Cargèse Summer School 2018

Bioenergetics and Geomicrobiology

(Are microbes better at thermodynamics than geochemists?)

Philippe Van Cappellen and Christina Smeaton

UNIVERSITY OF WATERLOO

Canada Excellence Research Chairs
Chaires d'excellence en recherche du Canada

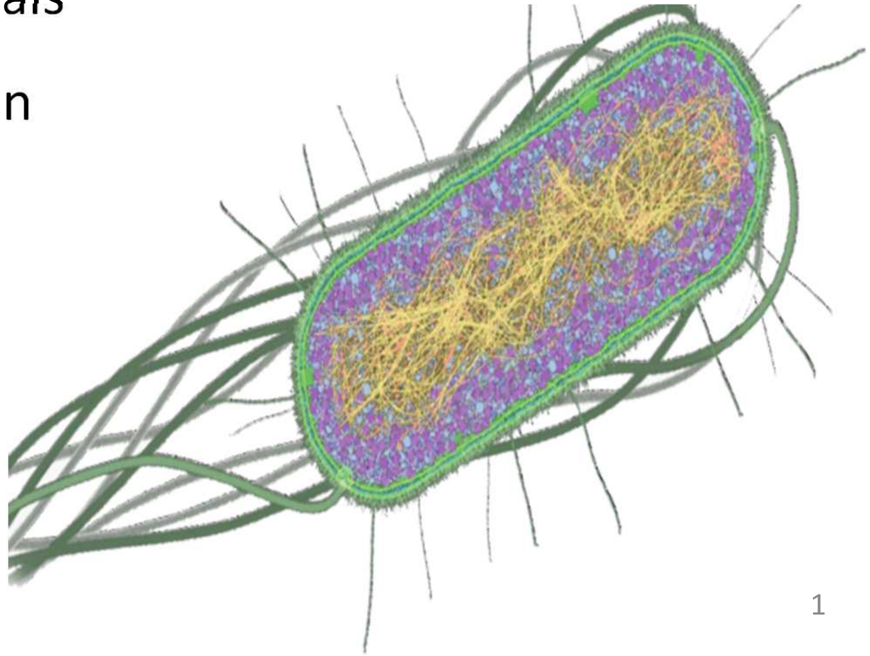
the water INSTITUTE
University of Waterloo

ecohydrology research group

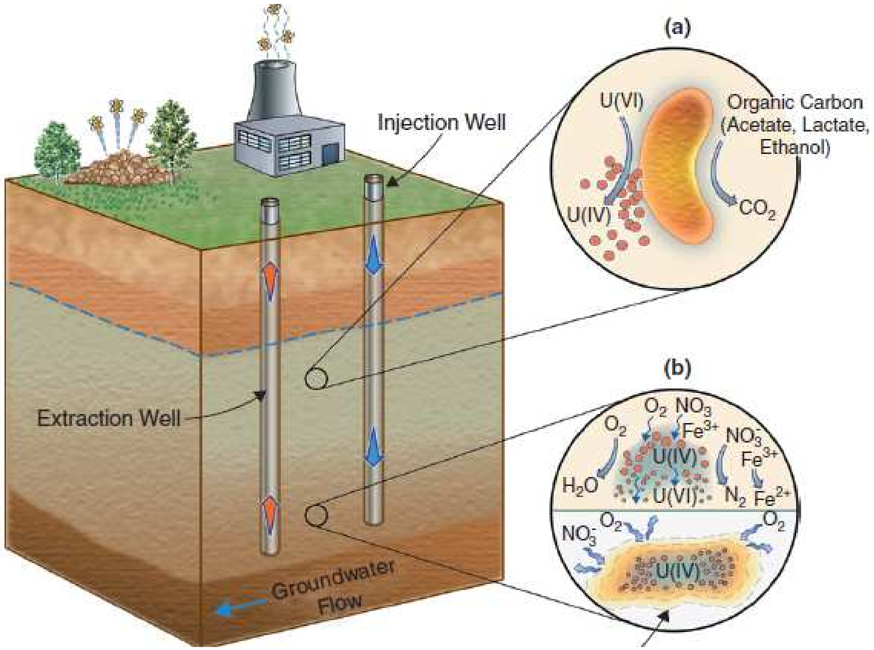
Geomicrobial Activity

- Cycling of carbon and nutrients (N, S, P, K, Si, Ca, Fe, …)
- Weathering, soil formation, soil fertility, water quality
- Organic matter decomposition, transformation and preservation
- Production greenhouse (CO_2, CH_4, N_2O) and other reactive gases
- Biomineralization, bio(nano)materials
- Natural attenuation, bioremediation
- Biotechnology
- Green (bio)chemistry

...

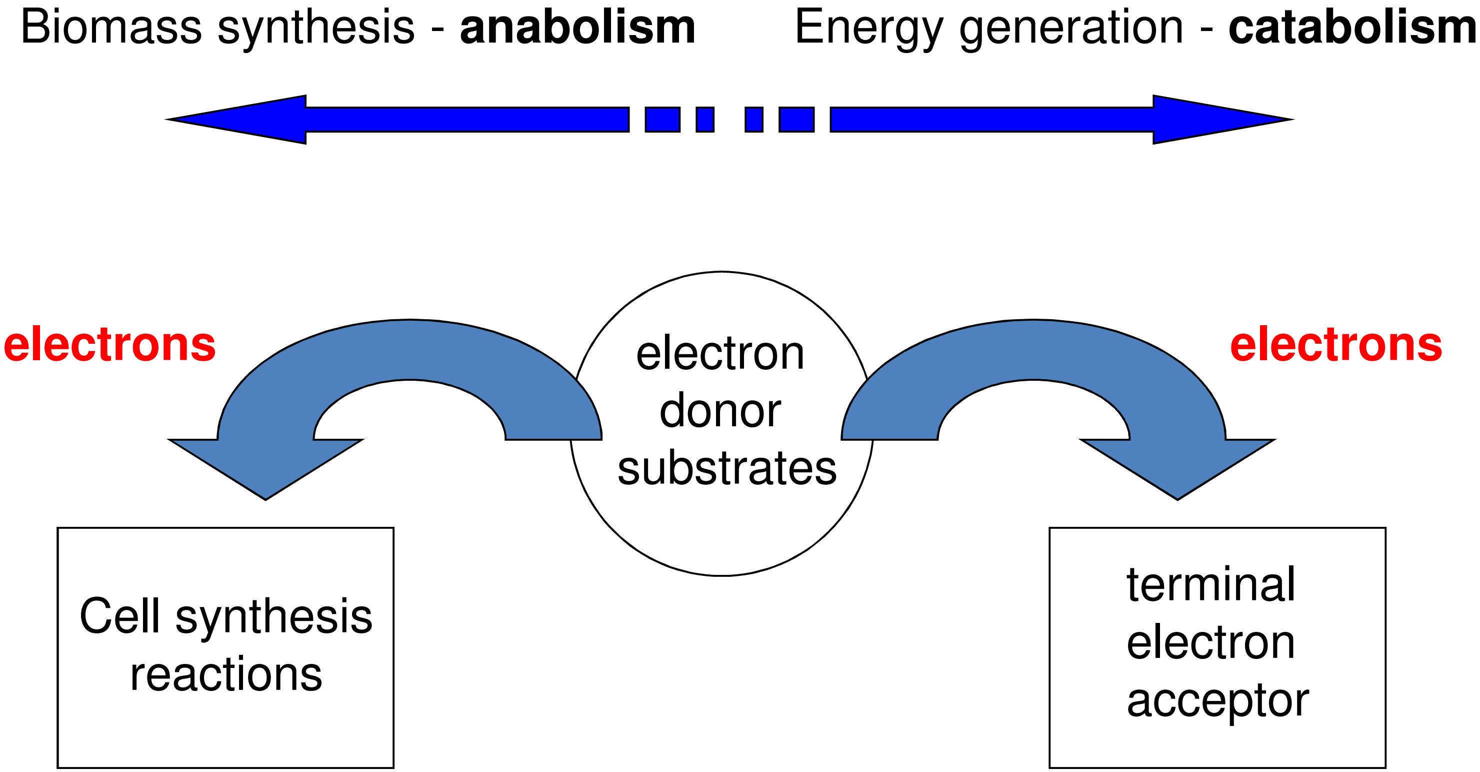


Geomicrobial Activity in the Subsurface



- Microbial ecosystems
- Ecological interactions
  - competition, syntrophy,
  - predation, energy flow
- Complex reaction networks
  - biotic-abiotic
- Energy-limited environments

