(+ NH_4^+)	(- NH_4^+)