

# Program

June 6: Lectures in the morning:

- 1. From Thermodynamics to microbial Lifestyles.
- 2. Thermodynamic Concepts applied to the Study of metabolic Interactions.

June 6 and 7: Exercises in the Evening: Predicting Processes in microbial Ecosystems.

June 8: Sulfur Lecture in the morning: Microbial Diversity in biogeochemically-driven Sulfur Cycling



# Contents

## Part 1

- Introduction to Bio-Thermodynamics
- The concept of "free energy"
- "Standard free energy" vs. "actual free energy"

#### Part 2

- Calculating "free energy" from electrochemical potential energy
- Summary of terms, relations and abbreviations
- Examples using Thermodyn



# From Bio-Thermodynamics to Bio-Energetics

Bio-Thermodynamics	Bio-Energetics
Application of physico-chemical principles to biological and biogeochemical processes	What cells need to grow.
How are (redox) reactions formulated?	Through which mechanisms do cells convert food energy?
What is energetically possible?	What are structural prerequisites?
How is available energy calculated?	What are the biochemical (enzymatic) processes?
How are diverse life styles defined	
energetically?	How do cells couple electron transport to ATP synthesis?
What is the predictive power of Bio-	
Thermodynamics? (applications)	How did cellular energy metabolism evolve?



# From Bio-Thermodynamics to Bio-Energetics

 $\Delta \text{Gr}$ 

How to best introduce thermodynamics.

- Start with Gf° and calculate ∆Gr° and Keq
- · Introduce Q and define non-equilibrium conditions
- Calculate  $\Delta Gr$  as deviation from  $\Delta Gr^{\circ}$
- Consider half-reactions
- Calculate the potential of electrons in half-reactions (Eh) from  $\Delta Gr$
- Combine electron donating and electron accepting half-reactions

Bio-Thermodynamics can **unify microbial diversity** with a set of basic physicochemical principles.

What are the unifying concepts for all life?

- 1. Thermodynamic rules
- 2. Directions of redox and acid-base reactions
- 3. Bioconversion of energy through electron transport and proton translocation (chemiosmotic gradients) to high-energy molecular bonds and cellular functions

1 + 2 + 3 define the basics of metabolic chemistry, cellular physiology.

Metabolic diversity lies in the kind and variations of electron transport components, enzymes, pathways, energy storage mechanisms, adaptation to trophic structure of habitats

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Objectives

The colonization of habitats, community composition and community dynamics depend on  $\ldots$ 

the stoichiometry of biomass formation and the presence and availability of the necessary chemical elements in the form of nutrients

the efficiency of the biochemical energy yield from dissimilation reactions and its coupling to biomass formation (growth)

the kinetics of growth

the environmental conditions which promote or hinder growth reactions

the presence and dominance of other organisms

The functioning of energy fluxes is dictated by thermodynamic laws

Biological energy conversion takes place via electron and/or proton transfer reactions

In this lecture we would like to address some basic thermodynamic approaches, apply them towards the understanding of microbial life styles and for the formulation and evaluation of geo-chemo-biological processes



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# Learning Objectives

Construct balanced redox reactions from half-cell reactions.

Predict energy yields from redox reactions.

Determine whether a redox reaction is spontaneous or non-spontaneous.

Convert between various energy units.

Identify types of metabolisms based on redox chemistry.



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# Bioenergetic Principles for Metabolism Different ways of "how to make a living" emerge from ...

- the kinds and presence of reductants and oxidants
- · the energy conversion mechanisms employed
- the actual conditions under which metabolic processes take place
- the biochemical machinery and cellular architecture involved in energy conversion
- · Free energy is due to chemical (redox) disequilibria
- Metabolism is basically redox (e- transfer) chemistry
- · Thermodynamics quantifies reaction energetics
- Enzymes determine kinetics by catalyzing electron transport, proton transfer and bond synthase reactions.
- Localization of enzymes, e.g. arrangements within the topology of membranes is directing electron flow and





measured, e.g. by incorporation of <sup>3</sup>H-leucine



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# Synthesis of "new" cells requires mass, energy and a "template"





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Assimilation requires:

- mass: nutrients
- "template": inoculum
- energy: since  $\Delta Gr$  is > 0
- catalytic converters: enzymes

Dissimilation requires:

- mass: oxidizable compounds
- · chemical oxidant
- release of energy, ∆Gr must be < 0
- energy converter: membranes, enzymes

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## **Biomass Yield from Stoichiometry**

(e.g. *Sporomusa termitida*, an anaerobic, hydrogenotrophic acetogen, present in the hindgut of the termite *Nasutitermes nigriceps*). What are its energy, carbon and nitrogen sources ?



#### Anaeobic Hindgut Microbiology in Termites











# Synthesis, Death and Degradation of Cell Mass





#### Elemental Analysis of *Chromatium okenii* Cell Biomass (DelDon biomass for phototrophic bacteria in Lago Cadagno)

Dead cells Biomass whose molecules consist of stoichiometric ratios of C, H, O, N, P, S, etc. atoms in biomacromolecules

Elemental analyses

C:H:O:N:P:S = 380 : 580 : 153 : 67 : 3 : 2.5

Oxidation states of atoms in biomass molecules S: -I, P: +V, N: -III, O: -II, H: +I, C<sub>calculated average</sub>: - 0.225

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# **Elemental Composition of Biomass**

Stoichiometric ratio of chemical elements which are present in the organic portion of biomass. Example: Organic components of bacterial biomass (P XII / 3)

C:H:O:N:P:S = 380 : 580 : 153 : 67 : 3 : 2.5

The 5 elements account for 86.7 % (w/w) of the biomass dry weight.

The composition includes cell mass without storage polymers which can vary depending of the growth status of a cell (e.g. Glycogen granules and sulfur droplets are not included in the stoichiometric ratio)



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Photosynthetic sulfido-autotrophic Formation of Biomass (Assimilation)



# Overview about Life Styles: All Organisms are Chemotrophs



Chemotrophy: ability to transform energy released through redox processes into biochemically useable energy, e.g. chemiosmotic potentials, reversed electron transport, motility, energy-rich bonds (e.g. phosphoanhydride), a.o.





# Overview about Life Styles: Some Organisms are ALSO Phototrophs



Phototrophy: ability to transform radiation energy (photon energy) into redox energy



# Overview about Life Styles: Carbon Autotrophy



# Overview about Life Styles: Is there Nitrogen Autotrophy?





# Overview about Life Styles: Is there Sulfur Autotrophy?



# Life Styles of Microorganisms: Summary

energy- conversion mechanism	electron- donor- source	carbon- donor- source		choice of oxidants (exogenous)
chemo-	litho-	auto-		O <sub>2</sub> NO <sub>3</sub> ', NO <sub>2</sub> '
	organo-	hetero-	M	MnO <sub>x(s)</sub> MnO(OH)
photo-	litho- auto-	trophic	SO <sub>4</sub> <sup>2-</sup> , S° <sub>(s)</sub> Fe(OH) <sub>3(c)</sub>	
	organo-	hetero-	-tropine	FeO(OH) <sub>(s)</sub> CO <sub>2</sub>
				H⁺ org. comp.





## Life style concepts

Free energy is due to chemical (redox) disequilibria

Metabolism is basically redox (e- transfer) biochemistry

Thermodynamics quantifies reaction energetics

Enzymes determine kinetics by catalyzing electron transport, proton transfer and bond synthase reactions.



# Bioenergetic Life Style Models - 1

Think "Stinky" and find answers to following steps (model building, Lee Kump, 130703)

- 1. Define the physico-chemical picture (sketch the situation conceptually)
- 2. Define the physical-chemical-biological processes and laws that underlie them and the boundaries of the model
- 3. Define and write down the (mathematical) biochemical laws and relationships that apply
- 4. Define the restrictive assumptions
- 5. Perform balances e.g. according to the laws of conservation (mass balance, electron balance, charge balance, energy balance)
- 6. Check units and make sure they are at the same level
- 7. Define the initial and the boundary conditions and the time steps of iteration
- 8. Solve the system of equations mathematically





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## Bioenergetic Life Style Models - 2

Think "Stinky" and find answers to following steps (model building, Lee Kump, 130703)

- 1. Define the physico-chemical picture (sketch the situation conceptually) of the system of interest → lateral gradients due to advective flow is a system determinant
- 2. Select the physical-chemical-biological processes / laws that define the system and the boundaries of the model → biochemical reactions obeying the laws of thermodynamics
- 3. Define and write down the (mathematical) biochemical laws and relationships that apply → stoichiometric process / reaction equations according to the rules of redox states (→Alex)
- 4. Define the restrictive assumptions  $\rightarrow$  dissimilation only or including assimilation as well ?
- 5. Perform balances according to the laws of conservation → electron balance, charge balance, mass balance = conservation stoichiometry
- 6. Check units and make sure they are at the same level  $\rightarrow$  all in mole mass / equal volume
- 7. Define the initial and the boundary conditions and the time steps of iteration  $\rightarrow$  lowest and highest concentrations / activities (accounts for interactions between molecules)
- 8. Solve the system of equations mathematically  $\rightarrow$  That's what Thermodyn is doing for us



## **Remember: Redox Reactions**

Oxidation: loss of electrons. half-reaction with e<sup>-</sup> on the right of the reaction equation Oxidant: electron acceptor

Arsenic-oxidation:  $As(III+) \rightarrow As(V+) + 2 e^{-}$ 

Reduction: gain of electrons. half-reaction with e<sup>-</sup> on the left of the reaction equation Reductant: electron donor

Denitrification:  $NO_3^- + 5 e^- + 6 H^+ \rightarrow \frac{1}{2}N_2 + 3 H_2O$ 



## Kluyver's global Concept of Metabolism: Redox Reactions



#### Diversity today:

kind of reductants / oxidants:  $H_2S$ ,  $S^\circ$ ,  $S_2O_3^{2-}$ ,  $H_2$ , Fe <sup>2+</sup>, organic compounds,  $CO_2$ ,  $SO_4^{2-}$ ,  $O_2$  etc. kind of electron transport coenzymes: Bpheo, FeS , Fd, UQ, PC, Cyt, NAD(P)<sup>+</sup>, etc. topology and architecture of the chemiosmotic membrane biochemical  $CO_2$ -fixation mechanism, catabolic and anabolic metabolic pathways



## Redox Concept applied to dissimilative Respiration



#### Diversity:

- + kind of oxidants:  $O_2$  ,  $SO_4^{2-}$  ,  $S^\circ$ ,  $S_2O_3^{2-}$ ,  $H^+$ ,  $Fe^{3+}$ ,  $NO_3^-$ ,  $CO_2$  etc.
- kind of transport coenzymes which direct electron flow to the final acceptor: NAD<sup>+</sup>, FAD, FeS , Fd, UQ, PC, Cyt, etc.
- topology and architecture of the membrane which allows to create and maintain a chemiosmotic potential difference





## Diversity during Mineralization (Chemotrophs)



# Van Niel's unifying Concept of Photosynthesis as a Redox Process

(van Niel, 1930)

How can all photosynthesis processes be represented as a common concept ?

 $2 H_2 O + CO_2 \xrightarrow{4} <HCHO> + O_2 + H_2O$ 

Redox concept of assimilative C-autotrophy

 $2 H_2 A$ + CO<sub>2</sub> <HCHO> + 2**A**  $+ H_2O$ e⁻-donor ..oxidized e--acceptor "reduced e-acceptor" e--donor" **B**<sub>ox</sub>  $\mathbf{C}_{\mathsf{red}}$  $\mathsf{A}_{\mathsf{red}}$ + D<sub>ox</sub>

Diversity

kind of reductants:  $H_2O$ ,  $H_2S$ , S°,  $S_2O_3^{2-}$ ,  $H_2$ ,  $C_{org.red.}$ kind and number of photosystems: PS I, PS II kind of light harvesting pigments: Chl, Carotenoids, Biliproteins kind of electron transport coenzymes: Bpheo, FeS , Fd, UQ, PC, Cyt, topology and architecture of phototrophic membranes biochemical  $CO_2$ -fixation pathways



## Things to think about

What distinguishes a purely chemotrophic life style from a phototrophic life style?

What are autotrophic life styles?

Karsten used the term "electrotrophic" life style. What does this term mean?



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# Sequence of energetically driven Events in Oxidant-limited Sediments







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# Oxidation States of Atoms in Molecules are defined employing Pauling's\* Electronegativity Scale

Exan	nples					
н						
2.2						
		В	С	Ν	0	F
		2.04	2.55	3.04	3.44	3.98
Na	Mg	AI	Si	Р	S	CI
0.93	1.31	1.61	1.9	2.19	2.58	3.16
к	Са			As	Se	Br
0.82	1.0			2.18	2.55	2.96



From www.arbuiso.com/diary/trendsdiary.pdf

\* Pauling, Linus, 1932. THE NATURE OF THE CHEMICAL BOND. IV. THE ENERGY OF SINGLE BONDS AND THE RELATIVE ELECTRONEGATIVITY OF ATOMS. Journal of the American Chemical Society, 54, 3570-3582

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# Oxidation States of Atoms in Molecules are defined employing Pauling's Electronegativity Scale



Periodic table of electronegativity using the Pauling scale

Electronegativity applied to organic molecules: <u>http://www.chemguide.co.uk/basicorg/bonding/eneg.html#top</u>

More about Electronegativity http://www.chemguide.co.uk/atoms/bonding/electroneg.html

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#### Exercise: Determine Oxidation State of Atoms in Molecules Carbon atoms and most bonds to hydrogen atoms are omitted in the structural formulas see special slide series on Redox Concepts



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## Prerequisites for Bio-Thermodynamics

How are oxidation states of atoms in molecules defined? How do we balance redox reactions? How do we define molecular species of substrates and products? How is the "energy content" of a molecule defined? How do we calculate the free energy of a reaction? What is the difference between free energy of a reaction under standard conditions and under actual conditions? How does Thermodyn calculate free energies of metabolic reactions? How can we study coupled reactions with Thermodyn?

What are half-reactions? How are electron potentials defined? How do we construct "redox towers"? How do we construct stability diagrams?

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## Objectives

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## Fermentation of Ethanol to Methane: "Methanobacillus omelianskii" Problem

Ethanol fermentation to methane is carried out by a co-culture

 $2 \text{ CH}_3\text{CH}_2\text{OH} + \text{HCO}_3^- \rightarrow 2 \text{ CH}_3\text{COO}^- + \text{H}^+ + \text{CH}_4 + \text{H}_2\text{O}$ ∆Gr°'<sub>25°</sub> = <u>99.90 [kJ mol⁻1]</u>  $\Delta Gr^{\circ}_{25^{\circ}} = -59.94 \text{ [kJ mol^{-1}]}$ Single cultures: Organism 1: Stain S (ethanolotrophic, hydrogenic, acetogenic fermenter  $2 \text{ CH}_3\text{CH}_2\text{OH} + 2 \text{ H}_2\text{O} \rightarrow 2 \text{ CH}_3\text{COO}^- + 2 \text{ H}^+ + 4 \text{ H}_2$ ∆Gr°'<sub>25°</sub> = + <del>89.3</del>7 lkJ mol⁻¹l  $\Delta Gr^{\circ}_{25^{\circ}} = + 169.28 \text{ [kJ mol^-1]}$ Organism 2: Strain MoH (hydrogenotrophic methanogen)  $4 \text{ H}_2 + \text{HCO}_3^- + \text{H}^+ \rightarrow \text{CH}_4 + 3 \text{ H}_2\text{O}$ ∆Gr°'<sub>25°</sub> = <del>189</del>.26 [kJ mol<sup>-1</sup>]  $\Delta Gr^{\circ}_{25^{\circ}} = -229.22 \text{ [kJ mol^{-1}]}$  $\Delta Gr^{\circ}_{25^{\circ}}$  = Free Gibbs energy of the reaction under standard conditions of all reactants  $\Delta Gr^{\circ}_{25^{\circ}}(Org1) + \Delta Gr^{\circ}_{25^{\circ}}(Org2) = \Delta Gr^{\circ}_{25^{\circ}}(Coculture) = + 169.28 - 229.22 = -59.94$ [kJ mol<sup>-1</sup>]  $\Delta Gr^{\circ}_{25^{\circ}}$  = Free Gibbs energy of the reaction under standard conditions of all reactants, except

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for protons, which are assumed to have an activity of 10<sup>-7</sup> (pH 7). Do NOT use !!