Life = Redox Chemistry



Biomass synthesis - **anabolism**

Energy generation - **catabolism**

electrons

electron donor substrates

electrons

Cell synthesis reactions

terminal electron acceptor

Example

Aerobic cell growth on citric acid: macrochemical reaction:

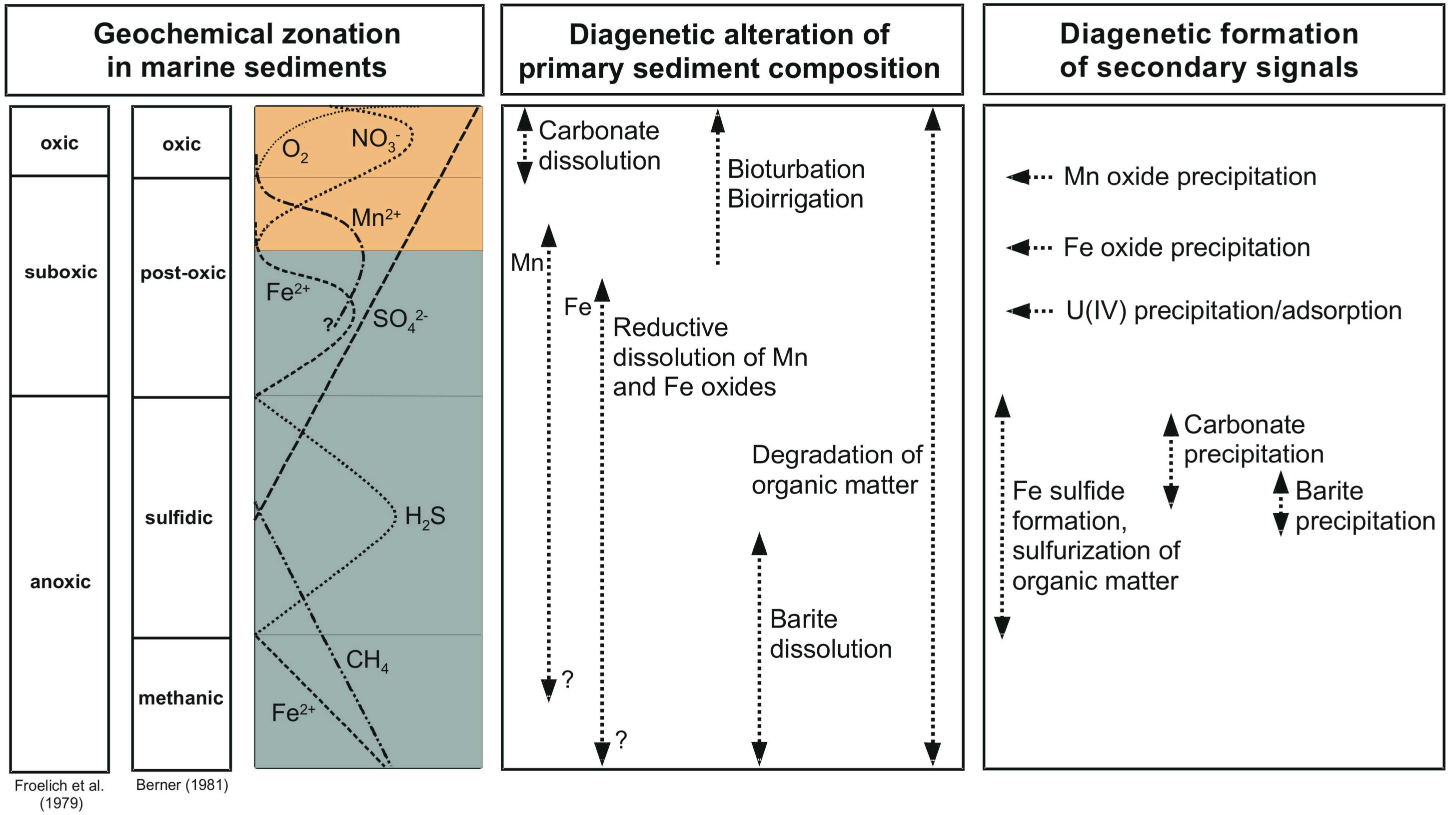
$$\overset{+1}{a\mathrm{C_6H_8O_7}} + b\mathrm{O_2} + c\mathrm{NH_4^+} \longrightarrow \underbrace{\overset{-0.2}{d\mathrm{CH_2O_{0.6}N_{0.2}}}}_{\text{new biomass}} + \overset{+4}{e\mathrm{CO_2}} + f\mathrm{H^+} + g\mathrm{H_2O}$$

Citric acid ($\mathrm{C_6H_8O_7}$): both electron donor for energy production and for biomass synthesis.

Growth yield:

Here: $Y_G = d/6a$ (mol C/mol C)

Redox Zonation



Geochemical zonation in marine sediments

Diagenetic alteration of primary sediment composition

Diagenetic formation of secondary signals

oxic
suboxic
anoxic

Froelich et al. (1979)

oxic
post-oxic
sulfidic
methanic

Berner (1981)

Carbonate dissolution

Bioturbation
Bioirrigation

Mn
?

Fe
?

Reductive dissolution of Mn and Fe oxides

Degradation of organic matter

Barite dissolution

Mn oxide precipitation

Fe oxide precipitation

U(IV) precipitation/adsorption

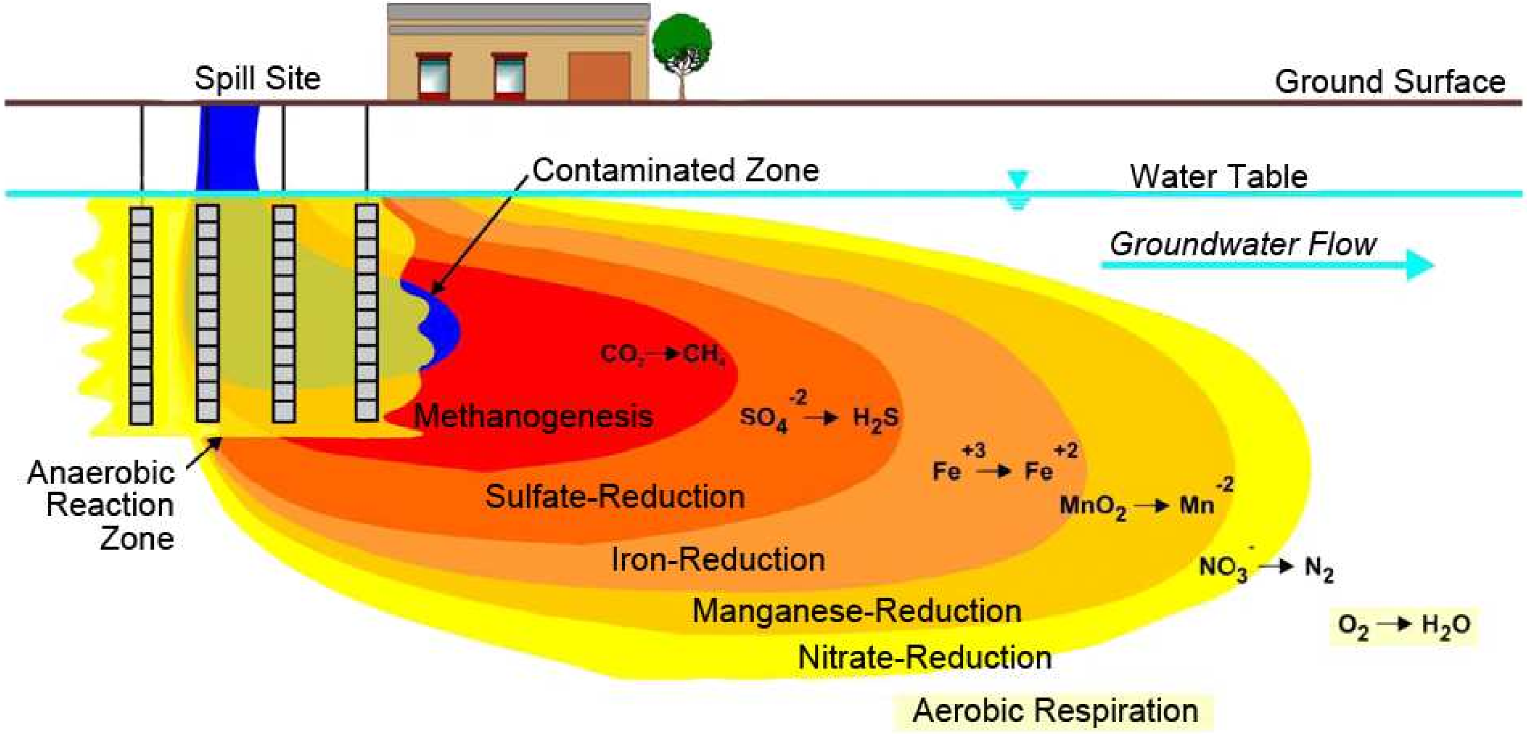
Fe sulfide formation, sulfurization of organic matter

Carbonate precipitation

Barite precipitation

http://www.awi.de/fileadmin/user_upload/Research/Research_Divisions/Geosciences/Marine_Geochemistry

Redox Zonation → Thermodynamics



Redox zonation:

subsurface microbial communities optimize catabolic energy production

https://clu-in.org/techfocus/default.focus/sec/Bioremediation/cat/Aerobic_Bioremediation_(Direct)/

Microbial Kinetics

Microbial growth: ***Monod kinetics***

X: biomass

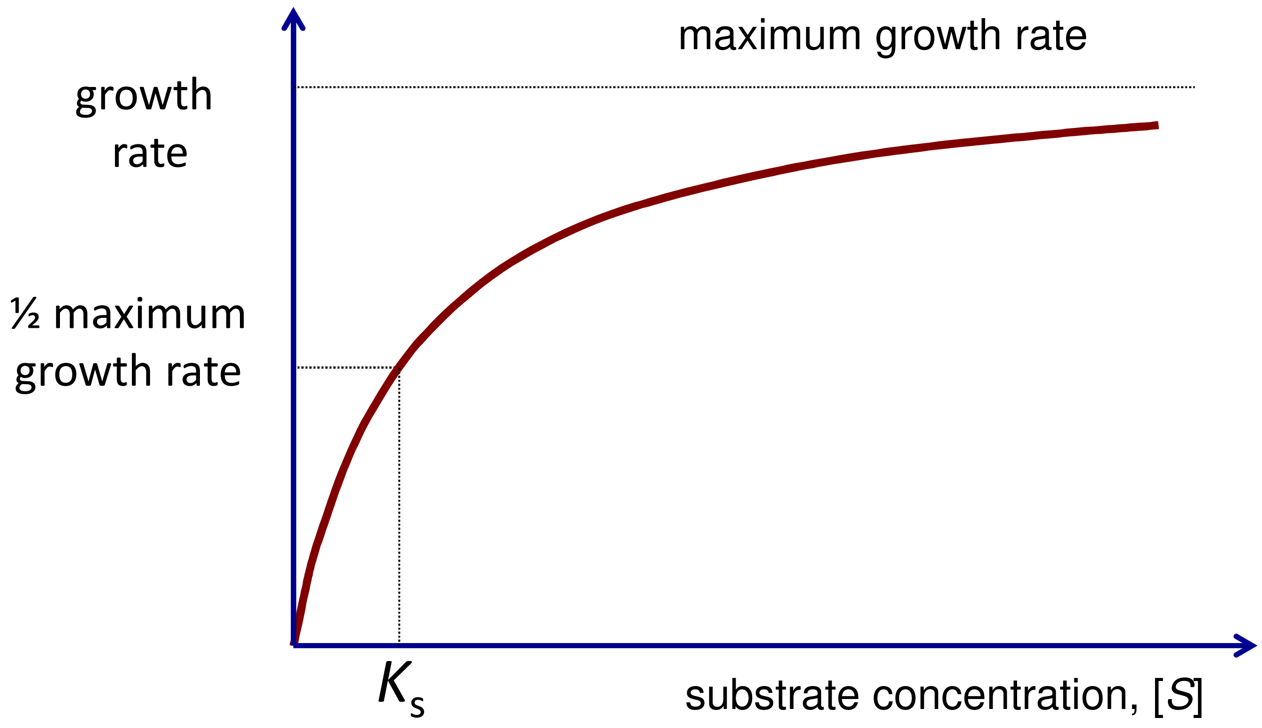
μ: specific growth rate

μ_{max}: maximum μ

S: limiting (growth) limiting

K_s: half-saturation constant

Saturation Kinetics

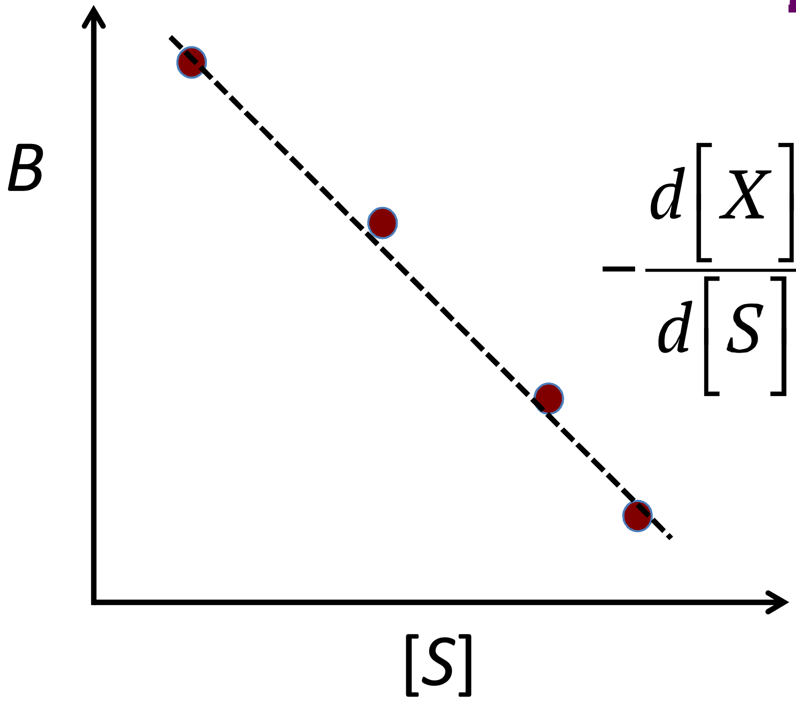


Growth Kinetics → Geochemical Kinetics

Michaelis-Menten kinetics
(substrate utilization):