## "Methanobacillus omelianskii" with Thermodyn®

Reaction 1: Organism 1: Stain S (ethanolotrophic, hydrogenic, acetogenic fermenter  $2 \text{ CH}_3\text{CH}_2\text{OH}_{aq} + 2 \text{ H}_2\text{O}_1 \rightarrow 2 \text{ CH}_3\text{COO}_{aq}^- + 2 \text{ H}_{aq}^+ + 4 \text{ H}_{2 \text{ aq}}$ 

Reaction 2: Organism 2: Strain MoH (hydrogenotrophic methanogen)  $4 H_{2 aq} + HCO_{3 aq} + H^{+}_{aq} \rightarrow CH_{4 aq} + 3 H_{2}O_{1}$ 

Reaction 3 = Reaction 1 + Reaction 2  $2 \text{ CH}_3\text{CH}_2\text{OH}_{aq} + \text{HCO}_3^{-}_{aq} \rightarrow 2 \text{ CH}_3\text{COO}_{aq}^{-} + \text{H}_{aq}^+ + \text{CH}_{4 aq}^+ + \text{H}_2\text{O}_1$ 

Boundary conditions for calculation Activities [mol equivalent / L]:

Conditions:

CH <sub>3</sub> CH <sub>2</sub> OH <sub>aq</sub> CH <sub>2</sub> COO <sup>-</sup> ag	10 <sup>-2</sup> 10 <sup>-4</sup>	T H <sub>2</sub> O	15°C 1.00
HCO <sub>3 an</sub>	5x10 <sup>-3</sup>	$H_{a0}^{2}$ (pH 7.5)	<b>10</b> <sup>-7.5</sup>
CH <sub>4 ag</sub>	2x10 <sup>-3</sup>	dy (i )	
$H_{2 a \alpha}$	10 <sup>-12</sup> ≤ v ≤ 1		





# "Methanobacillus omelianskii" with Thermodyn®

Reactior No.	(S,P)	Stoich. Coeff.	Enter formula	State	Special remarks	Activity	Variable IoN	te Compound	Formula E	rror
	s	2	C2H5OH	aq		1.00E-02		Ethanol	C2H5OH	Organism 1: Stein S
	s	2	H2O	1		1.00E+00		Water	H2O	Organishi T. Stain S
	р	2	CH3COO(-)	aq		1.00E-04		Acetate	CH3COO	(ethanolotrophic hydroge
	р	2	H(+)	aq		3.16E-08	hydrated	H+ Proton	H(+)	(ethanolotrophic, hydroge
	р	4	H2	aq			v	Hydrogen	H2	acetogenic fermenter
	s	4	H2	aq			v	Hydrogen	H2	Organism 2: Strain MoH
	s	1	HCO3(-)	aq		5.00E-03		Bicarbonate	HCO3(-)	Organishi z. Strain Worr
	s	1	H(+)	aq		3.16E-08	hydrated	H+ Proton	H(+)	(hydrogenotrophic
	р	1	CH4	aq		2.00E-03		Methane	CH4	(injulogonoulopino
	р	3	H2O	1		1.00E+00		Water	H2O	methanogen)
	s	2	C2H5OH	aq		1.00E-02	v	Ethanol	C2H5OH	Coculture of Organism 1
	s	1	HCO3(-)	aq		5.00E-03		Bicarbonate	HCO3(-)	
	р	2	CH3COO(-)	aq		1.00E-04		Acetate	CH3COO	(Stain S) + Organism 2
	р	1	H(+)	aq		3.16E-08	hydrated	H+ Proton	H(+)	(Chroin Mold)
	р	1	CH4	aq		2.00E-03		Methane	CH4	(Strain Mori)
	р	1	H2O	1		1.00E+00		Water	H2O	
Plot 1						Plot 2				
iemp. Ain. va Max. v	(T1) ariab ariab	(K) le: ole:	288.15 15 1.00E-12 1.00E+00	°C		Temp. (T2) Min. variabl Max. variab	288.15 1.00E-12 1.00E+00	15 °C		Conditions
.og pl	ot?:	×						Log plot?: x		
			<b>pK(T1)</b> pK(29	98) ∆Gr°(298) kJ/mol	∆Hr°(298) kJ/mol	∆Sr° (298) J/mol °K	∆Gr° % uncertaintv	pK(T2)	pH to activity co pH [	nversion H⁺]
show r	reac1	x	30.63 29.	66 169.280	159.620	-32.9	-0.09	30.63 Show reac1	7.50 3.162	E-08
show r	reac2	x	-41.62 -40.	16 -229.220	-239.920	-35.65	-0.03	-41.62 Show reac2	7.50 3.162	E-08 Standard values
show r	reac3		-10.99 -10.	50 -59.940	-80.300	-68.55	0.13	-10.99 Show reac3 x	7.50 3.162	E-08

# Individual Cultures of "Methanobacillus omelianskii" with Thermodyn®





## Coculture of "Methanobacillus omelianskii" with Thermodyn®



#### △Gr° for "Methanobacillus omelianskii" as a function of pH at 25°C and 100°C



#### Anaerobic Oxidation of Methane - Why are the $\Delta Gr^{\circ}$ values confusing

Table 2  $\Delta G^{\circ\prime}$  values for overall reactions using different electron acceptors

	Reaction	$\Delta G^{\circ\prime}$ (kJ mol <sup>-1</sup> CH <sub>4</sub> )
1:	$\overline{\text{CH}_4 + \text{SO}_4^{2-} \rightarrow \text{HCO}_3^- + \text{HS}^- + \text{H}_2\text{O}}$	-31.4
2:	$CH_4 + 4NO_3^- \rightarrow CO_2 + 4NO_2^- + 2H_2O$	-519.9
3:	$\begin{array}{c} \mathrm{CH_4} + 4\mathrm{MnO_2} + 7\mathrm{H^+} \rightarrow \mathrm{HCO_3^-} + 4\mathrm{Mn^{2+}} \\ + 5\mathrm{H_2O} \end{array}$	-511.6
4:	$\begin{array}{l} \mathrm{CH_4} + 8\mathrm{Fe(OH)_3} + 15\mathrm{H^+} \rightarrow \mathrm{HCO_3^-} + 8\mathrm{Fe^2} \\ ^+ + 21\mathrm{H_2O} \end{array}$	-1691.7

Overall reactions from Knittel et al. [41], Haroon et al. [32], and Beal et al. [6].  $\Delta G^{\circ\prime}$  values were calculated at pH 7, 25 °C, I value of 0.25 M using the formula supplied by Alberty [1]. The  $\Delta G^{\circ\prime}$  value for the iron reduction reaction was calculated assuming that ferric hydroxide dissociates into its respective ions

- 1. Calculate the chemical species that are actually participating in the reaction at pH 7
- 2. Formulate stoichiometries that are comparable
- 3. Assign physical states of reactants
- 4. Define actual conditions and correct for environmental variables that influence them

Methane oxidation by anaerobic archaea for conversion to liquid fuels. Thomas J. Mueller et al. 2015. J Industr. Microbiol. Biotechnol. 42:391-401



Anaerobic Oxidation of Methane - Corrected Equations for pH 7 / 25°C

1: 
$$CH_4 + SO_4^{2-} \rightarrow H_2CO_3 + HCO_3^{-} + H_2S + HS^{-} + H_2O_3^{-}$$

2: 
$$CH_4 + 4 NO_3^- \rightarrow H_2CO_3 + HCO_3^- + 4 NO_2^- + H_2O_3^-$$

3: 
$$CH_4 + 4 MnO_2 + 7 H^+ \rightarrow H_2CO_3 + HCO_3^- + Mn^{2+} + H_2O_3$$

4:  $CH_4 + 8 Fe(OH)_3 + 15 H^+ \rightarrow H_2CO_3 + HCO_3^- + 8 Fe^{2+} + H_2O_3$ 

Table 2  $\Delta G^{\circ\prime}$  values for overall reactions using different electron acceptors

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4:	$\begin{array}{l} \mathrm{CH_4} + 8\mathrm{Fe(OH)_3} + 15\mathrm{H^+} \rightarrow \mathrm{HCO_3^-} + 8\mathrm{Fe^2} \\ ^+ + 21\mathrm{H_2O} \end{array}$	-1691.7



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# How to read the following graphs

The reaction to be tested for energetic feasibility: 2 CO<sub>2</sub> + 4 H<sub>2</sub>  $\rightarrow$  CH<sub>3</sub>COO<sup>-</sup> + H<sup>+</sup> + H<sub>2</sub>O is presented in Thermodyn $^{\ensuremath{\mathbb{C}}}$  in the

following manner

Reactio n No. (S,P) Stoich. Coeff.	nter formula	Special Sremarks	Activity	Variable	∆Gr values in KiloJoule / mol (k calculated for chosen condition activities and range according t	J/mol) are s for pH, T to	,	
1 s 2 CC	)2	aq	1.00Ē	-03				
1 s 4 H2		aq		v	$\Delta Gr = \Delta Gr^0 + B \cdot T \cdot ln [CH_3COO] \cdot [H^*] \cdot [I$	$\frac{1}{2}O$ = $\Lambda Gr^{0}$ +	R.T.InC	
<b>1</b> p 1 CH	I3COO-	aq	1.00E	-04	$\Delta G = \Delta G + H + H + H = [CO]^2 \cdot [H]^4$			
<b>1</b> p 1 H+		aq	1.00E	-08				
1 p 2 H2	0	Ľ	1.00E-	+00	or $\Delta$ Gr = R · T · In $\frac{Q}{K}$			
H <sub>2</sub> is the redu	ctant who	se activity c	an be		κ <sub>eq</sub>		_	
vaneu				and p	resented as a logarithmic graph of fre	e energy		
Temp. (K)	373.15	27	3.15	∆Gr a	s a function of the activity of the redu			
Min. variable:	1.00E-08	1.00	E-08		Activity of reductant (hydrogen, H	2) 50 - O		
Max. variable:	1.00E+00	1.00E	E+00		-9 -8 -7 -6 5 -4 -3 -2			
R das consta	nt					-50 - uoiti		
-9 21/51.10	-3 [k l. mol-	1. 12-11			5 State 1998	-100 - 20 9		
=8.31451 · 10 <sup>-3</sup> [kJ · mol <sup>-1</sup> · K <sup>-1</sup> ] (concentration basis) =82.057844 · 10 <sup>-3</sup> [atm · L·mol <sup>-1</sup> · K <sup>-1</sup> ]								
-02.007044				If ∆Gi	$r > 0 \rightarrow$ reaction is endergonic	-200 L		
(pressure bas	515)			If ∆Gi	$r < 0 \rightarrow$ reaction is exergonic	T = 373.15 k	<	
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## Stoichiometric Balancing with Speciation of Oxidation Products

 $CH_3CHOHCOO^- + 4 NO_2^- + 5 H^+ \rightarrow 3 CO_2 + 2 N_2 + 5 H_2O$ 

Most of the CO<sub>2</sub> dissolves in water and becomes H<sub>2</sub>CO<sub>3</sub>\* and at pH 7.2 some of the H<sub>2</sub>CO<sub>3</sub> dissociates into HCO<sub>3</sub><sup>-</sup>. H<sub>2</sub>CO<sub>3</sub><sup>\*</sup> = H<sub>2</sub>CO<sub>3</sub> (actual acid) + CO<sub>2</sub> (as dissolved gas).

The above equation must thus be written as

 $CH_{3}CHOHCOO^{-} + 4 NO_{2}^{-} + (2+x) H^{+} \rightarrow x H_{2}CO_{3}^{*} + (3-x) HCO_{3}^{-} + 2 N_{2} + 2 H_{2}O_{3}^{-}$ 

Find the value for x, if the reaction conditions are pH 7.2, T =  $25^{\circ}$ C and ionic strength I = 1



## **Speciation of Oxidation Products**

Determine the ratio of  $H_2CO_3$  /  $HCO_3^-$  from the pK values of the carbonic acid / bicarbonate / carbonate acid/base reactions and the actual pH according to

$$[H_2CO_3^*] : [HCO_3^-] : [CO_3^{2-}] = \alpha_0 \times C_T : \alpha_1 \times C_T : \alpha_2 \times C_T$$

 $\alpha_0 + \alpha_1 + \alpha_2 = 1$ 

where  $\alpha_0 + \alpha_1 + \alpha_2 = 1$  and  $C_T = [H_2CO_3^*] + [HCO_3^-] + [CO_3^2^-]$  $C_{T}$  = 3 in our example for the complete oxidation of lactate

and

$$\alpha_{0} = \frac{\left[H^{+}\right]^{2}}{\left[H^{+}\right]^{2} + K_{1}\left[H^{+}\right] + K_{1} \cdot K_{2}} \qquad \alpha_{1} = \frac{K_{1}\left[H^{+}\right]}{\left[H^{+}\right]^{2} + K_{1}\left[H^{+}\right] + K_{1} \cdot K_{2}} \qquad \alpha_{2} = \frac{K_{1} \cdot K_{2}}{\left[H^{+}\right]^{2} + K_{1}\left[H^{+}\right] + K_{1} \cdot K_{2}}$$

 $K_1$  is equilibrium value for  $H_2CO_3^* \leftrightarrow HCO_3^- + H^+$ ,  $pK_{1(25^\circ C, I=1)} = 6.38$  $K_2$  is equilibrium value for  $HCO_3^ \leftrightarrow CO_3^{2-} + H^+$ ,  $pK_{2(25^\circ C, I=1)} = 10.32$ 

Proton concentration in  $\alpha_0$ ,  $\alpha_1$ ,  $\alpha_2$  can be replaced by [H+] = 10<sup>-pH</sup>, since pH = -<sub>10</sub>log[H+]

$$\alpha_{0} = \frac{10^{-2pH}}{10^{-2pH} + K_{1} \cdot 10^{-pH} + K_{1} \cdot K_{2}} \quad \alpha_{1} = \frac{K_{1} \cdot 10^{-pH}}{10^{-2pH} + K_{1} \cdot 10^{-pH} + K_{1} \cdot K_{2}} \quad \alpha_{2} = \frac{K_{1} \cdot K_{2}}{10^{-2pH} + K_{1} \cdot 10^{-pH} + K_{1} \cdot K_{2}}$$

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## Stoichiometry of Reaction Equation for desired pH

Calculated for the boundary conditions pH 7.2, T = 25°C and ionic strength I = 1  $a_0 = 0.133$   $a_1 = 0.866$   $a_2 < 0.001$ , neglected in calculation [H<sub>2</sub>CO<sub>3</sub>\*] = 0.399; calculate with x = 0.4 [HCO<sub>3</sub>] = 2.598; calculate with 3-x = 2.6 [CO<sub>3</sub><sup>2</sup>] < 0.003 And inserted into reaction equation CH<sub>3</sub>CHOHCOO<sup>-</sup> + 4 NO<sub>2</sub><sup>-</sup> + (2+x) H<sup>+</sup>  $\Rightarrow$  x H<sub>2</sub>CO<sub>3</sub>\* + (3-x) HCO<sub>3</sub><sup>-</sup> + 2 N<sub>2</sub> + 2 H<sub>2</sub>O yields CH<sub>3</sub>CHOHCOO<sup>-</sup> + 4 NO<sub>2</sub><sup>-</sup> + 2.4 H<sup>+</sup>  $\Rightarrow$  0.4 H<sub>2</sub>CO<sub>3</sub>\* + 2.6 HCO<sub>3</sub><sup>-</sup> + 2 N<sub>2</sub> + 2 H<sub>2</sub>O



## Summary: Complete Degradation of Lactate at pH 7.2 with different Oxidants

Nitrite and nitrate to dinitrogen oxide  $CH_3CHOHCOO^- + 6 NO_2^- + 4.4 H^+ \rightarrow 0.4 H_2CO_3^* + 2.6 HCO_3^- + 3 N_2O + 3 H_2O$ 

CH<sub>3</sub>CHOHCOO<sup>-</sup> + 3 NO<sub>3</sub><sup>-</sup> + 1.4 H<sup>+</sup> → 0.4 H<sub>2</sub>CO<sub>3</sub><sup>\*</sup> + 2.6 HCO<sub>3</sub><sup>-</sup> + 1.5 N<sub>2</sub>O + 1.5 H<sub>2</sub>O

Nitrite and nitrate to elemental nitrogen  $CH_3CHOHCOO^- + 4 NO_2^- + 2.4 H^+ \rightarrow 0.4 H_2CO_3^* + 2.6 HCO_3^- + 2 N_2 + 2 H_2O$ 

CH<sub>3</sub>CHOHCOO<sup>-</sup> + 2.4 NO<sub>3</sub><sup>-</sup> + 0.8 H<sup>+</sup> → 0.4 H<sub>2</sub>CO<sub>3</sub><sup>\*</sup> + 2.6 HCO<sub>3</sub><sup>-</sup> + 1.2 N<sub>2</sub> + 1.2 H<sub>2</sub>O

Nitrite and nitrate to ammonia CH<sub>3</sub>CHOHCOO<sup>-</sup> + 2 NO<sub>2</sub><sup>-</sup> + 2.4 H<sup>+</sup> + 2 H<sub>2</sub>O  $\rightarrow$  0.4 H<sub>2</sub>CO<sub>3</sub><sup>\*</sup> + 2.6 HCO<sub>3</sub><sup>-</sup> + 2 NH<sub>4</sub><sup>+</sup>

CH<sub>3</sub>CHOHCOO<sup>-</sup> + 1.5 NO<sub>3</sub><sup>-</sup> + 1.4 H<sup>+</sup> + 1.5 H<sub>2</sub>O → 0.4 H<sub>2</sub>CO<sub>3</sub><sup>\*</sup> + 2.6 HCO<sub>3</sub><sup>-</sup> + 1.5 NH<sub>4</sub><sup>+</sup>



## C / N Ratios: Complete Degradation of Lactate at pH 7.2 with different Oxidants

Nitrite and nitrate to dinitrogen oxide CH<sub>3</sub>CHOHCOO<sup>-</sup> + 6 NO<sub>2</sub><sup>-</sup> + 4.4 H<sup>+</sup> → 0.4 H<sub>2</sub>CO<sub>3</sub><sup>\*</sup> + 2.6 HCO<sub>3</sub><sup>-</sup> + 3 N<sub>2</sub>O + 3 H<sub>2</sub>O C / N<sub>oxidant</sub> = 0.5 CH<sub>3</sub>CHOHCOO<sup>-</sup> + 3 NO<sub>3</sub><sup>-</sup> + 1.4 H<sup>+</sup> → 0.4 H<sub>2</sub>CO<sub>3</sub><sup>\*</sup> + 2.6 HCO<sub>3</sub><sup>-</sup> + 1.5 N<sub>2</sub>O + 1.5 H<sub>2</sub>O  $C / N_{oxidant} = 1.0$ Nitrite and nitrate to elemental nitrogen CH<sub>3</sub>CHOHCOO<sup>-</sup> + 4 NO<sub>2</sub><sup>-</sup> + 2.4 H<sup>+</sup> → 0.4 H<sub>2</sub>CO<sub>3</sub><sup>\*</sup> + 2.6 HCO<sub>3</sub><sup>-</sup> + 2 N<sub>2</sub> + 2 H<sub>2</sub>O  $C / N_{oxidant} = 0.75$ CH<sub>3</sub>CHOHCOO<sup>-</sup> + 2.4 NO<sub>3</sub><sup>-</sup> + 0.8 H<sup>+</sup> → 0.4 H<sub>2</sub>CO<sub>3</sub><sup>\*</sup> + 2.6 HCO<sub>3</sub><sup>-</sup> + 1.2 N<sub>2</sub> + 1.2 H<sub>2</sub>O C / N<sub>oxidant</sub> = 1.25 Nitrite and nitrate to ammonia CH<sub>3</sub>CHOHCOO<sup>-</sup> + 2 NO<sub>2</sub><sup>-</sup> + 2.4 H<sup>+</sup> + 2 H<sub>2</sub>O → 0.4 H<sub>2</sub>CO<sub>3</sub><sup>\*</sup> + 2.6 HCO<sub>3</sub><sup>-</sup> + 2 NH<sub>4</sub><sup>+</sup>  $C / N_{oxidant} = 1.5$ CH<sub>3</sub>CHOHCOO<sup>-</sup> + 1.5 NO<sub>3</sub><sup>-</sup> + 1.4 H<sup>+</sup> + 1.5 H<sub>2</sub>O → 0.4 H<sub>2</sub>CO<sub>3</sub><sup>\*</sup> + 2.6 HCO<sub>3</sub><sup>-</sup> + 1.5 NH<sub>4</sub><sup>+</sup> C / N<sub>oxidant</sub> = 2.0 Ter 🖓 i-research & training MBL Woods Hole / Microbial Diversity Summer Course 2016 / Kurt Hanselmann / 57 / Bio-Thermodynamics 1

## Results

The hypothesis for regulating one pathway over the other is

high carbon to nitrate or nitrite ratios (nitrate or nitrite (oxidant) limited conditions) favor ammonia production.