$$-\frac{d[S]}{dt} = \frac{\mu_{\max}}{Y} \cdot X \cdot \frac{[S]}{K_s + [S]}$$

***Y*: growth yield**



$$-\frac{d\left[X\right]}{d\left[S\right]} = Y$$

Example: Sulfate Reducers Growing on H_2

Electron donor:

$$\left[H_2 \rightarrow 2H^+ + 2e^-\right] \text{ X 4}$$

Electron acceptor:

$$SO_4^{2-} + 8e^- + 10H^+ \rightarrow H_2S + 4H_2O$$

Catabolic reaction:

$$SO_4^{2-} + 4H_2 + 2H^+ \rightarrow H_2S + 4H_2O$$

Catabolic Reactions & Thermodynamics

$$SO_4^{2-} + 4H_2 + 2H^+ \rightarrow H_2S + 4H_2O$$

$\Delta G < 0:$ energy yielding reaction

$\Delta G \geq 0:$ energy demanding reaction

Catabolic reaction: $\Delta G < 0$

$$\Delta G = \Delta G^0 + RT \ln Q$$

Gibbs Energy of Reaction

$$\boxed{\Delta G = \Delta G^0 + RT \ln Q}$$

ΔG^0 : standard state Gibbs energy (a_i = 1)

$$\Delta G^0 = f(P,T)$$

$$\Delta G^0 = -RT \ln K_{eq}$$

Q: reaction quotient

$$Q = \frac{\Pi a_{\text{product } i}^{\nu_i}}{\Pi a_{\text{reactant j}}^{\nu_j}}$$

a: activity

ν: stoichiometric coefficient

Example: Sulfate Reducers Growing on H_2

$$SO_4^{2-} + 4H_2 + 2H^+ \rightarrow H_2S + 4H_2O$$

Aqueous solute: $a_{SO_4^{2-}} \approx \left[SO_4^{2-}\right]; \;\; a_{H_2S} \approx \left[H_2S\right]$

Hydrogen ion: $a_{H^+} = 10^{-pH}$

Volatile species: $a_{H_2} \approx P_{H_2}$

Pure solid: $a_{solid} = 1$ solvent: $a_{H_2O} \approx 1$

$$Q = \frac{[H_2S]}{[SO_4^{2-}]P_{H_2}^4(10^{-pH})^2}$$

Life = Chemical Disequilibrium

$$\Delta G = \Delta G^0 + RT\ln Q$$

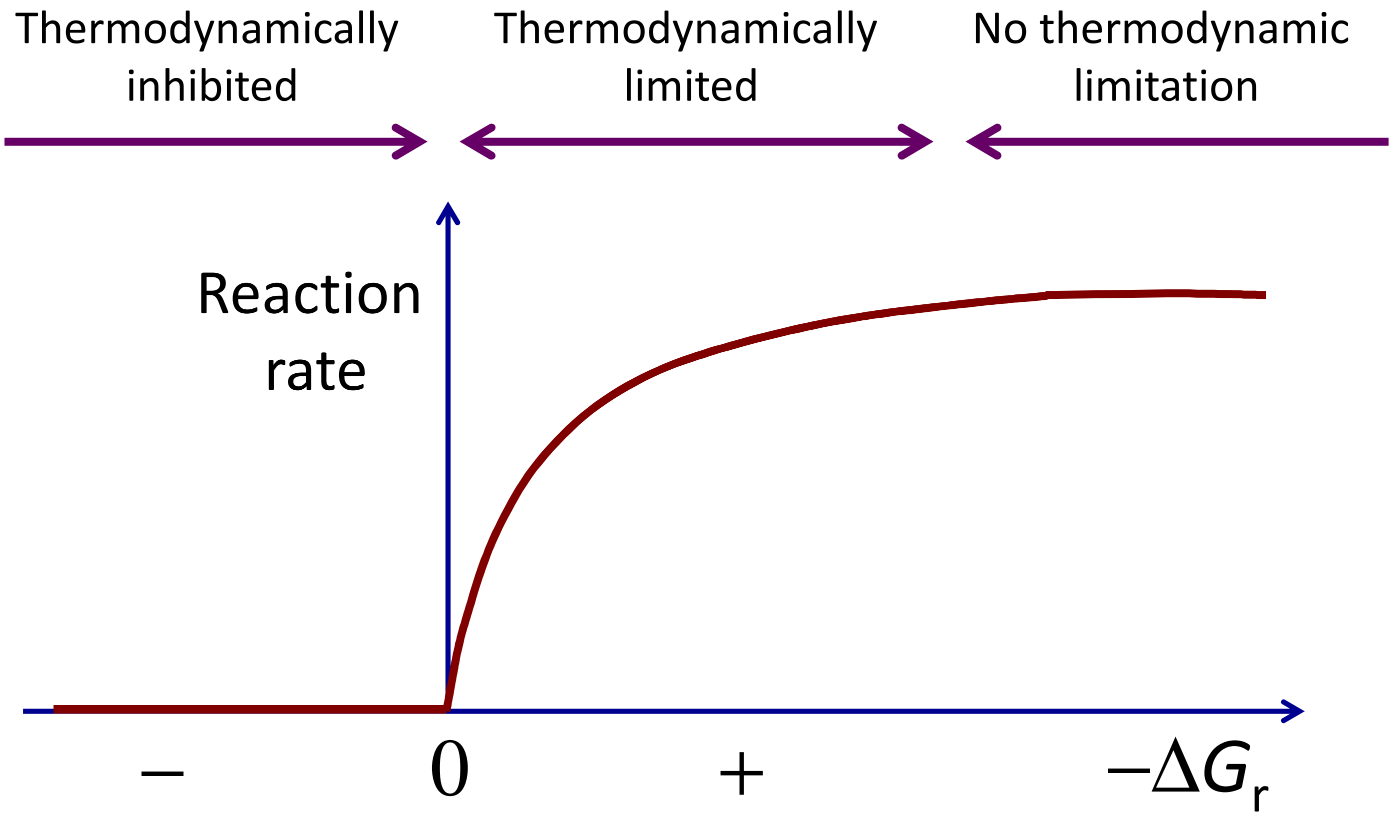
$$\Delta G^0 = -RT\ln K_{eq}$$

$$\Delta G = -RT\ln K_{eq} + RT\ln Q = RT\ln\frac{Q}{K_{eq}}$$

$$\Delta G < 0 \Rightarrow \frac{Q}{K_{eq}} < 1 \qquad 1 - \frac{Q}{K_{eq}}:$$

degree of
disequilibrium
[0,1]

Kinetics and Thermodynamics



Thermodynamic Limitation

Example: Sulfate reducing bacteria growing on H_2

Classical "Monod" kinetics:

$$-\frac{\mathrm{d}\left[\mathrm{SO}_4^{2-}\right]}{\mathrm{d}t}=\frac{\mu_{\max}\cdot X}{Y}\cdot\left\{\frac{\left[\mathrm{H}_2\right]}{K_{\mathrm{H}_2}+\left[\mathrm{H}_2\right]}\cdot\frac{\left[\mathrm{SO}_4^{2-}\right]}{K_{\mathrm{SO}_4}+\left[\mathrm{SO}_4^{2-}\right]}\right\}$$

With thermodynamic limitation:

$$-\frac{\mathrm{d}\left[\mathrm{SO}_4^{2-}\right]}{\mathrm{d}t}=\frac{\mu_{\max}\cdot X}{Y}\cdot\left\{\frac{\left[\mathrm{H}_2\right]}{K_{\mathrm{H}_2}+\left[\mathrm{H}_2\right]}\cdot\frac{\left[\mathrm{SO}_4^{2-}\right]}{K_{\mathrm{SO}_4}+\left[\mathrm{SO}_4^{2-}\right]}\right\}\boxed{\cdot F_T}$$

Thermodynamic Driving Force, F_T

$$\Delta G^*_{\mathrm{cat}} > 0: \quad F_T = 0$$

$$\Delta G^*_{\mathrm{cat}} < 0: \quad F_T \text{ between 0 and 1}$$

$$\Delta G^*_{\mathrm{cat}} << 0: \quad F_T \rightarrow 1$$

$\Delta G^*_{\mathrm{cat}} = \Delta G_{\mathrm{cat}} + \Delta G_{\mathrm{min}}$ where $\Delta G_{\mathrm{min}} > 0$

Model 1*: $\Delta G_{\mathrm{min}} = m \cdot \Delta G_{\mathrm{ATP}}$

Model 2#: $\Delta G_{\mathrm{min}} = \mathrm{F} \cdot \Delta \Psi$

*Jin Q. and Bethke C.M. (2003) *Appl. Environ. Microbiol.* **69**, 2340-2348

#LaRowe D.E. et al. (2012) *Geochim. Cosmochim. Acta* **90**, 96-109

Thermodynamic Driving Force, *F*

Model 1: Transition State Theory

$$\Delta G_{\mathrm{cat}} + m\Delta G_{\mathrm{ATP}} < 0: \qquad F_T = 1 - \exp\left(\frac{\Delta G_{\mathrm{cat}} + m\Delta G_{\mathrm{ATP}}}{\chi RT}\right)$$

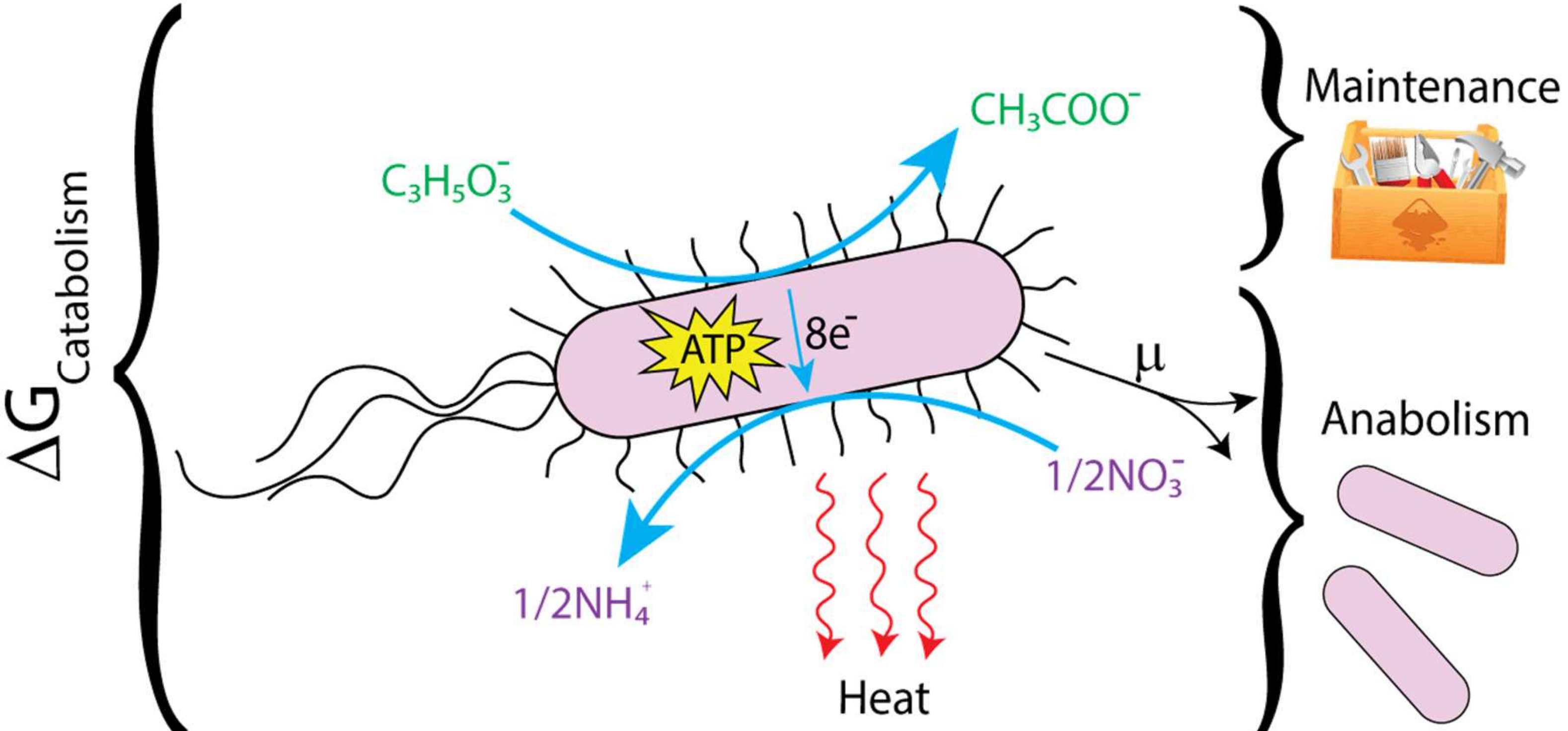
$$\Delta G_{\mathrm{cat}} + m\Delta G_{\mathrm{ATP}} \geq 0: \qquad F_T = 0$$

Model 2: Fermi-Dirac Statistics

$$F_T = \frac{1}{e^{\frac{E}{RT}} + 1}$$ where $$E = \Delta G_{\mathrm{cat}} + F\Delta\Psi$$

Active bacteria: $\Delta\Psi \geq 100\ \mathrm{mV}$

Cellular Energy Balance



Dissipation

Catabolic energy production = growth + dissipation + maintenance

Anabolic Energy Demand

Anabolism (**ANA**):

carbon source + nutrients (N, P, …) + energy → 1 C-mol biomass

Catabolism (**CAT**):