This can be supported by the stoichiometries.

High nitrite to nitrate ratios are not necessarily different, provided the nitrate to nitrite reduction step is not kinetically hindered.



Try to show how the pathways differ with regard to potential energy yields.

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## Summary: Calculating Gibbs free energies

Write a balanced chemical reaction

Consider the phases of each species

Choose a temperature and pressure

Obtain Gf<sup>o</sup> at that temperature and pressure for each species (Standard free energy of formation from the elements (Gf<sup>o</sup>).

Calculate ∆Grº

Calculate K<sub>eq</sub> (equilibrium distribution)

Determine actual conditions under which the reaction takes place

Calculate actual  $\Delta$ Gr as  $\Delta$ Gr<sup>o</sup> + RT InQ



# Contents

- Introduction to Bio-Thermodynamics
- The concept of "free energy"
- "Standard free energy" vs. "actual free energy"
- Calculating "free energy" from electrochemical potential energy
- Summary of terms, relations and abbreviations
- Examples using Thermodyn



# Setting the Scene

What you once knew, but never thought that it could be important and helpful in Microbial Ecology

Calculating half cell potentials using Nernst's equation and free Gibbs Energy Changes

Relations between  $\Delta Gr$  and E

# (Gibbs) Free energy Change of Reaction under Equilibrium Conditions $\Delta Gr^0$

 $aA + bB \iff cC + dD$ 

Equilibrium relation:

Difference of the sum of the free energies of formation of the products and the free energies of formation of the educts (substrates)

$$\Delta Gr^{0} = \sum Gf^{0}_{\text{products}} - \sum Gf^{0}_{\text{substrates}} \quad \left[ kJ \text{ mol}^{-1} \right]$$

or:

Difference of the enthalpy of the reaction and the temperature corrected entropy of the reaction

$$\Delta Gr^{0} = \Delta Hr^{0} - T \cdot \Delta Sr^{0} \qquad \left[ kJ \text{ mol}^{-1} \right]$$

Free energy of reaction for standard state equilibrium conditions  $\Delta Gr^0$ :

$$\Delta Gr^{0} = -R \cdot T \cdot \ln K_{eq} = -2.3026 \cdot R \cdot T \cdot \log K_{eq} = 0$$

$$\log K_{eq} = -\frac{\Delta G r^{0}}{2.3026 \cdot R \cdot T}$$
;  $K_{eq} = 10^{-\frac{\Delta G r^{0}}{2.3026 \cdot R \cdot T}}$ 

R gas constant =8.31451  $\cdot$  10<sup>-3</sup> [kJ  $\cdot$  mo<sup>I-1</sup>  $\cdot$  K<sup>-1</sup>] (concentration basis) =82.057844  $\cdot$  10<sup>-3</sup> [atm  $\cdot$  I  $\cdot$  mol<sup>-1</sup>  $\cdot$  K<sup>-1</sup>] (pressure basis)





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 $aA + bB \iff cC + dD$ 

Non-equilibrium relation:

Ratio of the algebraic product of the activities of the products divided by the algebraic product of the activities of the educts (substrates)

 $Q = \frac{\left\{C\right\}^{c} \cdot \left\{D\right\}^{d}}{\left\{A\right\}^{a} \cdot \left\{B\right\}^{b}} \text{ or } \frac{a_{C}^{c} \cdot a_{D}^{d}}{a_{A}^{a} \cdot a_{B}^{b}} \text{ or } a_{C}^{c} \cdot a_{D}^{d} \cdot a_{A}^{-a} \cdot a_{B}^{-b} \text{ or } \prod_{i} a_{i}^{vi}$ 

 $\left\{ \right\}$  and  $a_i^{vi}$  denote activities; i reactant A, B, C, D; vi stoichiometric factors a, b, c, d

Free energy of reaction for non-equilibrium conditions  $\Delta Gr$ : Deviation of free energy from a reference standard state  $\Delta Gr^0$ 

 $\Delta Gr = \Delta Gr^0 + R \cdot T \cdot InQ$ 



# The three Key Relationships in ecological Thermodynamics

## 1. Equilibrium relation:

Difference of the sum of the free energies of formation of the products and the free energies of formation of the educts (substrates)

$$\Delta Gr^{0} = \sum Gf^{0}_{\text{products}} - \sum Gf^{0}_{\text{substrates}} \quad \left[ kJ \text{ mol}^{-1} \right]$$

2. Free energy of reaction for standard state equilibrium conditions  $\Delta Gr^0$ :

$$\Delta Gr^{0} = -R \cdot T \cdot \ln K_{eq} = -2.3026 \cdot R \cdot T \cdot \log K_{eq} = 0$$
  
$$\log K_{eq} = -\frac{\Delta Gr^{0}}{2.3026 \cdot R \cdot T} ; \quad K_{eq} = 10^{-\frac{\Delta Gr^{0}}{2.3026 \cdot R \cdot T}}$$

3. Free energy of reaction under actual conditions  $\Delta Gr$ :  $\Delta Gr = \Delta G^{\circ} + R \cdot T \cdot InQ$ 



## Summary: Non-equilibrium Thermodynamics applied to microbial Processes

$$a A + b B \Leftrightarrow c C + d D$$

$$Q = \frac{\begin{bmatrix} C \end{bmatrix}^{\circ} \begin{bmatrix} D \end{bmatrix}^{d}}{\begin{bmatrix} A \end{bmatrix}^{a} \begin{bmatrix} B \end{bmatrix}^{b}}$$

$$\Delta Gr = \Delta Gr^{\circ} + RT \ln Q$$

$$\Delta Gr^{\circ} = -RT \ln K_{eq} = \sum Gf^{\circ}(P) - \sum Gf^{\circ}(S)$$

$$\Delta Gr = RT \ln \frac{Q}{K_{eq}}$$

$$E_{1/2} = -\frac{\Delta Gr}{n F} = -\frac{1}{n F} \left( \Delta Gr^{\circ} + RT \ln Q \right) = -\frac{R T}{n F} \ln \frac{Q}{K_{eq}}$$

R = 8.31451 10<sup>-3</sup> [kJ mol<sup>-1</sup> °K<sup>-1</sup>], F= 96.485309 [kJ mol<sup>-1</sup> V<sup>-1</sup>]



## Distinguish $\Delta Gr^{\circ}$ from $\Delta Gr$ and do not use $\Delta Gr^{\circ}$

 $\Delta Gr^\circ$  is a chemist's view of free energy of a reaction in equilibrium, standardized to molar conditions:

 $\Delta Gr^{0} = -R \cdot T \cdot lnK_{eq} = -2.3026 \cdot R \cdot T \cdot logK_{eq} = 0$ 

 $K_{eq}$  = equilibrium coefficient (ratio)

 $\Delta Gr$  is a biologist's view of free energy available to microbes in a particular environment.

The actual conditions in the environment determine how much energy is available while the reaction proceeds to equilibrium

 $\Delta Gr = \Delta Gr^{\circ} + RT \ln Q$ 

Q = non - equilibrium ratio $R = 8.31451 \ 10^{-3} \left[ kJ \ mol^{-1} \ ^{\circ}K^{-1} \right]$ 



## Thermochemical Relations that you might see in the Literature

 $\Delta Gr^{o'}$  under standard conditions at pH 7 at standard conditions, 1 M and 1 atm

 $\begin{array}{l} \Delta Gr^{o^{\prime}}=\Delta G^{o}+m\;\Delta G'_{f}\left(H^{+}\right)\\ m=\text{number of protons produced}\\ \Delta G'_{f}(H^{+})=\text{free energy of formation of }H^{+}\;\text{at pH 7}=-39.96\;\text{kJ/mol}\end{array}$ 

(there is no need to use  $\Delta Gr^{\circ}$ '. pH must be treated as one of the environmental reaction conditions and calculations can be made at any pH)



## What is the Difference between $\Delta Gr$ and $\Delta Gr^{\circ}$ ?

 $CH_{3}COO^{-}_{(aq)} + H^{+}_{(aq)} = CO_{2(aq)} + CH_{4(aq)}$ 

 $\Delta Gr^{o}$  = - 2.303 R T log K<sub>eq</sub>

 $-\log K_{eq} = pK_{eq} = \dots$ 

 $\Delta Gr = \Delta Gr^{\circ} + 2.303 \text{ R T} (\log a_{CO2} + \log a_{CH4} - \log a_{Ac-} - \log a_{H+})$ 



# What is the difference between $K_{eq}$ and Q ?

$$\Delta Gr = \Delta Gr^{\circ} + RT \ln Q_{r}$$
  
$$\Delta Gr^{\circ} = - RT \ln K_{eq}$$
  
$$\Delta Gr = - RT \ln K_{eq} + RT \ln Q_{r} = RT \ln (Q_{r}/K_{eq})$$

What is Q<sub>r</sub>? The activity product of reaction 'r'

Example

 $CH_3COO^-_{(aq)} + H^+_{(aq)} \longrightarrow CH_{4(aq)} + CO_{2(aq)}$ 







# Conditions for thermochemical Reactions




#### Abbreviations for Terms used

- ratio of actual activity products of reactants Q
- gas constant =8.31451 10<sup>-3</sup> [kJ mol<sup>-1</sup> K<sup>-1</sup>], (concentration basis) R =82.057844 10<sup>-3</sup>[atm Liter mol<sup>-1</sup> K<sup>-1</sup>] (pressure basis)
- Т thermodynamic temperature in [K]
- Faraday's constant = 96.485309 [kJ mol<sup>-1</sup> V<sup>-1</sup>] F
- number of electrons transferred n
- Gf⁰ standard free energy of formation [kJ/mol]
- standard enthalpy of formation [kJ/mol] Hf⁰
- Sf⁰ standard entropy of formation [J/K mol]
- ∆Gf⁰ standard free energy change of formation [kJ/mol]
- standard enthalpy change of formation, [kJ/mol]  $\Delta H f^{\circ}$
- ∆Gr⁰ change of Gibbs free energy of reaction at standard conditions = -R T InK<sup>o</sup>, [kJ/mol]
- change of Gibbs free energy of reaction at standard conditions except for one reactant ∆Gr°' (e.g. at pH 0), [kJ/mol]
- change of Gibbs free energy of reaction with actual conditions, [kJ/mol] ∆Gr
- K<sub>eq</sub>= K<sup>o</sup> thermondynamic equilibrium coefficient
- $\log K_{eq}$  - $\Delta Gr^{o}$  / 2.3026 R T
- Q disequilibrium ratio
- $\Delta E$ electrochemical potential =emf,  $\Delta Er = \Delta Gr / n F$  [Volt]
- $\Delta Er^{o}$ standard emf= electrochemical reference potential = -\Delta Gro / n F [Volt]

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# Definition of Terms for Standard Conditions

#### $\Delta Gr^{\circ} = \sum Gf^{\circ}(products) - \sum Gf^{\circ}(educts)$

The Gibbs free energy ( $\Delta$ Gr<sup>o</sup>) tells us in which direction a reaction will take place.  $\Delta$ Gr° is the sum of the Gibbs Free Energies of formation of the products on the right hand side of a reaction, minus the sum of the Gibbs Free Energies of formation of the substrates on the left hand side of the reaction.

If  $\Delta Gr^{\circ} < 0$ , the reaction will proceed to the right (in the direction as written in the stoichiometric reaction equation); if  $\Delta Gr^{\circ} > 0$ , the reaction will proceed to the left.

#### $\Delta$ Hr<sup>o</sup> = $\Sigma$ Hf<sup>o</sup>(products) - $\Sigma$ Hf<sup>o</sup>(educts)

The enthalpy of reaction ( $\Delta$ Hr<sup>o</sup>) tells us how much heat will flow in or out of the reaction system.

If  $\Delta$ Hr<sup>o</sup> < 0, the reaction is exothermic -- it releases heat. For example, degradation of organic carbon compounds (C<sub>org.</sub> + O<sub>2</sub> --> CO<sub>2</sub> + H<sub>2</sub>O) gives off heat. If  $\Delta$ Hr<sup>o</sup> > 0, the reaction is endothermic -- it consumes heat. Melting ice [H2Os(ice) --> H2OI(water)] is endothermic -- it takes up heat and cools the environment.

#### $\Delta Sr^{\circ} = \sum Sf^{\circ}(products) - \sum Sf^{\circ}(educts)$

The entropy of a reaction ( $\Delta Sr^{0}$ ) tells us whether the products (substrates, reactants) are more or less disordered than the educts. For example, the reaction of liquid water to steam  $[H_2O_{I(water)} \rightarrow H_2O_{g(vapor)}]$  has a large entropy. The gaseous water molecules are more dispersed, are less well bonded together, and have greater kinetic energy.



#### Hierarchy of thermodynamic Terms: Define the terms





### Hierarchy of thermodynamic Terms: Define the Terms (Solution)



Basic thermochemical values

Thermochemical values of compounds calculated from basic thermochemical values

Gibbs free energies and electron potentials of reactions at standard state equilibrium calculated from thermochemical values of reactants

Gibbs free energies and electron potentials of reactions at nonequilibrium conditions in relation to standard state equilibrium conditions calculated from activities of reactants

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#### Thermodynamic Relationships to remember

 $\Delta Gr = \text{free reaction energy at disequilibrium [kJ/Mol]}$   $\Delta Gr^{\circ} = \text{free reaction energy at equilibrium and for standard conditions (T = 298K)}$   $\Delta Gr^{\circ} = - R^*T^* \ln K_{eq} [kJ/Mol]$   $\Delta Gr = \Delta Gr^{\circ} + R^*T^* \ln Q [kJ/Mol] \text{ or } \Delta Gr = R^*T^* \ln [Q/Keq], \text{ if you replace } \Delta Gr^{\circ} \text{ with } - R^*T^* \ln K_{eq}$  Q disequilibrium ratio of reactants for a given set of conditions  $At equilibrium \Delta Gr = 0, \text{ since } Q = K_{eq}$   $\Delta Gr = R^*T^* \ln [Q/Keq] \text{ is exergonic, if } Q < Keq$   $K_{eq} \text{ equilibrium coefficient}$   $R = \text{gas constant } 8.31451^* 10^{-3} [kJ^* \text{Mol}^{-1*} \text{K}^{-1}]$  kJ = Kilojoule T in K = temperature in Kelvin

In (Y) =  $2.3026 \log (Y)$  = conversion of natural logarithms to logarithms to the base 10



# Check the following Statements ! Are they correct?

A chemical reaction will always proceed in the direction towards which its reactants can attain lower Gibbs free energies of formation, i.e.  $\Sigma Gf_{\text{substrates}}^{\circ} < \Sigma Gf_{\text{substrates}}$ 

A chemical reaction will always proceed in the direction towards which its Gibbs free energy is negative, i.e.  $\Delta$ Gr < 0

Products and educts are in equilibrium when the Gibbs free energies of formation are equal, i.e.  $\sum Gf_{\text{products}} = \sum Gf_{\text{substrates}}$ 

Products and educts are in equilibrium when the Gibbs free energy of a reaction is zero, i.e.  $\Delta Gr = 0$ 



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#### Check – Are the Statements correct?