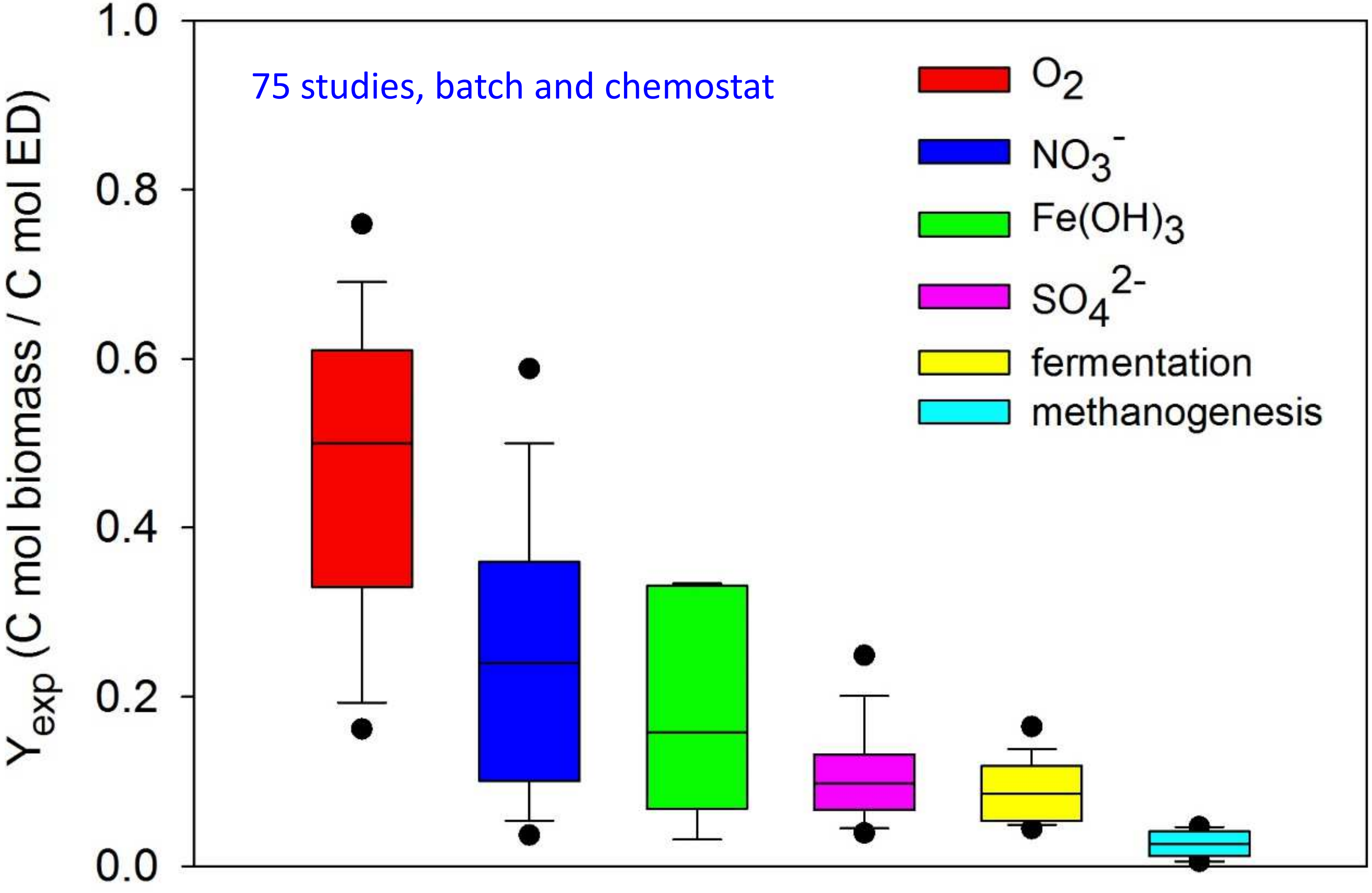
electron donor + electron acceptor → products + energy

Metabolic reaction (**MET**): $\mathrm{MET} = \mathrm{ANA} + \lambda_{cat} \bullet \mathrm{CAT}$

λ_{cat}: number of times the catabolic reaction must proceed in order to build 1 C-mol biomass

λ_{cat} is directly related to the growth yield!

Thermodynamics → Growth Yields



Smeaton C. and Van Cappellen P. (2018) GCA – under review.

Example: Iron Reducers Growing on Acetate

Anabolic reaction (ANA):

$$\underset{\text{acetate}}{0.525\,C_2H_3O_2^-} + 0.2NH_4^+ + 0.275H^+ \rightarrow \underset{\text{biomass}}{CH_{1.8}O_{0.5}N_{0.2}} + 0.05HCO_3^- + 0.4H_2O$$

ΔG^0_{ANA} = -17.8 kJ/C-mol biomass (at 35°C)

ν = 0.525 mol acetate/C-mol biomass

Catabolic reaction (CAT):

$$8Fe(OH)_3 + C_2H_3O_2^- + 15H^+ \rightarrow 8Fe^{2+} + 2HCO_3^- + 20H_2O$$

ΔG^0_{CAT} = -475.9 kJ/mol acetate (at 35°C)

Thermodynamics → Growth Yield

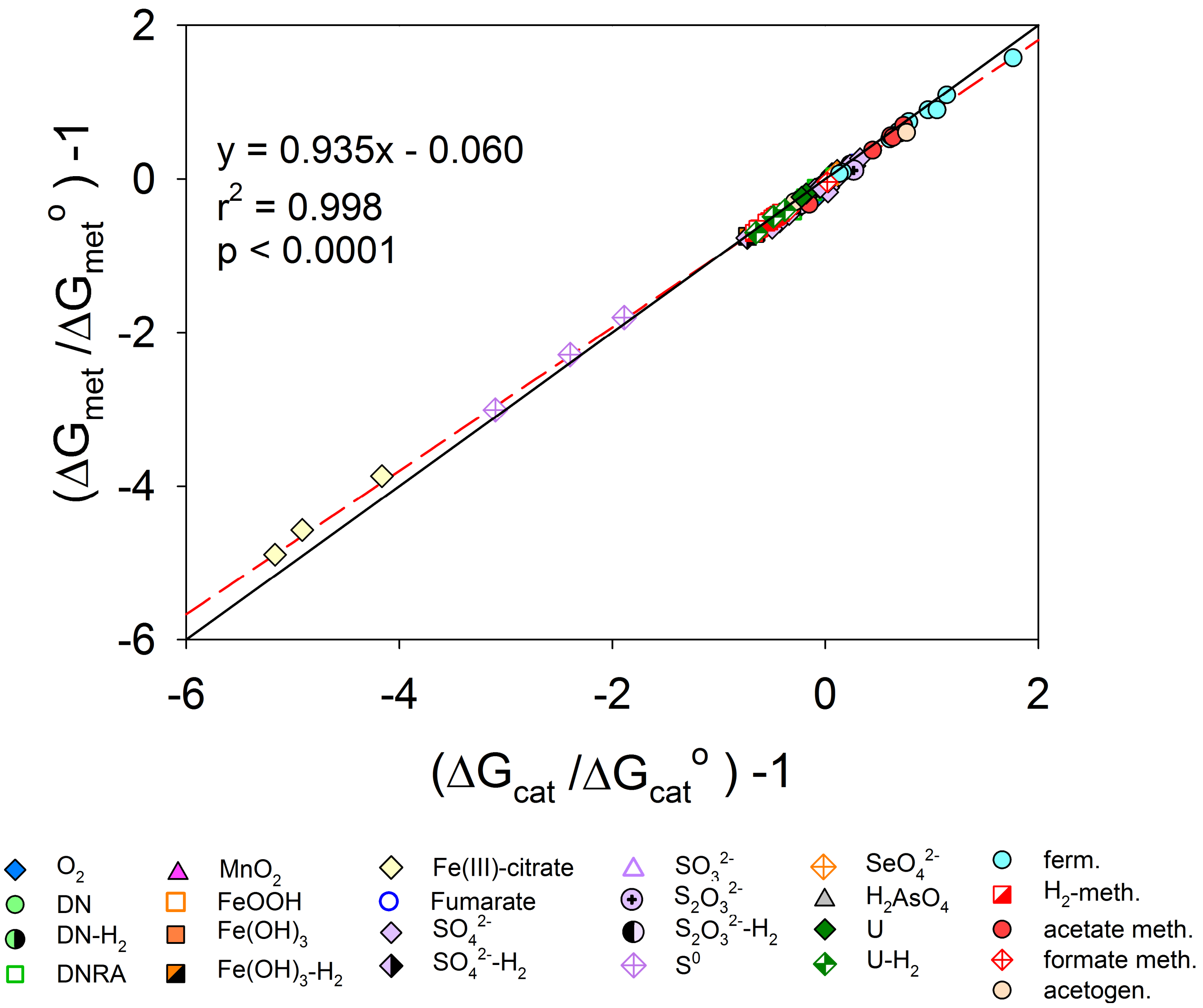
$$\lambda_{\mathrm{cat}} \left[\text{mol acetate/C-mol biomass}\right]$$