Under what conditions are products and educts at equilibrium?

If products and educts are not at equilibrium how can one predict the direction in which the reaction would proceed?

How will a reaction system behave if it is left to proceed entirely by itself?

Illustrate with thermodynamic terms the saying "You can't shovel manure into the rear end of a horse and expect to get hay to out of its mouth"



### **Chemical Properties of Elements and Molecules**

http://www.webelements.com

Element specific, e.g. Carbon: http://www.webelements.com/carbon/

Compound specific within C: <u>http://www.webelements.com/carbon/compounds.html</u>



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### Aerobic and anaerobic Ammonium Oxidation as Life Styles - 1

Define the conditions under which these life styles are thermodynamically favorable

 $4 \text{ NH}_4^+ + 3 \text{ O}_2 \longrightarrow 2 \text{ N}_2 + 4 \text{ H}^+ + 6 \text{ H}_2\text{O}$  $NH_4^+ + NO_2^- \longrightarrow N_2 + 2H_2O$  $5 \text{ NH}_4^+ + 3 \text{ NO}_3^- \longleftrightarrow 4 \text{ N}_2 + 2 \text{ H}^+ + 9 \text{ H}_2\text{O}$  $2 \text{ NH}_4^+ + 3 \text{ N}_2\text{O} \iff 4 \text{ N}_2 + 2 \text{ H}^+ + 3 \text{ H}_2\text{O}$ 

Boundary conditions for Thermodyn: Reductant:  $[NH_4^+] = 10^{-3}$  [Mol/l]; variable oxidant:  $10^{-10} < [oxidant] < 10^{-1} [Mol/l];$ N<sub>2</sub> = 10<sup>-6</sup> [Mol/l] pH = 7.6  $T = 25^{\circ}C$  (298.15 K)





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### Aerobic and anaerobic Ammonium Oxidation - 2

Example: Anaerobic Methane Oxidation in a Consortium

 $CH_{4 aq} + SO_{4}^{2-}aq \rightarrow HCO_{3}^{-}aq + HS^{-}aq + H_{2}O_{1}$ 

For Thermodyn®





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#### Nitrite-driven anaerobic Methane Oxidation by oxygenic Bacteria

Ettwig, K.F. et al. 2010. Nature 464, 543-548, March 25

#### Abstract

Only three biological pathways are known to produce oxygen: photosynthesis, chlorate respiration and the detoxification of reactive oxygen species. Here we present evidence for a fourth pathway, possibly of considerable geochemical and evolutionary importance. The pathway was discovered after metagenomic sequencing of an enrichment culture that couples anaerobic oxidation of methane with the reduction of nitrite to dinitrogen. The complete genome of the dominant bacterium, named "*Candidatus Methylomirabilis oxyfera*", was assembled.

This apparently anaerobic, denitrifying bacterium encoded, transcribed and expressed the well-established aerobic pathway for methane oxidation, whereas it lacked known genes for dinitrogen production. Subsequent isotopic labeling indicated that "*M. oxyfera*" bypassed the denitrification intermediate nitrous oxide (N<sub>2</sub>O, laughing gas) by the conversion of two nitric oxide (NO) molecules to dinitrogen and oxygen, which was used to oxidize methane. These results extend our understanding of hydrocarbon degradation under anoxic conditions and explain the biochemical mechanism of a poorly understood freshwater methane sink. Because nitrogen oxides were already present on early Earth, our finding opens up the possibility that oxygen was available to microbial metabolism before the evolution of oxygenic photosynthesis.



### Is Dismutation of Nitric Oxide thermodynamically feasible?



#### Is oxygenic Denitrification thermodynamically feasible?

 $1 \text{ CH}_4 + 8 \text{ NO}_2^- + 8 \text{ H}^+ \implies 1 \text{ CO}_2 + 4 \text{ N}_2 + 4 \text{ O}_2 + 6 \text{ H}_2\text{O}$ 



#### Mixed Denitrification of Methane

In the issue of March 25, 2010 of Nature<sup>\*</sup>, we find an interesting new process that links the oxidation of methane under anoxic conditions to the denitrification of nitrite. Concomitant to the release of  $N_2$ , the researchers describe the production of  $O_2$  as a novel oxygenic process. The bacterial species that carries it out was named *Candidatus Methylomirabilis oxyfera*, which means "strange oxygen-producing methane consumer".

Check the mixed oxidation process from a thermodynamic perspective by

- 1. Writing the stoichimetrically balanced overall equation for methane oxidation with nitrite and the production of oxygen and elemental nitrogen gas.
- 2. Assuming reasonable activities for the reactants for thermodynamic calculations.
- 3. Carrying out the calculations with the aid of Thermodyn<sup>©</sup>
- 4. Discussing the graphical presentation of the thermodynamic calculations

Can you think of reasons why mixed denitrification did not work in this organism when nitrate was offered as an electron acceptor for methane oxidation?

*Methylomirabilis oxyfera* was isolated from anoxic sediments in ditches of agricultural lands. Can you think of geological situations in Earth history or on other planets, where oxygen could have been produced this way?

\* Ettwig, K.F. et al. 2010. Nitrite-driven anaerobic methane oxidation by oxygenic bacteria. Nature 464, 543-548, March 25



### Oxygenic Denitrification of Methane in 2 Steps - Calculations with Thermodyn 1





Microbial Ecolo



#### Oxygenic Denitrification of Methane in 2 Steps

### Denitrification and Methane Oxidation by Methylomirabilis oxyfera





Microbial Ecol

#### Nitrogen, Oxygen and Nitric Oxide

Electronic configuration N: 1s2 2s2 2p3

Electronegativity N: 3.04 (Pauling scale)

Electronic configuration O: 1s2 2s2 2p4

Electronegativity O: 3.44 (Pauling scale)

The nitric oxide molecule is a highly reactive free radical.



NO exposed to  $O_2$  reacts to nitrous oxide 2 NO +  $O_2 \rightarrow 2 NO_2$ 

NO can be formed from  $O_2$  and  $N_2$  by an endothermic reaction at temperatures > 2000°C (lightning)

 $N_2 + O_2 \rightarrow 2 \text{ NO}$ 

The reverse reaction would be highly exothermic, energetically exergonic, i.e. favourable.

catalytic converters in cars minimize NO emission by conversion of NO to  $O_2$  and  $N_2$ 



#### Is Nitric Oxide Dismutase an additional Enzyme in the Nitrogen Cycle?





### Phylogeny of 'Methylomirabilis oxyfera' pmoA protein sequences





# Coupling of methane oxidation and nitrite reduction in enrichment cultures of *'Methylomirabilis oxyfera'*





∛i-r₊t

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Microbial Ed



# Facultative Denitrifyers

Use Thermodyn to define conditions under which aerobic respiration and denitrification can proceed simultaneously ?

Why can sulfate reduction not compete energetically with denitrification and aerobic respiration ?

Choose the dissimilation of succinate under the following boundary conditions:

Succinate (C <sub>4</sub> H <sub>4</sub> O <sub>4</sub> <sup>2-</sup> )	1 Millimol / Liter [millimolar = mM]
Oxygen	1 $\mu$ M $\leq$ O <sub>2</sub> $\leq$ 500 $\mu$ M
Nitrate	1 $\mu$ M $\leq$ NO <sub>3</sub> <sup>-</sup> $\leq$ 500 $\mu$ M
Sulfate	1 $\mu$ M $\leq$ SO <sub>4</sub> <sup>2-</sup> $\leq$ 500 $\mu$ M
Hydrogencarbonate Hydrogensulfide Nitrogen gas Proton concentration Temperature	$HCO_3^-$ constant at 10 mM $HS^-$ constant at 100 $\mu M,$ how can this remain constant ? $N_2$ constant at 100 $\mu M$ pH 7.5 $25^\circ C$





Microbial E

# Alternative Oxidants



# Could oxygenic Dismutation Reactions of N and S-oxides have played a Role in the Production of $O_2$ during early Earth?

Check the following reactions for thermodynamic feasibility:

- 2 NO<sub>(g)</sub> N<sub>2(g)</sub> + O<sub>2(g)</sub>
- $N_2O_{(g)}$   $N_{2(g)}$  +  $1/_2O_{2(g)}$
- $NO_{2(g)}$   $^{1/2}N_{2(g)} + O_{2(g)}$
- $SO_{2(g)}$   $S^{o}_{(s)}$  +  $O_{2(g)}$
- $SO_{3 (g)}$   $S^{o}_{(s)}$  +  $1^{1}/_{2} O_{2 (g)}$





Microbial Ecolo

#### Bio-energetic Question: NH₄<sup>+</sup> Oxidation

Under which conditions can NH<sub>4</sub><sup>+</sup> be oxidized aerobically and anaerobically?

- · Define oxidants and conditions that lead to exergonic reactions.
- · Where in the ocean could the anaerobic processes occur?
- · Check with actual measurements of conditions.



#### Ammonia Oxidation by Nitrogen Oxides

Ammonia oxidation with NO<sub>x</sub> (NO + NO<sub>2</sub>), N<sub>2</sub>O and NO<sub>2<sup>-</sup></sub> as oxidants What makes a process proceed in the direction as written? e- transferred  $4 \text{ NH}_4^+ + 3 \text{ NO}_2 \rightarrow 3^{1/2} \text{ N}_2 + 6 \text{ H}_2 \text{O} + 4 \text{ H}^+$ 12  $2 \operatorname{NH}_4^+ + 3 \operatorname{NO} \rightarrow 2^{1/2} \operatorname{N}_2 + 3 \operatorname{H}_2 \operatorname{O} + 2 \operatorname{H}^+$ 6  $2 \text{ NH}_4^+ + 3 \text{ N}_2 \text{O} \rightarrow 4 \text{ N}_2 + 3 \text{ H}_2 \text{O} + 2 \text{ H}^+$ 6  $1 \text{ NH}_4^+ + 1 \text{ NO}_2^- \rightarrow 1 \text{ N}_2 + 2 \text{ H}_2\text{O}$ 3

Make reactions comparable and determine conditions for which the processes are thermodynamically feasible a- transforrad

1 NH <sub>4</sub> +	+	¾ NO <sub>2</sub>	$\rightarrow$	<sup>7</sup> / <sub>8</sub> N <sub>2</sub>	+	1½ H <sub>2</sub> O + 1 H <sup>+</sup>	3
1 NH <sub>4</sub> +	+	1½ NO	$\rightarrow$	$1\frac{1}{4}N_2$	+	1½ H <sub>2</sub> O + 1 H <sup>+</sup>	3
1 NH <sub>4</sub> +	+	1½ N <sub>2</sub> O	$\rightarrow$	2 N <sub>2</sub>	+	1½ H <sub>2</sub> O + 1 H <sup>+</sup>	3
1 NH <sub>4</sub> +	+	1 NO <sub>2</sub> -	$\rightarrow$	1 N <sub>2</sub>	+	2 H <sub>2</sub> O	3

Thermodyn: "NH4-oxidation.xlsx" and "NH4-oxidation\_pH8.xlsx"





Microbial Ecol

Under which  $O_2$  concentrations (22  $\mu$ M, 10  $\mu$ M, <1  $\mu$ M) in the OMZ will aerobic respiration be energetically less favorable than denitrification?

- Define organic substrates (DOC) that can be degraded by both life styles.
- Define oxidants for denitrification.
- Write comparable degradation processes as stoichiometric reaction equations.
- Define conditions that are representative for OMZs.
- · Check energetic yields with actually measured conditions in OMZs.



# Bio-energetic Question: Sequential Use of Oxidants

What are oxidants that are available for microbial degradation of DOM in oxic and anoxic waters?

In which sequence are they being consumed by microbes?

- Assume an organic substrates (DOC) that is being degraded by the organisms.
- Define the sequence of oxidants for degradation.
- Define conditions that are representative for oxic and anoxic waters.
- Write comparable degradation processes as stoichiometric reaction equations.
- Check energetic yields.



#### Bio-energetic Question: Enrichment of sulfate-reducing Bacteria

Why is it thermodynamically more favorable to enrich sulfate-reducers with sulfite, rather than sulfate?

- Define an organic substrates (DOC) that is known to be used by sulfate reducers.
- Write two degradation equations, one with sulfate, the other with sulfite as oxidant Define enrichment conditions.
- Calculate energetic yields for the two processes.
- Find the explanation for the result by studying the biochemical mechanism for sulfate reduction.



Bio-energetic Question: How Nitrogen and Sulfur Cycles can be linked Can reduced Sulfur Compounds act as Electron Donors for Denitrification?



Formulate possible reactions that could be mediated by microorganisms and define the conditions that could support their lifestyles

Microbial F

Bio-energetic Question: How Nitrogen and Sulfur Cycles can be linked Can oxidized Sulfur Compounds act as Electron Acceptors for Nitrification?



Formulate possible reactions that could be mediated by microorganisms and define the conditions that could support their lifestyles



#### Bio-energetic Question: aerobic and denitrifying Oxidation of Sulfur Compounds

These are reactions and corresponding standard free energies from Pancho's presentation on *"Ecología molecular del bacterioplancton oxidante de compuestos reducidos de azufre en las aguas deficientes de oxígeno del Océano Pacífico Sur Oriental"* 

HS <sup>-</sup> + 2O <sub>2</sub> → SO <sub>4</sub> <sup>2-</sup> + 2H <sup>+</sup>	$\Delta G^{\circ}$ = -798 kJ mol <sup>-1</sup>
$S_2O_3^{2-} + H_2O + 2O_2 \longrightarrow 2SO_4^{2-} + 2H^+$	$\Delta G^{\circ}$ = -818 kJ mol <sup>-1</sup>
$S^{\circ} + H_2O + 1.5O_2 \longrightarrow SO_4^{2-} + 2H^+$	$\Delta G^{\circ}$ = -587 kJ mol <sup>-1</sup>
$HS^{-} + NO_{3}^{-} \longrightarrow SO_{4}^{2-} + N_{2}$	$\Delta G^{\circ}$ = -679.3 kJ mol <sup>-1</sup>
$S_2O_3^{2-} + NO_3^{-} \longrightarrow 2SO_4^{2-} + N_2 + H^+$	$\Delta G^{\circ}$ = -907.2 kJ mol <sup>-1</sup>

- Are their stoichiometry correct?
- · Why are the thermodynamic values not comparable?
- How would thermodynamically comparable reactions look like?
- What are actual conditions for substrates and products in the OMZ?
- Calculate actual ∆Gr values and draw conclusions about the occurrence in the OMZ.



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#### Bio-energetic Question: aerobic and denitrifying Oxidation of Sulfur Compounds

These are reactions and corresponding standard free energies from Pancho's presentation on *"Ecología molecular del bacterioplancton oxidante de compuestos reducidos de azufre en las aguas deficientes de oxígeno del Océano Pacífico Sur Oriental"* 

 $HS^{-} + 2O_{2} \longrightarrow SO_{4}^{2-} + 2H^{+} \qquad \Delta G^{\circ} = -798 \text{ kJ mol}^{-1}$   $S_{2}O_{3}^{2-} + H_{2}O + 2O_{2} \longrightarrow 2SO_{4}^{2-} + 2H^{+} \qquad \Delta G^{\circ} = -818 \text{ kJ mol}^{-1}$   $S^{\circ} + H_{2}O + 1.5O_{2} \longrightarrow SO_{4}^{2-} + 2H^{+} \qquad \Delta G^{\circ} = -587 \text{ kJ mol}^{-1}$   $HS^{-} + NO_{3}^{-} \longrightarrow SO_{4}^{2-} + N_{2} \qquad \Delta G^{\circ} = -679.3 \text{ kJ mol}^{-1}$   $S_{2}O_{3}^{2-} + NO_{3}^{-} \longrightarrow 2SO_{4}^{2-} + N_{2} + H^{+} \qquad \Delta G^{\circ} = -907.2 \text{ kJ mol}^{-1}$ 

- Can you think of other reactions in the coupled N-S-cycle that might occur?
- Under what conditions are they thermodynamically favorable?
- Did you include disproportionation of S° and dismutation of thiosulfate?
- Are disproportionation of S° and dismutation of thiosulfate thermodynamically exergonic?

#### Bio-energetic Question: Can Sulfate assure the anaerobic Oxidation of Ammonia to N-oxides?

$$NH_4^+ + SO_4^{2-} \longrightarrow NO_3^- + HS^- + H^+ + H_2O$$

$$NH_4^+ + {}^{3}/_4 SO_4^{2-} \longrightarrow NO_2^- + {}^{3}/_4 HS^- + {}^{1}/_4 H^+ + H_2O$$

$$NH_4^+ + \frac{5}{8}SO_4^2 \longrightarrow NO + \frac{5}{8}HS^- + \frac{3}{8}H^+ + \frac{11}{2}H_2O$$

 $NH_4^+ + \frac{1}{2}SO_4^{2-} \longrightarrow \frac{1}{2}N_2O + \frac{1}{2}HS^- + \frac{1}{2}H^+ + \frac{11}{2}H_2O$ 

Are the proposed processes thermodynamically feasible ?



Microbial Ecolo

# Bio-energetic Question: Combine Sulfate Reduction with Denitrification and Anammox to achieve anaerobic Oxidation of Ammonia to N<sub>2</sub>



#### Bio-energetic Question: How Dinitrogen Oxide (N<sub>2</sub>O) can be cycled

Under which conditions can N<sub>2</sub>O be an oxidant, how can it be a reductant?

- Define stoichiometric reactions for N<sub>2</sub>O as reductant as well as oxidant.
- Choose conditions, which are representative for a natural habitat.
- Check energetic yields and define thermodynamically exergonic and endergonic reactions.
- Can N<sub>2</sub>O be disproportionated in an energetically favorable way for chemolithotrophic microbes?



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# Bio-energetic Question: Can Dinitrogen Oxide (N<sub>2</sub>O) be an Electron Donor for anoxigenic Photosynthesis

What is the redox potential of  $N_2O$  electrons? Can  $N_2O$  electrons be transferred into a photosystem of phototrophic bacteria?

- Define stoichiometric half reactions for N<sub>2</sub>O oxidation.
- Calculate the potential in mV for electrons released from  $N_2O$ .
- Find components of photosystems that could accept N<sub>2</sub>O electrons i.e. that could oxidize N<sub>2</sub>O.
- Compare N<sub>2</sub>O as electron donor for photosynthesis with nitrite, ammonia and nitrous oxide.
- Compare N<sub>2</sub>O as electron donor for photosynthesis with non-nitrogen electron donors like hydrogensulfide, ferrous iron, di- and trihydrogenarsenite.
- Compare  $N_2O$  as electron donor for photosynthesis with  $H_2O$  as electron donor.



Thermodynamics for the synthesis of biomass





Microbial Ec

# Synthesis of "new" cells requires mass, energy and a "template"



### Thermochemical values for biomass monomers

How much energy is needed to "synthesize" a cell?

- Can we calculate energy needs for the synthesis of a cell?
- We need to know the composition of a cell.
- Gf° values allow one to calculate △Gr° of cellular monomers.
- · Is there a minimum energy need for the replication of a cell?

Synthesis model	Model	Hf <sup>° (1)</sup>	Gf <sup>° (1)</sup>					
monomers for	structure	[kJ/mol]	[kJ/mol]					
carbohydrates	$C_4H_8O_4$	-790.51	-611.9					
proteins	$C_6H_8O_3N_2$	-1147.8	-989.39					
lipids	$C_8H_{16}O$	-316.84	-76.45					
P-compounds	CH <sub>5</sub> O <sub>5</sub> P	-1401.25	-1318.58					
S-compounds	$C_2H_4O_2S$	-158.7	-91.81					
<sup>(1)</sup> values for STP, solvatation correction included								





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# What cells are made off (building cells): the chemical elemental composition of living cells





# Thermodynamics of biomass production

Assimilation of C, H, O into cell mass  $<C_4H_7O_3>$  (= approximate composition) $4 \ HCO_3^- + 21 \ H^+ + 17 \ e^ <C_4H_7O_3> + 9 \ H_2O$  $\Delta Gr_1$  $17 \ Fe(II+)$  $\rightarrow$  $17 \ Fe(III+) + 17 \ e^ \Delta Gr_2$  $4 \ HCO_3^- + 21 \ H^+ + 17 \ Fe(II+)$  $\rightarrow$  $<C_4H_7O_3> + 9 \ H_2O + 17 \ Fe(III+)$  $\Delta Gr_1 - \Delta Gr_2$  $4 \ HCO_3^- + 21 \ H^+ + 17 \ Fe(II+)$  $\rightarrow$  $<C_4H_7O_3> + 9 \ H_2O + 17 \ Fe(III+)$  $\Delta Gr_1 - \Delta Gr_2$  $d \ Gr_1 - \Delta Gr_2$  $n \ [Fe(II+) \ m \ fe(II+) \ for \ fe(II+) \ fe(II+) \ for \ fe(II+) \$ 

 $[Fe(II+) + {}^{1}/_{4}O_{2} + H^{+} \longrightarrow Fe(III+) + {}^{1}/_{2}H_{2}O] \qquad n[\Delta Gr_{3} - \Delta Gr_{2}]$ 

How would you calculate the  ${\scriptstyle \Delta Gr}$  values for assimilation and dissimilation reactions with Thermodyn ?



Microbial F

#### Calculation method illustrated for $\alpha$ –D–Glucose

Assumption: Molecules are synthesized from the elements:

 $6C + 6H_2 + 3O_2 \rightarrow C_6H_{12}O_6$ Enthalpy, entropy, free energy (Gibbs energy) of formation are defined as:

 $Hf^{\circ} = E_{el} + E_{vib}(T) + E_{trans}(T) + E_{rot}(T) + E_{sol}(T) + RT$   $Sf^{\circ} = S_{vib}(T) + S_{trans}(T) + S_{rot}(T)$  $Gf^{\circ} = Hf^{\circ} - TSf^{\circ}$ 



Calculations with the G3MP2 method provide high quality precision (error of about 6 kJ/mol) within an optimal calculation time for moderate size molecules.



Molecule in H <sub>2</sub> O	Hf°	Gf°	Method of
	[kJ/mol]	[kJ/mol]	calculation
glc-D	-1215	-907	g3mp2
g6p2-	-2816	-2504	b3lyp
f6p2-	-2761	-2312	b3lyp
fdp4-	-4434	-4699	b3lyp
dhap2-	-2194	-2088	g3mp2
ga3p2-	-2210	-2101	g3mp2
bpg4-	-4598	-4485	g3mp2
3pg3-	-2886	-2775	g3mp2
2pg3-	-2526	-2395	g3mp2
рер3-	-1679	-1604	g3mp2
pyr1-	-584	-501	g3mp2
ADP3-	-5545	-5154	b3lyp
ATP4-	-7594	-7223	b3lyp
mNAD1+	-812	-953	g3mp2
mNADH	-835	-674	g3mp2

#### Standard Hfº and Gfº for glycolysis intermediates





Microbial F

# Calculate the stoichiometric composition and the oxidation state of carbon in biomass that is derived from the oxidation states of the atoms in biomolecules

According to calculations by Verde et al. (2004) the dry organic biomass of autotrophs has a C:N:P ratio of 113:14:1.

#### Calculate the average oxidation state of the C atoms in biomass, which consists of

30% (w/w)	Carbohydrates: polysaccharides, hexoses (HCHO) <sub>m</sub> , nucleotide pentoses	0
35% (w/w)	Proteins: amino acids $(C_{16}H_{24}O_5N_4)_n$	- 0.125
6% (w/w)	Nucleic acid: RNA, DNA, purines, pyrimidines,	
	pentoses, phosphate (CHONP)	
12% (w/w)	Lipids: glycerol, fatty acids, phosphate (CHOPS)	
16% (w/w)	Neutral lipids (C <sub>8</sub> H <sub>16</sub> O) <sub>p</sub>	- 1.750
0.75% (w/w)	Small molecules: nucleotides, coenzymes (CHONPS)	
Average		- 0.2375

Verde T. et al. (2004) FUNDAMENTAL CONNECTIONS AMONG ORGANISM C:N:P STOICHIOMETRY, MACROMOLECULAR COMPOSITION, AND GROWTH. Ecology, 85, 1217–1229

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Microbial Eco

#### Why thermodynamics allows microorganisms to make a living

#### Gibbs free Energy ( $\Delta G$ ):

is a function of

- Temperature
- Pressure
- Chemical Composition

#### $\Delta G_r = \Delta G_r^{o} + RT \ln Q$

ΔG<sup>°</sup><sub>r</sub>: standard state (reference frame)

Q : chemical composition which defines deviation from equilibrium



#### How to present thermochemical calculation results - Terms to remember

аA	+	b B	сC	+	d D
Su	bst	rates	Products	s	

Re call :

 $\Delta Gr = \Delta Gr^{0} + R \cdot T \cdot \ln Q = -R \cdot T \cdot \ln K_{eq} + R \cdot T \cdot \ln Q = R \cdot T \cdot \ln \frac{Q}{K_{eq}}$ 

$$Q = \frac{\left[C\right]^{c} \cdot \left[D\right]^{d}}{\left[A\right]^{a} \cdot \left[B\right]^{b}}$$

#### We woud like to present $\Delta Gr$ as a function of one of the reactants



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#### How to present thermochemical calculation results (activity is always $\leq$ 1)



How to present thermochemical calculation results (product-dependence)

аA	+	b B	$\longleftrightarrow$	с С	+	d D
Su	bst	rates		Pro	odu	cts

Verify the graphical presentation of  $\Delta Gr$  for a product as variable:  $\Delta Gr = u_P + m_P \cdot \log[P]$  for  $[P] > 0, [P] \neq 0$  and P = products C or D



 $u_P$  for product C as variable = ?  $m_P$  for product C as variable = ?



#### Semilogarithmic presentation of $\Delta$ Gr for product formation



Thermodyn<sup>©™</sup>



# With Thermodyn<sup>©</sup> we can compare up to 5 consumption and production reactions as long as we choose the same variable for each of them



### Comparing reactions with large and small free energies



Aerobic oxidation of glucose yields high free energies

#### Comparing reactions with large and small free energies



4

### When do we use the logarithmic presentation mode

Reactior No.	(S,P)	Stoich. coeff.	Enter formula	State	Special remarks Activity	Variable				
1	s	4	H2	aq		v				
1	s	1	CO2	aq	0.001					
1	р	1	CH4	aq	0.001					
1	р	2	H2O	I	1					
2	s	4	H2	aq		v				
2	s	1	SO42-	aq	0.001		Plot top		Plot bottom	
2	s	1	H+	aq	0.000001		Tomp (K)	208 15	Tomp (K)	208 15
2	n	1	HS-	20	0.001		Min variable	1 00E-14	Min variable	1 00F-1
2	p n	4	H2O	1	1 00F+00		Max variable	1.00E-14	Max variable	1.00E-1
_	٣	-								
							Log plot?:	x	Log plot?:	
3	s	1	Ethanol	aq	1.00E-03					
3	s	1	H2O	1	1.00E+00		Show react.1	x	Show react.1	х
3	р	1	acetate	aq	1.00E-03		Show react.2	х	Show react.2	х
3	р	1	H+	aq	1.00E-07		Show react.3	х	Show react.3	x
3	р	2	H2	aq		v	Show react.4	x	Show react.4	х
							Show react.5	x	Show react.5	x
4	s	1	n-butyrate	aq	1.00E-03					
4	s	2	H2O	1	1					
4	р	2	acetate	aq	1.00E-03					
4	р	1	H+	aq	1.00E-07					
4	р	2	H2	aq		v				
5	s	1	propionate	aq	1.00E-03					
5	s	2	H2O	, i	1.00E+00					
5	р	1	acetate	aq	1.00E-03					
5	р	1	CO2	aq	1.00E-03					
5	p	3	H2	aq		v				

Logarithmic vs. linear mode of presentation 150 100 л 50 kJ/mol reaction -12 -14 -10 -2 -50 -100 Plot bottom Temp. (K) Min. variable Max. variable 298.15 298.15 1.00E-14 1.00E-02 1.00E-11 1.00E-09 -150 80 Log plot?: -200 60 Show react.1 Show react.2 Show react.3 X X X X X 40 Show react.4 Show react.5 20 kJ/mol reaction 0 2.1E-10 1E-11 1E-10 -20 -40 -60 -80

Plot top Temp. (K) Min. variable Max. variable

Log plot?:

Show react.1 Show react.2

Show react.3 Show react.4 Show react.5 x

X X X X X

-100 MBL Woods Hole / Microbial Diversity Summer Course 2016 / Kurt Hanselmann / 134 / Bio-Thermodynamics 1

Microbial E

#### Summary: Present thermochemical calculation results



### Check the following statements ! Are they correct?

A chemical reaction will always proceed in the direction towards which its reactants can attain lower Gibbs free energies of formation, i.e.  $\sum Gf_{products}^{o} < \sum Gf_{substrates}^{o}$ 

A chemical reaction will always proceed in the direction towards which its Gibbs free energy is negative, i.e.  $\Delta Gr < 0$ 

Products and educts are in equilibrium when the Gibbs free energies of formation are equal, i.e.  $\sum Gf_{\text{products}} = \sum Gf_{\text{substrates}}$ 

Products and educts are in equilibrium when the Gibbs free energy of a reaction is zero, i.e.  $\Delta Gr = 0$ 





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1.115



### **Electromicrobiology Live Wires**

Discoveries of microbial communities that transfer electrons between cells and across relatively long distances are launching a new field of microbiology.



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### **Electromicrobiology Live Wires**



El-Naggar, Mohamed Y. and Steven E. Finkel. 2013. Live Wires. The Scientist Magazine, May 1. Image by Thom Graves

### Contents

- Introduction to Bio-Thermodynamics
- The concept of "free energy"
- "Standard free energy" vs. "actual free energy"
- Calculating "free energy" from electrochemical potential energy
- Summary of terms, relations and abbreviations
- Examples using Thermodyn



Microbial E

#### Remember: Redox Reactions

**Oxidation:** loss of electrons half-reaction with e- on the right of the reaction equation Needs to be linked to an oxidant = electron acceptor

 $As(III+) \rightarrow As(V+) + 2 e^{-1}$ Arsenic-oxidation:

Generalized equilibrium oxidation half-reaction

$$A_{red}$$
  $A_{ox} + n e^- + q_{ox} H^+ q = n$ 

Reduction: gain of electrons.

half-reaction with e- on the left of the reaction equation Needs to be linked to an reductant = electron donor

 $NO_3^- + 5 e^- + 6 H^+ \rightarrow \frac{1}{2}N_2 + 3 H_2O$ Denitrification:

Generalized equilibrium oxidation half-reaction

B <sub>ox</sub> +	n e <sup>-</sup> + q <sub>red</sub> H <sup>+</sup>	<b>B</b> <sub>red</sub>	q = n+1	
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#### **Remember: Redox Reactions**





Microbial Ecol
#### **Remember: Redox Reactions**

Oxidation: loss of electrons half-reaction with e<sup>-</sup> on the right of the reaction equation Needs to be linked to an oxidant = electron acceptor

As(III+)  $\leftarrow \rightarrow$  As(V+) + 2 e<sup>-</sup> Arsenic-oxidation:

Generalized equilibrium halfreaction

 $A_{ox} + n e^{-} + q_{ox} H^{+}$ A<sub>red</sub>



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#### **Remember: Redox Reactions**

Oxidation: loss of electrons half-reaction with e- on the right of the reaction equation Needs to be linked to an oxidant = electron acceptor

Arsenic-oxidation:  $As(III+) \rightarrow As(V+) + 2 e^{-}$  $\frac{1}{2}$  As(III+)  $\rightarrow \frac{1}{2}$  As(V+) + 1 e<sup>-1</sup>

Standard Redox Potential  $(E_h^0) \rightarrow$  per electron under STP





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#### **Remember: Redox Reactions**







# Midpoint Potential

Redox reactions consist of two half reactions, an oxidizing and a reducing one.

Oxidation is the loss of electrons from orbitals of an atom, reduction is the gain of electrons.

Together, oxidation and reduction reaction form the combined redox reaction in which electrons are balanced.

Oxidation and reduction potentials ( $\Delta Eh$ ) express the tendency for a chemical species to be oxidized or reduced.

The potential (energy) of the electron that is released or taken up by a half reaction is measured in volts.

The standard reduction potential ( $\Delta Eh^{\circ}red$ ) is the likelihood (tendency) for a chemical species that it will be reduced under standard state conditions (298 K, 1 atm, and with 1 M solutions). It is written in the form of a reduction half reaction. The more positive the potential is the more likely it will be reduced under standard state conditions.

The standard oxidation potential ( $\Delta Eh^{\circ}ox$ ) is the likelihood (tendency) for a chemical species that it will be oxidized under standard state conditions (298 K, 1 atm, and with 1 M solutions). It is written in the form of an oxidation half reaction.



# The concept of metabolic redox half-reactions e.g. oxidation of ferric iron by *Acidothiobacillus ferrooxidans*

4 Fe <sup>2+</sup> + O <sub>2</sub> + 10 H <sub>2</sub> O	 4 Fe(OH) <sub>3</sub> + 8 H <sup>+</sup>
4 Fe <sup>2+</sup> + 12 H <sub>2</sub> O	 4 Fe(OH) <sub>3</sub> + 4 e <sup>-</sup> + 12 H <sup>+</sup>
O <sub>2</sub> + 4 e <sup>-</sup> + 4 H <sup>+</sup>	 2 H <sub>2</sub> O

Generalized equilibrium halfreactions





#### Electron flow in electrochemical cells



Electrons (e<sup>-</sup>) flow from the anode (electron donor) to the cathode (electron acceptor). An electrochemically more negative couple can reduce a less negative couple

adapted from: Anderson, G.M. 1996. Thermodynamics of natural systems, John Wiley, New York

Iron nail in aqueous

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#### **Redox potential**

$$\Delta Er = -\frac{\Delta Gr}{n \cdot F} \text{ and } \Delta Er^{\circ} = -\frac{\Delta Gr^{\circ}}{n \cdot F} = \frac{R \cdot T}{n \cdot F} \cdot \ln K^{\circ}$$
$$\Delta Er = -\frac{1}{n \cdot F} \cdot (\Delta Gr^{\circ} + R \cdot T \cdot \ln Q) = \Delta Er^{\circ} - \frac{R \cdot T}{n \cdot F} \cdot \ln Q$$
$$\Delta Er = -\frac{R \cdot T}{n \cdot F} \cdot \ln \frac{Q}{K^{\circ}}$$

R = 8.31451 •  $10^{-3}$  [kJ • Mol<sup>-1</sup> • K<sup>-1</sup>], F= 96.485309 [kJ • mol<sup>-1</sup> • V<sup>-1</sup>] T = 273.15 [K] in Kelvin degrees, K<sup>o</sup> = K<sub>eq</sub> = equilibrium coefficient



#### Redox potential definition by IUPAC

- Any oxidation-reduction (redox) reaction can be divided into two half reactions: one in which a chemical species undergoes oxidation and one in which another chemical species undergoes reduction.
- If a halfreaction is written as a reduction, the driving force is the reduction potential.
- If the half-reaction is written as oxidation, the driving force is the oxidation potential related to the reduction potential by a sign change.
- The redox potential is the reduction/oxidation potential of a compound measured under standards conditions against a standard reference half-cell (E<sup>o</sup>), normally H<sup>+</sup>/H<sub>2</sub>.
- Today: Actual redox potentials for processes occurring in a microbial habitat (Er in [volt]) are calculated for the prevailing conditions and the activities of the chemical species involved at a particular time and referred to the conditions against the standard reference half-cell H<sup>+</sup>/H<sub>2</sub> (E°).
- Earlier: In biochemical systems the standard redox potential was defined at pH 7.0 versus the hydrogen electrode and
  partial pressure of hydrogen = 1 bar (E°). This is no longer needed, since it has become easy to calculate redox potentials
  for any set of conditions.