$$Y \left[\text{C-mol biomass/mol acetate}\right]$$

$$\lambda_{\mathrm{cat}} = \frac{1 - Y \cdot \nu}{Y} \qquad \Delta G_{\mathrm{met}} = \Delta G_{\mathrm{ana}} + \lambda_{\mathrm{cat}} \Delta G_{\mathrm{cat}}$$

$$\boxed{Y = \frac{\Delta G_{\mathrm{cat}}}{\Delta G_{\mathrm{met}} + \Delta G_{\mathrm{cat}} \cdot \upsilon - \Delta G_{\mathrm{ana}}}}$$

Departure from Standard State Conditions



Gibbs Energy Dynamic Yield Method (GEDYM)

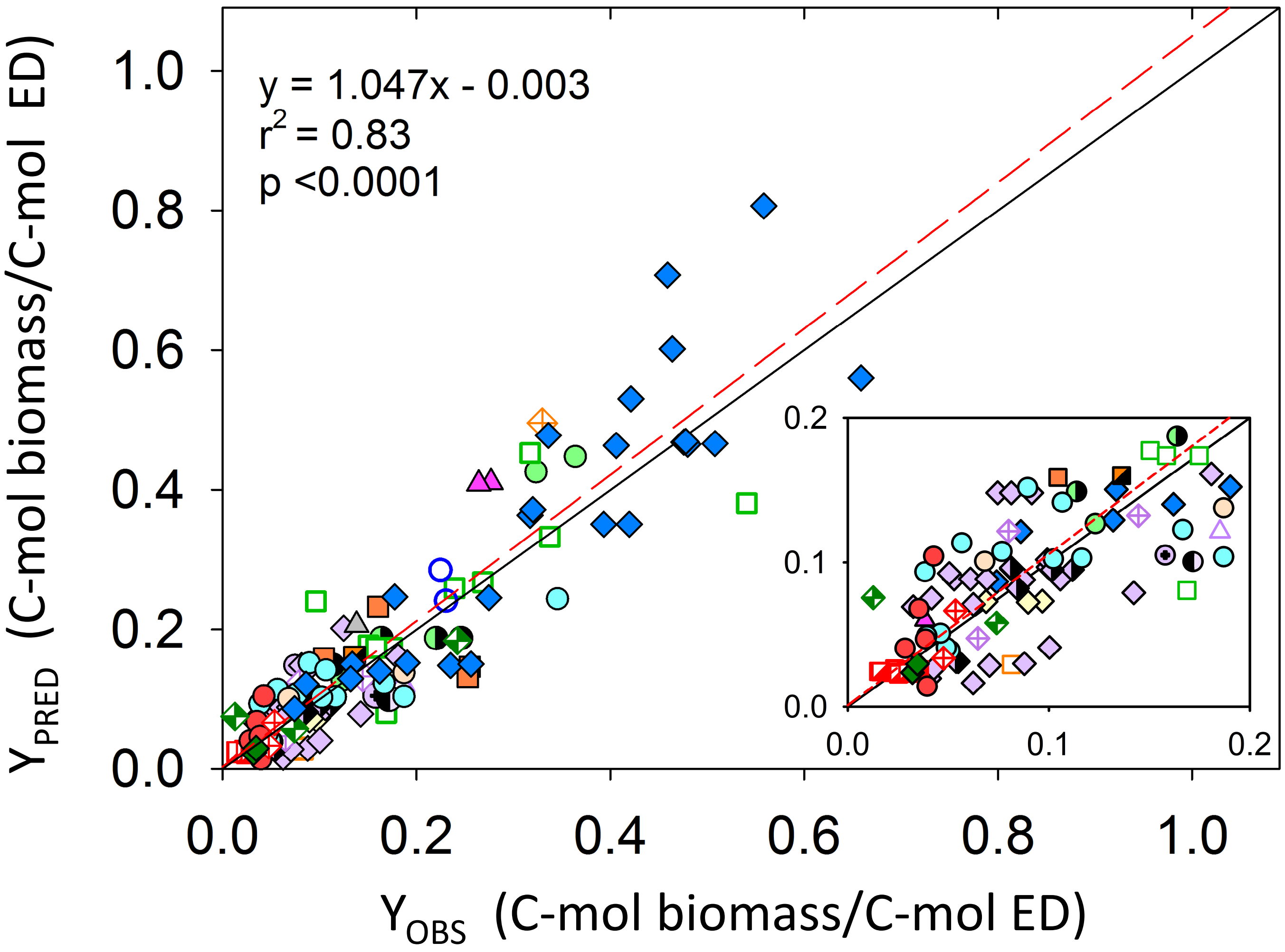
Empirical relationship:

$$\left(\frac{\Delta G_{\mathrm{met}}}{\Delta G^{0}_{\mathrm{met}}}-1\right) = m\cdot\left(\frac{\Delta G_{\mathrm{cat}}}{\Delta G^{0}_{\mathrm{cat}}}-1\right)+b$$

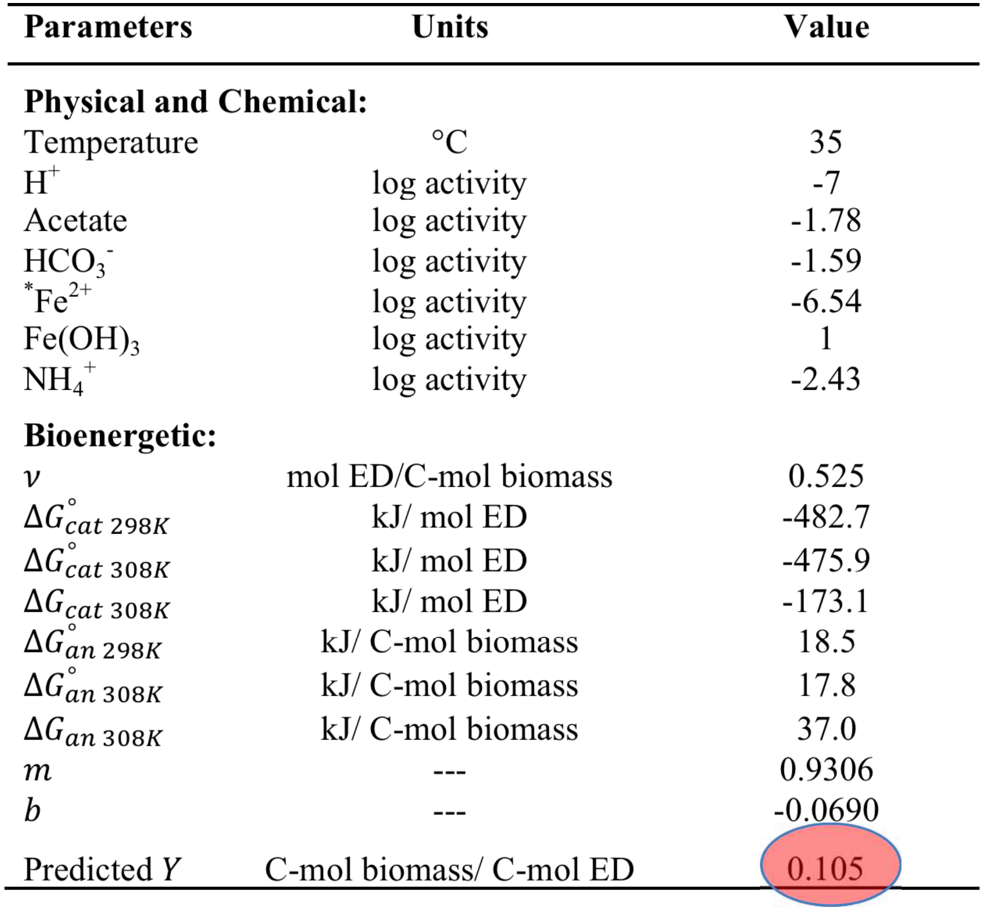
Obtain ΔG_{met} and calculate:

$$Y = \frac{\Delta G_{\mathrm{cat}}}{\Delta G_{\mathrm{met}}+\Delta G_{\mathrm{cat}}\cdot\upsilon-\Delta G_{\mathrm{ana}}}$$

Gibbs Energy Dynamic Yield Method (GEDYM)



Experimental Data: Acetate/$Fe(OH)_3$



Parameters	Units	Value
Physical and Chemical:		
Temperature	°C	35
H^+	log activity	-7
Acetate	log activity	-1.78
HCO_3^-	log activity	-1.59
$^{*}Fe^{2+}$	log activity	-6.54
$Fe(OH)_3$	log activity	1
NH_4^+	log activity	-2.43
Bioenergetic:		
ν	mol ED/C-mol biomass	0.525
$\Delta G^{\circ}_{cat\ 298K}$	kJ/ mol ED	-482.7
$\Delta G^{\circ}_{cat\ 308K}$	kJ/ mol ED	-475.9
$\Delta G_{cat\ 308K}$	kJ/ mol ED	-173.1
$\Delta G^{\circ}_{an\ 298K}$	kJ/ C-mol biomass	18.5
$\Delta G^{\circ}_{an\ 308K}$	kJ/ C-mol biomass	17.8
$\Delta G_{an\ 308K}$	kJ/ C-mol biomass	37.0
m	---	0.9306
b	---	-0.0690
Predicted Y	C-mol biomass/ C-mol ED	0.105

Experimental conditions: Caccavo et al. (1994) *Geobacter sulfurreducens* sp. nov., a hydrogen- and acetate-oxidizing dissimilatory metal-reducing microorganism. *Applied and Environmental Microbiology* **60**, 3752-3759.

Metabolic (Macrochemical) Reaction

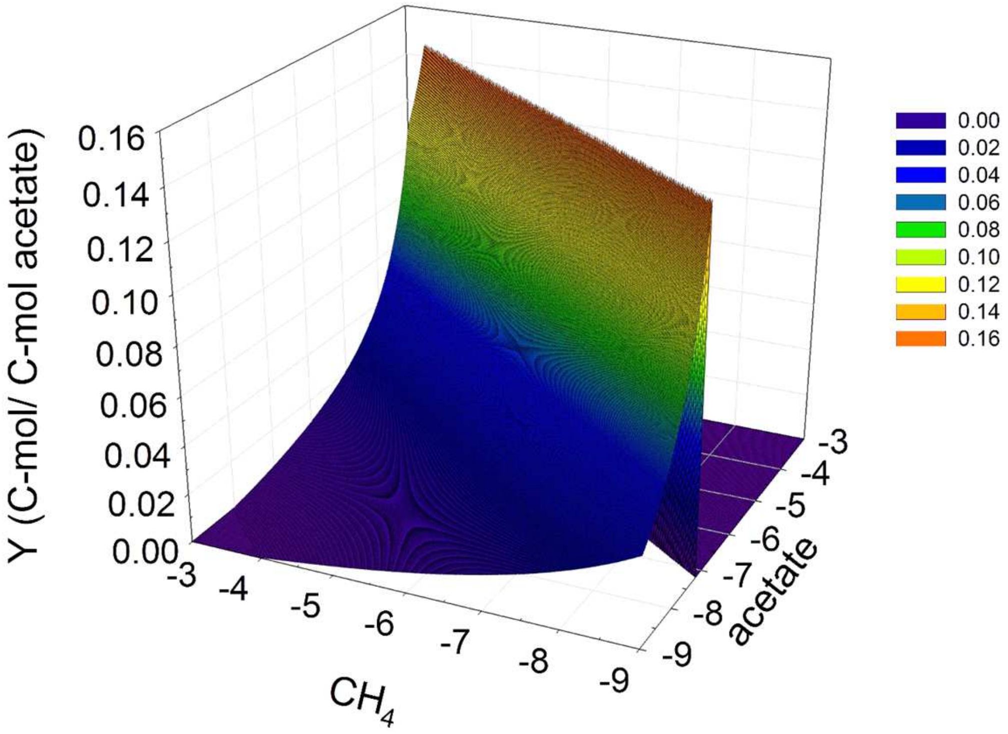
$$Y = 0.105 \text{ C-mol biomass (mol acetate)}^{-1}$$

$$\begin{aligned}&6.67\ \mathrm{Fe(OH)_3} + \mathrm{C_2H_3O_2^-} + 0.064\ \mathrm{NH_4^+} + 12.59\ \mathrm{H^+}\\ &\quad\rightarrow 0.318\ \mathrm{CH_{1.8}O_{0.5}N_{0.2}} + 6.67\ \mathrm{Fe^{2+}} + 1.68\ \mathrm{HCO_3^-} + 16.79\ \mathrm{H_2O}\end{aligned}$$

$$\Delta G_{\text{met}} = -132.5 \text{ kJ (mol acetate)}^{-1}$$

Dynamic Growth Yields

Acetotrophic
Methanogenesis
(12°C, pH 7)



$$C_2H_3O_2^- + H_2O \rightarrow CH_4 + HCO_3^-$$

Growth and Maintenance

Pirt Equation:

$$\boxed{r_s = \frac{\mu \cdot X}{Y} + m_s}$$

where

r_s : substrate utilization rate

μ : specific growth rate

X : biomass

Y : growth yield

m_s : maintenance requirement (rate)

Bioenergetics and Reactive Transport

1. F_T: thermodynamic limitation on catabolic reaction

2. λ_{cat}: coupling catabolism and anabolism

3. m_e: maintenance requirement

The End

Source: http://www.jantoo.com/cartoon/12265265