IUPAC Compendium of Chemical Terminology 2003

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#### Thermodynamics of electrochemical half reactions: Redox potentials

Substrate<sub>reduced</sub> 
$$\longrightarrow$$
 Product<sub>oxidized</sub> + n electrons  
 $S_{red}$   $\longrightarrow$   $P_{ox}$  + n electrons  
 $E = \frac{-\Delta Gr}{n \cdot F}$  or  $E = \frac{-\Delta Gr^{\circ}}{n \cdot F} - \frac{R \cdot T}{n \cdot F} \cdot \ln Q$   
and since  $\frac{-\Delta Gr^{\circ}}{n \cdot F} = E^{\circ}$ , and  $\Delta Gr^{\circ} = -R \cdot T \cdot \ln K_{eq}$   
 $E = E^{\circ} - \frac{R \cdot T}{n \cdot F} \cdot \ln Q$  or  $E = \frac{R \cdot T}{n \cdot F} \cdot \ln \frac{K_{eq}}{Q}$   
E redox potential, E° redox potential under standard conditions [V]  
n number of electrons transferred  
R (gas constant) = 8.31451 10<sup>-3</sup> [kJ mol<sup>-1</sup> °K<sup>-1</sup>]  
F (Farady constant) = 96.485309 [kJ mol<sup>-1</sup> V<sup>-1</sup>]



# **Definition of Terms**

Actual oxidation and reduction potentials ( $\Delta$ Eh) express the potential difference to the standard reference potentials ( $\Delta$ Eh°). Standard potentials are measured in volts at standard conditions. Gaseous hydrogen has universally been recognized as having standard reduction and oxidation potentials of zero.

Actual reduction and oxidation potentials are expressed as the difference in the potential from the standard potential of the corresponding half reaction.

????? The less positive the potential of a reaction is, the more likely it will be oxidized.

#### What is a "redox tower"?

In common presentations of a "redox tower" the substances at the top have a greater likelihood of being oxidized while the ones at the bottom have a greater likelihood of being reduced. When an electron donating substance that is positioned at the top is coupled with an electron acceptor substance positioned below, the one at the top will become oxidized while the one at the bottom will become reduced. The combination of the oxidation reaction with the reduction reaction yields the redox reaction.

The redox tower is a list of standard reduction potentials in descending order of the tendency for chemical species to be reduced.

Species at the top are more likely to be oxidized while species at the bottom are more likely to be reduced.

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# Definition of Terms

Evaluate the statement:

"A standard reduction potential expresses the tendency for a given chemical species to be reduced / oxidized."

How are standard reduction potential and standard oxidation potential related to each other?

The standard oxidation potential expreses the tendency for a given chemical species to be oxidized as opposed to be reduced. For the same chemical species the standard reduction potential and standard oxidation potential are opposite in sign.

How are standard electron potential and actual electron potential related to each other?

Standard electron potentials are defined for conditions of 298K (25°C), 1atm, and solute activities of 1M (standard conditions). Actual electron potentials include the deviation from the standard potential with regard to temperature, pressure and activity.

#### How are electron reduction potentials measured?

Standard reduction potentials are measured relative to the potential of hydrogen which has been universally set to zero (= 0 Volts)

How can you calculate the standard reduction potential of H2O, if you know the oxidation potential of O2?

The standard oxidation potential and standard reduction potential are always opposite in sign for the same species. The oxidation potential of O2 is +0.77V.



# Check



Determine the values in general terms for the reaction

# Summary: Non-equilibrium thermodynamics applied to microbial processes

$$a A + b B \Leftrightarrow c C + d D$$

$$Q = \frac{\left[C\right]^{c} \left[D\right]^{d}}{\left[A\right]^{a} \left[B\right]^{b}}$$

$$\Delta Gr = \Delta Gr^{o} + RT \ln Q$$

$$\Delta Gr^{o} = -RT \ln K_{eq} = \sum Gf^{o}(P) - \sum Gf^{o}(S)$$

$$\Delta Gr = RT \ln \frac{Q}{K_{eq}}$$

$$E_{1/2} = -\frac{\Delta Gr}{n F} = -\frac{1}{n F} \left(\Delta Gr^{o} + RT \ln Q\right) = -\frac{R T}{n F} \ln \frac{Q}{K_{eq}}$$

$$R = 8.31451 \ 10^{-3} \ \left[kJ \ mol^{-1} \ ^{o}K^{-1}\right], F = 96.485309 \ [kJ \ mol^{-1} \ V^{-1}]$$





# Electron transfer in Chemolithotrophy



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# Text book "electron towers" are often based on E°

#### Energetic calculations with actual redox potentials: aerobic oxidation of ferrous iron

$Fe^{2+} + \frac{1}{4}$	O <sub>2</sub> + 1	$1/_{2} H_{2}O$	 Fe(OH)	2 <sup>+</sup> + H <sup>+</sup>	∆Er = ? [Volt]
Habitat co 10 <sup>-6</sup> 0.2	nditions *10 <sup>-3</sup>	; 1	10 <sup>-6</sup>	pH 8	
Fe(OH) <sub>2</sub> <sup>+</sup> 10 <sup>-6</sup>	+ 1 e <sup>-</sup> 10 <sup>-3</sup>	+ 2H+ pH 8	 Fe <sup>2+</sup> + 10 <sup>- 6</sup>	2 H <sub>2</sub> O 1	Er = -0.127 [Volt]
<sup>1</sup> / <sub>4</sub> O <sub>2</sub> 0.2*10 <sup>-3</sup>	+ 1 e⁻ 10 <sup>-3</sup>	+ 1H <sup>+</sup> pH 8	 <sup>1</sup> / <sub>2</sub> H <sub>2</sub> O 1		Er = +0.567 [Volt]

#### Anything more positive can oxidize anything less positive

Fe(III)/Fe(II) O <sub>2</sub> /H <sub>2</sub> O	Er = -0.127 [V] Er = +0.567 [V]
∆Er	= $Er_{accepting couple}$ - $Er_{donating couple}$ = 0.567 - (-0.127) = 0.694 [V]
∆Gr	= - n F ∆Er = - (1) ( 96485 Joule mol <sup>-1</sup> V <sup>-1</sup> ) (+0.694V) = - 66.96 kJoule mol <sup>-1</sup>
•	



Microbial

# Standard electrode potentials of metabolic reactions (1 Mol/I, 1 atm, pH 7)

Couple	E°´[mV]	e <sup>-</sup> transferred
CO <sub>2</sub> /CO	- 540	2
SO <sub>4</sub> <sup>2-</sup> / HSO 3 <sup>-</sup>	- 516	2
CO <sub>2</sub> / Formate	- 430	2
6 CO <sub>2</sub> / Glucose	- 430	24
2H <sup>+</sup> / H <sub>2</sub>	- 418	2
S <sub>2</sub> O <sub>3</sub> <sup>2-</sup> /HS <sup>-</sup> + HSO <sub>3</sub> <sup>-</sup>	- 400	2
CO <sub>2</sub> / Methanol	- 380	6
2 CO <sub>2</sub> / Acetate	- 280	8
S°/H <sub>2</sub> S	- 280	2
CO <sub>2</sub> /CH <sub>4</sub>	- 240	8
SO <sub>4</sub> <sup>2-</sup> /H <sub>2</sub> S	- 220	8
Acetaldehyde / Ethanol	- 197	2
Dihydroxyaceton -P/Glycerin -P	- 190	
Pyruvate / Lactate	- 186	2
Oxaloacetate / Malate	- 170	2
HSO <sub>3</sub> <sup>-</sup> / S <sub>3</sub> O <sub>6</sub> <sup>2-</sup>	- 170	???????
HSO3 / HS	- 116	6
Cyt b 5580x / Cyt b 558red	- 75 to - 45	1
APS/AMP + HSO3	- 60	2
Acrylyl -CoA / Propionyl -CoA	- 15	2 ???
Glycin / Acetat + NH 4+	- 10	2 ???
$S_4O_6^{2-}/2S_2O_3^{2-}$	+ 24	2
Fumarate / Succinate	+ 33	2
AsO <sub>4</sub> <sup>3-</sup> / AsO <sub>3</sub> <sup>3-</sup>	+ 139	2
Dimethylsulfoxid / Dimethylsulfid	+ 160	????
Fe(OH) <sub>3</sub> + HCO <sub>3</sub> / FeCO <sub>3</sub>	+ 200	2
Fe <sup>3+</sup> / Fe <sup>2+</sup>	+ 200	1
S <sub>3</sub> O <sub>6</sub> <sup>2-</sup> / S <sub>2</sub> O <sub>3</sub> <sup>2-+</sup> + HSO <sub>3</sub> <sup>-</sup>	+ 225	1
NO2 <sup>-</sup> / NO	+ 360	1
NO <sub>3</sub> <sup>-</sup> / NO <sub>2</sub> <sup>-</sup>	+ 421	2
NO <sub>2</sub> -/NH <sub>3</sub>	+ 440	6
SeO <sub>4</sub> <sup>2-</sup> / SeO 3 <sup>2-</sup>	+ 475	2
$NO_{3}^{-} / \frac{1}{2}N_{2}$	+ 740	5
Fe <sup>3+</sup> / Fe <sup>2+</sup>	+ 763	1 pH 2
Mn <sup>4+</sup> / Mn <sup>2+</sup>	+ 798	2
<sup>1</sup> / <sub>2</sub> O <sub>2</sub> / H <sub>2</sub> O	+ 818	2
NO / <sup>1</sup> / <sub>2</sub> N <sub>2</sub> O	+ 1180	1
N <sub>2</sub> O / N <sub>2</sub>	+ 1360	2

With the Thermodyn you can calculate electrochemical potentials for any actual condition of redox pairs whose thermochemical values are available in the data base



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Microbial Ecol

# Standard electrode potentials of coenzymes (1 Mol/I, 1 atm, pH 7)

Couple	E°´[mV]	e <sup>-</sup> transferred
Ferredoxin <sub>ox</sub> / Ferredoxin <sub>red</sub>	- 432	1
2H <sup>+</sup> / H <sub>2</sub>	- 418	2
Ferredoxin <sub>ox</sub> / Ferredoxin <sub>red</sub>	- 410 to - 390	1 Clostridium
Flavodoxin <sub>ox</sub> / Flavodoxin <sub>red</sub>	- 370	2 ???
NAD <sup>+</sup> / NADH	- 320	2
FeS-Protein <sub>ox</sub> / FeS -Protein <sub>red</sub>	- 305	1 high potential
Cyt c <sub>3ox</sub> / Cyt c <sub>3red</sub>	- 290	1
Lipoic / Dihydrolipoic	- 290	2
FAD / FADH 2	- 220	2
FMN / FMNH 2	- 190	2
Flavodoxin <sub>ox</sub> / Flavodoxin <sub>red</sub>	- 120	2 ???
Menaquinone <sub>ox</sub> / Menaquinone <sub>red</sub>	- 74	2
Cyt b <sub>558ox</sub> / Cyt b <sub>558red</sub>	- 75 to - 45	1
Rubredoxin ox / Rub redoxin red	- 57	2 ????
Cyt b <sub>556ox</sub> / Cyt b <sub>556red</sub>	+ 35 to + 120	1
Ubiquinone <sub>ox</sub> / Ubiquinone <sub>red</sub>	+ 110	2
Cyt b <sub>562ox</sub> / Cyt b <sub>562red</sub>	+ 125 to + 260	1
Cyt c <sub>ox</sub> / Cyt c <sub>red</sub>	+ 230 to + 250	1
Cyt d <sub>ox</sub> / Cyt d <sub>red</sub>	+ 260 to + 280	1
FeS-Protein <sub>ox</sub> / FeS - Protein red	+ 280	1 low potential
Cyt a <sub>ox</sub> / Cyt a <sub>red</sub>	+ 290	1
Cyt c <sub>555ox</sub> / Cyt c <sub>555red</sub>	+ 355	1
Cyt aa3 <sub>ox</sub> / Cyt aa3 <sub>red</sub>	+ 388	1
<sup>1</sup> / <sub>2</sub> O <sub>2</sub> / H <sub>2</sub> O	+ 818	2









Microbial Ecology

# Calculating Eh with Thermodyn - 2





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#### Electron potentials of half reactions with different states and variable pH









#### Introducing pe, electron activity

Influence of changes in solute concentrations or  $\ /$  and pH on disequilibrium electron activity:

Analogy:  $pH = -\log [H^+]$  pe = -log [e<sup>-</sup>]

$$\begin{array}{rcl} \mathsf{A}_{\mathsf{red}} & \leftrightarrow & \mathsf{A}_{\mathsf{ox}} + \mathsf{ne}^{-} + \mathsf{q}_{\mathsf{ox}} \; \mathsf{H}^{+} \\ \mathsf{B}_{\mathsf{ox}} + \mathsf{ne}^{-} + \mathsf{q}_{\mathsf{red}} \; \mathsf{H}^{+} & \leftrightarrow & \mathsf{B}_{\mathsf{red}} \end{array}$$

Solve for oxidation reaction

$$K^{\circ} = \frac{[A_{ox}] \cdot [e^{-}]^{n} \cdot [H^{+}]^{q_{ox}}}{[A_{red}]}$$

$$[e^{-}]^{n} = \frac{K^{\circ} \cdot [A_{red}]}{[A_{ox}] \cdot [H^{+}]^{q_{ox}}}$$

$$-n \cdot \log [e^{-}] = -\log K^{\circ} - \log [A_{red}] + \log [A_{ox}] + q_{ox} \log [H^{+}]$$

 $pe^{\circ} = \frac{1}{n}pK^{\circ} - \frac{1}{n}\log\frac{[A_{red}]}{[A_{ox}]} - \frac{q_{ox}}{n}pH$ 





#### Interpreting pe



#### peº for reduction half-reaction

$$A_{red} \iff A_{ox} + ne^{-} + q_{ox} H^{+}$$

$$B_{ox} + ne^{-} + q_{red} H^{+} \iff B_{red}$$

$$K^{\circ} = \frac{[B_{red}]}{[B_{ox}] \cdot [e^{-}]^{n} \cdot [H^{+}]^{q_{red}}}$$

$$[e^{-}]^{n} = \frac{[B_{red}]}{[B_{ox}] \cdot K^{\circ} \cdot [H^{+}]^{q_{red}}}$$

$$-n \cdot \log [e^{-}] = \log K^{\circ} - \log [B_{red}] + \log [B_{ox}] + q_{red} \log [H^{+}]$$

$$pe^{\circ} = -\frac{1}{n}pK^{\circ} + \frac{1}{n}\log \frac{[B_{ox}]}{[B_{red}]} - \frac{q_{red}}{n}pH$$

Draw pe-pH stability diagram and interpret changes in he concentrations of reduced and oxidized species

What are pe-pH stability diagrams useful for ?

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# pe for generalized reduction half-reaction

$$A_{ox} + C + ne^- + q_{red} H^+ \longrightarrow A_{red} + B$$

$$\Delta Gr = \Delta Gr^{\circ} + R T \ln Q = \Delta Gr^{\circ} + R T 2.3026 \log Q$$
$$Q = \frac{\left[A_{red}\right] \left[B\right]}{\left[A_{ox}\right] \left[C\right] \left[e^{-}\right]^{n} \left[H^{+}\right]^{q}}$$

try to derive

$$pe = \frac{\Delta Gr^{\circ} - \Delta Gr}{2.3026 n R T} + \frac{1}{n} \log \frac{\left[A_{red}\right] \left[B\right]}{\left[A_{ox}\right] \left[C\right]} - \frac{q}{n} pH$$

$$pe = \frac{\Delta Gr^{\circ} - \Delta Gr}{2.3026 n R T} + \frac{1}{n} \log Q' - \frac{q}{n} pH$$

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# pe for generalized oxidation half-reaction

 $A_{ox}$  + C + ne<sup>-</sup> + q H<sup>+</sup>  $\leftarrow$   $A_{red}$  + B

$$\Delta Gr = \Delta Gr^{\circ} + R T \ln Q = \Delta Gr^{\circ} + R T 2.3026 \log Q$$
$$Q = \frac{\left[A_{ox}\right] \left[C\right] \left[e^{-}\right]^{n} \left[H^{+}\right]^{q}}{\left[A_{red}\right] \left[B\right]}$$

try to derive

$$pe = \frac{\Delta Gr^{\circ} - \Delta Gr}{2.3026 \text{ n R T}} - \frac{1}{n} \log \frac{\left[A_{\text{red}}\right] \left[B\right]}{\left[A_{\text{ox}}\right] \left[C\right]} - \frac{q}{n} \text{ pH}$$

$$pe = \frac{\Delta Gr^{\circ} - \Delta Gr}{2.3026 \text{ n R T}} - \frac{1}{n} \log Q' - \frac{q}{n} \text{ pH}$$





#### Relations between E and pe - 1

$$E = \frac{2.3026 \cdot R \cdot T \cdot p_e}{F}$$

$$p_e = \frac{F}{2.3026 \cdot R \cdot T} \cdot E \qquad \text{replace E with } E = \frac{R \cdot T}{n \cdot F} \cdot 2.3026 \cdot \log \frac{K_{eq}}{Q}$$

$$p_e = \frac{F}{2.3026 \cdot R \cdot T} \cdot \frac{R \cdot T}{n \cdot F} \cdot 2.3026 \cdot \log \frac{K_{eq}}{Q}$$

$$p_e = \frac{1}{n} \cdot \log \frac{K_{eq}}{Q} \qquad p_e = p_e^0 - \frac{1}{n} \cdot \log Q$$

follows from Q = 1 (equilibrium conditions)

 $p_e^0 = \frac{1}{n} \cdot logK_{eq}$ 

- n number of electrons transferred
- Q ratio of actual activity products of reactants
- R gas constant =8.31451 10<sup>-3</sup> [kJ mol<sup>-1</sup> K<sup>-1</sup>] =82.057844 10<sup>-3</sup>[atm Liter mol<sup>-1</sup> K<sup>-1</sup>]
- T thermodynamic temperature in [K]

In Y = 2.3026 <sub>10</sub>logY

F Faraday conversion constant, = 96.485309 [kJ mol<sup>-1</sup> V<sup>-1</sup>]



#### Relations between E and pe - 2



Check Thermochemical energy yield from substrate oxidation with different oxidants

Boundary conditions: (concer	ntrations are given in [mol/l])
Ethanol	10-4
HCO <sub>3</sub> -	5*10 <sup>-3</sup>
oxidant	0.5*10 <sup>-3</sup> in general
reduced oxidant	≤ 0.3*10 <sup>-3</sup>
рН	6.5 ≤ pH ≤ 8.5

Formulate the dissimilation reactions for the fllowing oxidants:  $SO_4^{2^-}$ ,  $SO_3^{2^-}$ ,  $S_2O_3^{2^-}$ ,  $S^o$ ,  $HCO_3^-$ ,  $H^+$ ,  $O_2$ ,  $NO_3^$ and calculate the thermochemical energy yield for the boundary conditions given above.

See also Exercises section





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Microbial Eco

# Why thermodynamics allows microorganisms to make a living

Gibbs free Energy ( $\Delta G$ ): is a function of

- Temperature
- Pressure
- Chemical Composition

# $\Delta G_r = \Delta G_r^{o} + RT \ln Q$

 $\Delta G_r^{o}$  : standard state (reference frame) Q : chemical composition which defines deviation from equilibrium





How to present thermochemical calculation results - Terms to remember

аA	+	b B	сC	+	d D
Su	bst	rates	Products	5	

Re call :

$$\Delta Gr = \Delta Gr^{0} + R \cdot T \cdot \ln Q = -R \cdot T \cdot \ln K_{eq} + R \cdot T \cdot \ln Q = R \cdot T \cdot \ln \frac{Q}{K_{eq}}$$

$$Q = \frac{\left[C\right]^{c} \cdot \left[D\right]^{d}}{\left[A\right]^{a} \cdot \left[B\right]^{b}}$$

#### We woud like to present $\Delta Gr$ as a function of one of the reactants



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#### Electron potentials of half reactions with different states and variable pH







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Terration of the second second

# Apparent Eh anomaly 1: The problem

Thermodyn calculates values for  $E^{\circ}h(H_2)$  under standard conditions (activities = 1, pH = 0, T= 25°C) which are different from 0 Volt (the reference value for the hydrogen electrode) and E °h(O<sub>2</sub>) which differ from the expected value of +1.233 Volt. Why ?

**Explanation:** We use the aqueous states  $H_{2aq}$  and  $O_{2aq}$  for most physiologically relevant applications. The physicochemical reference values, however, are calculated for the gaseous state  $H_{2g}$  and  $O_{2g}$ . The thermochemical values for the two states differ which leads to different E°h values.

Compound name	Formula	aState	Gf(0) (298) [kJ/mol/l]	Hf(0) (298) [kJ/mol/l]	Sf(0) (298) ] [J/K/m	nol]
Element: Hydrogen						
Electron	e-	aq		0	0	65.25
Electron	e-	g		0	0	65.25
Proton	H+	aq		0	0	0
Hydrogen	H2	g		0	0	130.5
Hydrogen	H2	aq	17.5	5 -4	4.16	57.7
Element: Oxygen						
Oxygen	02	g		0	0	205
Oxygen	02	aq	16.	4 -	11.7	110.9
Water	H2O		-237.	2 -2	85.8	69.9
Water	H2O	g	-228.	6 -24	41.8	188.7





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#### Apparent Eh anomaly 2: Calculations with Thermodyn



# Apparent Eh anomaly 3: Comparison of values for Eh(H<sub>2</sub>)

2 H <sup>+</sup> + 2e <sup>-</sup>		$\rightarrow$ H <sub>2</sub>	
Eh(H <sub>2</sub> ) <sub>gas</sub>	<sub>eous</sub> [Volt]	Eh(H <sub>2</sub> ) <sub>age</sub>	eous [Volt]
pH 0	pH 7	pH 0	pH 7
0	- 0.414	- 0.091	- 0.505





# Apparent Eh anomaly 4: Comparison of values for E°h(O<sub>2</sub>)

$4 H^+ + 4e^- + O_2 \longrightarrow H_2O$			
$E^{\circ}h(O_2)_{gas}$	seous [Volt]	$E^{\circ}h(O_2)_{aq}$	eous [Volt]
pH 0	pH 7	pH 0	рН 7
+ 1.229	+ 0.815	+ 1.272	+ 0.858





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#### Phototrophy with Ferro-iron

Define the conditons which allow ferro-iron to act as an electron donor in anoxygenic photosynthesis.

Can you think of other redox pairs which could act as electron donors in anoxygenic photosynthesis ?









# Electron Flow in oxygenic Phototrophs





# Contents

- Introduction to Bio-Thermodynamics
- The concept of "free energy"
- "Standard free energy" vs. "actual free energy"
- Calculating "free energy" from electrochemical potential energy
- Summary of terms, relations and abbreviations
- Examples using Thermodyn



# Summary of thermodynamic relationships

$$a A + b B \Leftrightarrow c C + d D$$

$$Q = \frac{\left[C\right]^{c} \cdot \left[D\right]^{d}}{\left[A\right]^{a} \cdot \left[B\right]^{b}}$$

$$\Delta Gr = \Delta Gr^{o} + R \cdot T \cdot \ln Q$$

$$\Delta Gr^{o} = -R \cdot T \cdot \ln K^{o} = \sum Gf^{o}(P) - \sum Gf^{o}(S)$$

$$\Delta Gr = R \cdot T \cdot \ln \frac{Q}{K^{o}}$$

$$\Delta Er = -\frac{\Delta Gr}{n \cdot F} = -\frac{1}{n \cdot F} \cdot \left(\Delta Gr^{o} + R \cdot T \cdot \ln Q\right) = -\frac{R \cdot T}{n \cdot F} \cdot \ln \frac{Q}{K^{o}}$$

$$R = 8.31451 \cdot 10^{-3} \left[kJ \cdot Mol^{-1} \cdot {}^{o} K^{-1}\right], F = 96.485309 \left[kJ \cdot mol^{-1} \cdot V^{-1}\right]$$



#### Hierarchy of thermodynamic terms: Define the terms



# Hierarchy of thermodynamic terms: Define the terms (solution)



Basic thermochemical values

Thermochemical values of compounds calculated from basic thermochemical values

Gibbs free energies and electron potentials of reactions at standard state equilibrium calculated from thermochemical values of reactants

Gibbs free energies and electron potentials of reactions at nonequilibrium conditions in relation to standard state equilibrium conditions calculated from activities of reactants

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# Example Calculations with Thermodyn©

#### Kurt Hanselmann

Swiss Federal Institute of Technology, ETH Zürich Department of Earth Sciences – Geology Institute

#### Contents

- Introduction to Bio-Thermodynamics
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5 cm

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#### Summary: Calculating Gibbs free energies

Write a balanced chemical reaction

Consider the phases of each species

Choose a temperature and pressure

Obtain Gf<sup>o</sup> at that temperature and pressure for each species (Standard free energy of formation from the elements (Gf<sup>o</sup>).

Calculate ∆Grº

Calculate K<sub>eq</sub> (equilibrium distribution)

Determine actual conditions under which the reaction takes place

Calculate actual  $\triangle$ Gr as  $\triangle$ Gr<sup>o</sup> + RT InQ



# The following slides summarize thermodynamic presentations of chemolithotrophic life styles

Define the life style by extracting the dissimilation reactions from the Thermodyn Excel sheet and define the conditions under which the life style can exist.



# Chemolithotrophic microorganisms

Formulate chemotrophic dissimilation reactions for the following organisms, write the proper stoichiometries and evaluate the thermodynamic feasibility of the proposed reactions using Thermodyn®

Aerobically	<ul> <li>Hydrogen oxidizing Alcaligenes eutrophus</li> <li>CO-oxidizing Pseudomonas carboxydovorans</li> <li>Ammonium oxidizing Nitrosomonas europaea</li> <li>Nitrite oxidizing Nitrobacter winogradskyi</li> <li>Sulfur oxidizing Sulfolobus acidocaldarius</li> <li>Hydogensulfide oxidizing Beggiatoa mirabilis</li> <li>Thiosulfate oxidizing Thiobacillus novellus</li> <li>Manganese oxidizing Leptothrix sp.</li> <li>Ferrous iron oxidizing Thiobacillus ferrooxidans</li> <li>Methane oxidizing Methylococcus capsulatus</li> </ul>
Anaerobically	<ul> <li>H<sub>2</sub>S oxidizing <i>Thioploca sp.</i></li> <li>S<sup>o</sup> oxidizing <i>Thiomargaritha sp.</i></li> <li>NH<sub>4</sub><sup>+</sup> oxidizing <i>Brocadia anammoxidans</i></li> <li>S<sub>2</sub>O<sub>3</sub><sup>2-</sup> oxidizing <i>Thiobacillus denitrificans</i></li> <li>S<sup>o</sup> disproportionating <i>Desulfocapsa sulfoexigens</i></li> <li>S<sub>2</sub>O<sub>3</sub><sup>2-</sup> dismutating <i>Desulfovibrio sulfodismutans</i></li> </ul>
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# Free energies of aerobic chemolithotrophic dissimilation reactions 1

$NH_4^+ + 1^{1/2}CO_2 + 1^{1/2}H_2O$ e.g. <i>Nitrosomonas sp.</i>	$\longrightarrow$ 1 <sup>1</sup> / <sub>2</sub> <hcho> + NO<sub>2</sub><sup>-</sup> + 2H<sup>+</sup></hcho>
$NO_2^- + \frac{1}{2}CO_2 + \frac{1}{2}H_2O$ e.g. <i>Nitrobacter sp.</i>	$\longrightarrow$ <sup>1</sup> / <sub>2</sub> <hcho> + NO<sub>3</sub><sup>-</sup></hcho>
H <sub>2</sub> S + 2 CO <sub>2</sub> + 2 H <sub>2</sub> O e.g. <i>Thiobacillus sp.</i>	$\longrightarrow$ 2 <hcho> + SO<sub>4</sub><sup>2-</sup> + 2H<sup>+</sup></hcho>
Fe <sup>2+</sup> + <sup>1</sup> / <sub>4</sub> CO <sub>2</sub> + H <sup>+</sup> e.g. <i>Thiobacillus ferrooxidans</i>	$\rightarrow$ 1/ <sub>4</sub> <hcho> + Fe<sup>3+</sup> + 1/<sub>4</sub> H<sub>2</sub>O , Leptothrix sp.</hcho>
$Mn^{2+} + \frac{1}{2}CO_2 + 2 H^+$ e.g. Bacillus sp., Aeromonas	$\longrightarrow$ 1/ <sub>2</sub> <hcho> + Mn<sup>4+</sup> + 1/<sub>2</sub> H<sub>2</sub>O sp., Hyphomicrobium sp.</hcho>
$H_2 + \frac{1}{2} CO_2$ e.g. <i>Paracoccus sp., Hydroge</i>	$\xrightarrow{1}_{2} <\text{HCHO}> + \frac{1}_{2} H_{2}O$



#### How Thermodyn works (chemolithotrophic dissimilation reactions)

- 1:  $CH_4 + 2O_2 \implies CO_2 + 2H_2O$  2:  $H_2 + 0.5O_2 \implies H_2O$
- 4: H<sub>2</sub>S + 2 O<sub>2</sub>  $\implies$  SO<sub>4</sub><sup>2-</sup> + 2 H <sup>+</sup>

Reaction	No.	(S,P)	Stoich. Coeff.	Enter formula	State	Special remarks	Activity	Variable
	1	s	1	CH4	aq		1,00E-06	
	1	s	2	02	aq			v
	1	р	1	CO2	aq		1,00E-06	
	1	р	2	H2O	I		1,00E+00	
	2	s	1	H2	aq		1,00E-06	
	2	s	0,5	02	aq			۷
	2	р	1	H2O	I		1,00E+00	
	3	s	1	со	aq		1,00E-06	
	3	s	0,5	O2	aq			v
	3	р	1	CO2	aq		1,00E-06	
	4	s	1	H2S	aq		1,00E-06	
	4	s	2	O2	aq			v
	4	р	1	SO42-	aq		1,00E-06	
	4	p	2	H+	aq		1,00E-08	
	5	s	1	SO	s	rhombic	1,00E+00	
	5	s	1,5	02	aq			v
	5	s	1	H2O	Ľ		1,00E+00	
	5	р	1	SO42-	aq		1,00E-06	
	5	p	2	H+	aq		1,00E-08	

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# Free energies of aerobic chemolithotrophic dissimilation reactions 1

Reaction No.	(S,P)	Stoich. Coeff.	Enter formula	State	Special remarks	Activity Activity	Plot 1: delta-G as a function of log of         a	it 100°C
1	s	1	CH4	aq		1.00E-06	- <u>A</u> -3: Oxygen	200
1	s	2	02	aq		V	$ 4$ : Oxygen $\Delta - \Delta $	.200 T
1	р	1	CO2	aq		1,00E-06		-300 +
1	р	2	H2O	I		1,00E+00	E .	-400 -
2	s	1	H2	aq		1,00E-06	ic	-500 -
2 2	s p	0,5 1	02 H2O	aq I		1.00E+00		-600 -
	r					,		-700 -
3	S	1	CO	aq		1,00E-06	E E	-800 -
3	s n	0,5	CO2	aq		1 00E-06	2	000
	٢		002	uq		1,002.00	_	-900 ±
4	s	1	H2S	aq		1,00E-06	Plot 2: delta-G as a function of log of	at 0°C
4	S	2	02	aq		4 00F 00		aluc
4	p	1	S042-	aq		1,00E-06		
	ρ	2	пт	ay		1,00E-06		-100 <sup>@</sup>
5	s	1	S0	s	rhombic	1,00E+00	- 4: Oxygen	-200 +
5	s	1,5	02	aq		v	$-\Box$ 5: Oxygen $\Delta \Delta \Delta$	-300 -
5	s	1	H2O	1		1,00E+00	Ę	100
5	р	1	SO42-	aq		1,00E-06	ti ti	-400 +
5	р	2	H+	aq		1,00E-08		-500 +
							<b>e D-D-D-D-D-D-D-D-D-D-D-</b> D-D-D-D-D-D-D-D-	-600 +
								-700 +
							E	800
								-000 -
								-900 -





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#### Free energies of aerobic chemolithotrophic dissimilation reactions 2



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# Free energies of anaerobic chemolithotrophic dissimilation reactions

Reaction No.	(S,P)	Stoich. Coeff.	Enter formula	State	Special remarks Ad	tivity	Variable	Prot 1: deita-G as a function of log of    1: Hydrogen    2: Hydrogen
1	s	1	H2	aq		-	v	- 3: Hydrogen 50 -
1	s	1	a-Fe2O3	s		1,00E+00		
1	s	4	H+	aq		1,00E-02		
1	р	3	H2O	- Ľ		1,00E+00		
1	p	2	Fe2+	aq		1,00E-06		5 -10 - pH 8 -50 -
2	s	1	H2	aq			v	
2	s	1	a-Fe2O3	s		1,00E+00		2 pH 5 -100 -
2	s	4	H+	aq		1,00E-05		
2	р	3	H2O	L.		1,00E+00		
2	р	2	Fe2+	aq		1,00E-06		PH 2 -150 -
3	s	1	H2	ad			v	-200-
3	s	1	a-Fe2O3	s		1.00E+00		-200
3	s	4	H+	ad		1.00E-08		Plot 2: delta-G as a function of log of
3	p	3	H2O	1		1.00E+00		-
3	p	2	Fe2+	aq		1,00E-06		-■-  - <u>□</u> -□- <u>□</u> - <u>□</u>
4	s	1	NH4+	aq			v	Dismutation -50
4	s	0,6	NO3-	aq		1,00E-06		
4	р	0,8	N2	aq		1,00E-06		-100
4	р	1,8	H2O	1		1,00E+00		Ę
4	р	0,4	H+	aq		1,00E-08		-150
5	s	1	S2O32-	aq			v	ů Ú
5	s	1	H2O	- É		1,00E+00		-200
5	р	1	HS-	aq		1,00E-06		
5	P	1	SO42-	aq		1,00E-06		É Anammox
5	р	1	H+	aq		1,00E-08		3
o <u>t 1</u> mp. (K n. vari Ix. var	() iable: riable	:	298,15 1,00E-14 1,00E-04		<u>Plot 2</u> Temp. (K) Min. variable: Max. variable:	298 1,00E 1,00E	,15 -14 -04	-300

#### Chemolithotrophic Denitrification of reduced Sulfur Compounds Example: Dissimilation by denitrifying *Thiomargarita sp.*

Intermediate storage of elemental sulfur and loss of N as N<sub>2</sub>O 4 H<sub>2</sub>S + 2 NO<sub>3</sub><sup>-</sup> + 2H<sup>+</sup>  $\rightarrow$  4 S° + N<sub>2</sub>O + 5 H<sub>2</sub>O 4 S° + 6 NO<sub>3</sub><sup>-</sup> + H<sub>2</sub>O  $\rightarrow$  4 SO<sub>4</sub><sup>2-</sup> + 3 N<sub>2</sub>O + 2H<sup>+</sup> 4 H<sub>2</sub>S + 8 NO<sub>3</sub><sup>-</sup>  $\rightarrow$  4 SO<sub>4</sub><sup>2</sup> + 4 N<sub>2</sub>O + 4 H<sub>2</sub>O (H<sub>2</sub>S + 2 NO<sub>3</sub><sup>-</sup>  $\rightarrow$  SO<sub>4</sub><sup>2</sup> + N<sub>2</sub>O + H<sub>2</sub>O)

Intermediate storage of elemental sulfur and loss of N as N<sub>2</sub>

$5 H_2 S + 2 NO_3^- + 2H^+$ $5 S^\circ + 6 NO_3^- + 2H_2 O$	${\rightarrow}$	$5 S^{\circ} + N_2 + 6 H_2O$ $5 SO_4^{2-} + 3 N_2 + 4H^+$
5 H <sub>2</sub> S + 8 NO <sub>3</sub> <sup>-</sup>	$\rightarrow$	$5 \text{ SO}_4^2 + 4 \text{ N}_2 + 2\text{H}^+ + 4 \text{ H}_2\text{O}$
(H <sub>2</sub> S + 1 % NO <sub>3</sub> -	$\rightarrow$	$SO_4^2 + \% N_2 + \% H^+ + \% H_2O$

Which nitrate concentrations in the cytoplasm of *Thiomargarita sp*. lead to energetically most favorable reactions?

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#### Summary Exercises: Virtual experiments employing Thermodyn

We compare the anaerobic metabolism of ethanol ( $CH_3CH_2OH$ ) employing different sulfur compounds as oxidants and demonstrate how energy yield can vary. The virtual experiments are carried out in chemostats with constant operating conditions (= boundary conditions)

Boundary conditions (concentrations in [mol/l])

Ethanol variable  $10^{-2} - 10^{-10}$ , HCO<sub>3</sub><sup>-</sup>, 5\*10<sup>-3</sup>, oxidant 0.5\*10<sup>-3</sup> in general, reduced oxidant  $\le 0.3*10^{-3}$ , pH 6.5  $\le$  pH  $\le 8.5$ , T = 25°C (298.15 K)

Problems

1. Formulate the dissimilation reactions for complete (to  $HCO_3^-$ ) oxidation of ethanol with the following oxidants:  $SO_4^{2-}$ ,  $SO_3^{2-}$ ,  $S_2O_3^{2-}$ ,  $S^{\circ}$ , and compare with  $HCO_3^-$ ,  $H^+$ ,  $O_2^-$ ,  $NO_3^-$  as oxidants.

2. Explain the differences in thermochemical energy yields.



# Thermodynamic comparison of sulfate, sulfite, thiosulfate and sulfur as electron acceptors (oxygen for comparison)



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#### Thermodynamics for alternative electron acceptors to sulfate: bicarbonate, protons, nitrate and oxygen



# Check yourself: Incomplete propionate degradation

The conversion of propionate to acetate by *Syntrophobacter wolinii* follows the stoichiometric reaction equation :

 $CH_{3}CH_{2}COO^{-} + 3 H_{2}O \rightarrow CH_{3}COO^{-} + HCO_{3}^{-} + 3H_{2} + H^{+}$ 

Which of the following statements about the thermodynamics of this reaction is correct?

- A At higher pH the reaction would become more exergonic
- B At lower pH the reaction would become more endergonic





#### Bio-energetic question that came up during the course: NH<sub>4</sub><sup>+</sup> oxidation

Under which conditions can NH<sub>4</sub><sup>+</sup> be oxidized aerobically and anaerobically?

- · Define oxidants and conditions that lead to exergonic reactions.
- · Where in the ocean could the anaerobic processes occur?
- · Check with actual measurements of conditions.



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#### Bio-energetic question: Aerobic respiration vs. denitrification

Under which  $O_2$  concentration (22  $\mu$ M, 10  $\mu$ M, <1  $\mu$ M) in the OMZ will aerobic respiration be energetically less favorable than denitrification?

- Define organic substrates (DOC) that can be degraded by both life styles.
- · Define oxidants for denitrification.
- Write comparable degradation processes as stoichiometric reaction equations.
- Define conditions that are representative for OMZs.
- · Check energetic yields with actually measured conditions in OMZs.



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### Bio-energetic question: Sequential use of oxidants

What are oxidants that are available for microbial degradation of DOM in oxic and anoxic waters?

In which sequence are they being consumed by microbes?

- Assume an organic substrates (DOC) that is being degraded by the organisms.
- Define the sequence of oxidants for degradation.
- Define conditions that are representative for oxic and anoxic waters.
- Write comparable degradation processes as stoichiometric reaction equations.
- Check energetic yields.



## Bio-energetic question: Enrichment of sulfate-reducing bacteria

Why is it thermodynamically more favorable to enrich sulfate-reducers with sulfite, rather than sulfate?

- Define an organic substrates (DOC) that is known to be used by sulfate reducers.
- Write two degradation equations, one with sulfate, the other with sulfite as oxidant Define enrichment conditions.
- Calculate energetic yields for the two processes.
- Find the explanation for the result by studying the biochemical mechanism for sulfate reduction.



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#### Bio-energetic question: aerobic and denitrifying oxidation of sulfur compounds

These are reactions and corresponding standard free energies from Pancho's presentation on "Ecología molecular del bacterioplancton oxidante de compuestos reducidos de azufre en las aguas deficientes de oxígeno del Océano Pacífico Sur Oriental"

 $HS^{-} + 2O_2 \longrightarrow SO_4^{2-} + 2H^+$  $\Delta G^{\circ} = -798 \text{ kJ mol}^{-1}$  $S_2O_3^{2-} + H_2O + 2O_2 \longrightarrow 2SO_4^{2-} + 2H^+ \Delta G^\circ = -818 \text{ kJ mol}^{-1}$  $S^{\circ} + H_2O + 1.5O_2 \longrightarrow SO_4^{2-} + 2H^+$  $\Delta G^{\circ} = -587 \text{ kJ mol}^{-1}$  $HS^{-} + NO_{3}^{-} \longrightarrow SO_{4}^{2-} + N_{2}$ ∆G° = -679.3 kJ mol<sup>-1</sup>  $S_2O_3^{2-} + NO_3^{-} \longrightarrow 2SO_4^{2-} + N_2 + H^+ \Delta G^\circ = -907.2 \text{ kJ mol}^{-1}$ 

- Are their stoichiometry correct?
- Why are the thermodynamic values not comparable?
- How would thermodynamically comparable reactions look like?
- What are actual conditions for substrates and products in the OMZ?
- Calculate actual 
  \u03c4 Gr values and draw conclusions about the occurrence in the OMZ.

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## Bio-energetic guestion: aerobic and denitrifying oxidation of sulfur compounds

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HS<sup>-</sup> + 2O<sub>2</sub> → SO<sub>4</sub><sup>2-</sup>+ 2H<sup>+</sup>  $\Delta G^{\circ} = -798 \text{ kJ mol}^{-1}$  $S_2O_3^{2-} + H_2O + 2O_2 \longrightarrow 2SO_4^{2-} + 2H^+ \Delta G^\circ = -818 \text{ kJ mol}^{-1}$  $S^{\circ} + H_2O + 1.5O_2 \longrightarrow SO_4^{2-} + 2H^+$  $\Delta G^{\circ} = -587 \text{ kJ mol}^{-1}$  $HS^{-} + NO_{3}^{-} \longrightarrow SO_{4}^{2-} + N_{2}^{2-}$ ∆G° = -679.3 kJ mol<sup>-1</sup>  $S_2O_3^{2-} + NO_3^{-} \longrightarrow 2SO_4^{2-} + N_2 + H^+ \Delta G^\circ = -907.2 \text{ kJ mol}^{-1}$ 

- Can you think of other reactions in the coupled N-S-cycle that might occur?
- · Under what conditions are they thermodynamically favorable?
- Did you include disproportionation of S° and dismutation of thiosulfate?
- Are disproportionation of S° and dismutation of thiosulfate thermodynamically exergonic?





## Bio-energetic question: Combine sulfate reduction with denitrification and anammox to achieve anaerobic oxidation of ammonia to $N_2$



Schrum H.N. et al. 2009. Geology, 37/10, 939-942, October





### Bio-energetic question: How dinitrogen oxide $(N_2O)$ can be cycled

Under which conditions can  $N_2O$  be an oxidant, how can it be a reductant?

- Define stoichiometric reactions for N<sub>2</sub>O as reductant as well as oxidant.
- Choose conditions, which are representative for a natural habitat.
- · Check energetic yields and define thermodynamically exergonic and endergonic reactions.
- Can N<sub>2</sub>O be disproportionated in an energetically favorable way for chemolithotrophic microbes?



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## Bio-energetic question: Can dinitrogen oxide (N<sub>2</sub>O) be an electron donor for anoxigenic photosynthesis

What is the redox potential of N<sub>2</sub>O electrons? Can N<sub>2</sub>O electrons be transferred into a photosystem of phototrophic bacteria?

- Define stoichiometric half reactions for N<sub>2</sub>O oxidation.
- Calculate the potential in mV for electrons released from N<sub>2</sub>O.
- Find components of photosystems that could accept N<sub>2</sub>O electrons i.e. that could oxidize  $N_2O$ .
- Compare N<sub>2</sub>O as electron donor for photosynthesis with nitrite, ammonia and nitrous oxide.
- Compare N<sub>2</sub>O as electron donor for photosynthesis with non-nitrogen electron donors like hydrogensulfide, ferrous iron, di- and trihydrogenarsenite.
- Compare N<sub>2</sub>O as electron donor for photosynthesis with H<sub>2</sub>O as electron donor.



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## Hydrogensulfide as electron donor for photosynthesis







#### Electron donors and acceptors in phototrophy

Which redox pairs are possible electron donors and where could they enter the photon driven electron flow ?



#### Standard electrode potentials of metabolic reactions (1 Mol/I, 1 atm, pH 7)

Couple	E°' [mV]	e <sup>-</sup> transferred
CO <sub>2</sub> / CO	- 540	2
SO42- / HSO3	- 516	2
CO <sub>2</sub> / Formate	- 430	2
6 CO <sub>2</sub> / Glucose	- 430	24
2H <sup>+</sup> / H <sub>2</sub>	- 418	2
S <sub>2</sub> O <sub>3</sub> <sup>2-</sup> / HS <sup>-</sup> + HSO <sub>3</sub> <sup>-</sup>	- 400	2
CO <sub>2</sub> / Methanol	- 380	6
2 CO <sub>2</sub> / Acetate	- 280	8
S° / H <sub>2</sub> S	- 280	2
CO <sub>2</sub> / CH <sub>4</sub>	- 240	8
SO <sub>4</sub> <sup>2-</sup> / H <sub>2</sub> S	- 220	8
Acetaldehyde / Ethanol	- 197	2
Dihydroxyaceton-P / Glycerin-P	- 190	
Pyruvate / Lactate	- 186	2
Oxaloacetate / Malate	- 170	2
HSO <sub>3</sub> <sup>-</sup> / S <sub>3</sub> O <sub>6</sub> <sup>2-</sup>	- 170	??????
HSO3 <sup>-</sup> / HS <sup>-</sup>	- 116	6
Cyt b <sub>558ox</sub> / Cyt b <sub>558red</sub>	- 75 to - 45	1
APS / AMP + HSO3 <sup>-</sup>	- 60	2
Acrylyl-CoA / Propionyl-CoA	- 15	2 ???
Glycin / Acetat + NH4 <sup>+</sup>	- 10	2 ???
S <sub>4</sub> O <sub>6</sub> <sup>2-</sup> / 2 S <sub>2</sub> O <sub>3</sub> <sup>2-</sup>	+ 24	2
Fumarate / Succinate	+ 33	2
AsO <sub>4</sub> <sup>3-</sup> / AsO <sub>3</sub> <sup>3-</sup>	+ 139	2
Dimethylsulfoxid / Dimethylsulfid	+ 160	????
Fe(OH) <sub>3</sub> + HCO <sub>3</sub> / FeCO <sub>3</sub>	+ 200	2
Fe <sup>3+</sup> / Fe <sup>2+</sup>	+ 200	1
S <sub>3</sub> O <sub>6</sub> <sup>2-</sup> / S <sub>2</sub> O <sub>3</sub> <sup>2-+</sup> + HSO <sub>3</sub> <sup>-</sup>	+ 225	1
NO <sub>2</sub> <sup>-</sup> / NO	+ 360	1
NO <sub>3</sub> <sup>-</sup> / NO <sub>2</sub> <sup>-</sup>	+ 421	2
$NO_2^{-1}/NH_3$	+ 440	6
SeO <sub>4</sub> <sup>2-</sup> /SeO <sub>3</sub> <sup>2-</sup>	+ 475	2
$NO_{3}^{-}/ \frac{1}{2}N_{2}$	+ 740	5
Fe <sup>3+</sup> /Fe <sup>2+</sup>	+ 763	1 pH 2
Mn <sup>**</sup> / Mn <sup>**</sup>	+ 798	2
$1/2 O_2 / H_2 O_2$	+ 818	2
NO / '/ <sub>2</sub> N <sub>2</sub> O	+ 1180	1
N <sub>2</sub> O / N <sub>2</sub>	+ 1360	2





### Standard electrode potentials of coenzymes (1 Mol/I, 1 atm, pH 7)

Couple	E°' [mV]	e <sup>-</sup> transferred
Ferredoxin <sub>ox</sub> / Ferredoxin <sub>red</sub>	- 432	1
$2H^+/H_2$	- 418	2
Ferredoxin <sub>ox</sub> / Ferredoxin <sub>red</sub>	- 410 to - 390	1 Clostridium
Flavodoxin <sub>ox</sub> / Flavodoxin <sub>red</sub>	- 370	2 ???
NAD <sup>+</sup> / NADH	- 320	2
FeS-Protein <sub>ox</sub> / FeS-Protein <sub>red</sub>	- 305	1 high potential
Cyt c <sub>3ox</sub> / Cyt c <sub>3red</sub>	- 290	1
Lipoic / Dihydrolipoic	- 290	2
FAD / FADH <sub>2</sub>	- 220	2
FMN / FMNH <sub>2</sub>	- 190	2
Flavodoxin <sub>ox</sub> / Flavodoxin <sub>red</sub>	- 120	2 ???
Menaquinone <sub>ox</sub> / Menaquinone <sub>red</sub>	- 74	2
Cyt b <sub>558ox</sub> / Cyt b <sub>558red</sub>	- 75 to - 45	1
Rubredoxin <sub>ox</sub> / Rubredoxin <sub>red</sub>	- 57	2 ????
Cyt b <sub>556ox</sub> / Cyt b <sub>556red</sub>	+ 35 to + 120	1
Ubiquinone <sub>ox</sub> / Ubiquinone <sub>red</sub>	+ 110	2
Cyt b <sub>562ox</sub> / Cyt b <sub>562red</sub>	+ 125 to + 260	1
Cyt c <sub>ox</sub> / Cyt c <sub>red</sub>	+ 230 to + 250	1
Cyt d <sub>ox</sub> / Cyt d <sub>red</sub>	+ 260 to + 280	1
FeS-Protein <sub>ox</sub> / FeS-Protein <sub>red</sub>	+ 280	1 low potential
Cyt a <sub>ox</sub> / Cyt a <sub>red</sub>	+ 290	1
Cyt c <sub>555ox</sub> / Cyt c <sub>555red</sub>	+ 355	1
Cyt aa3 <sub>ox</sub> / Cyt aa3 <sub>red</sub>	+ 388	1
'/ <sub>2</sub> O <sub>2</sub> / H <sub>2</sub> O	+ 818	2

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Microbial Ecology

## Phototrophy with Ferro-iron

Define the conditons which allow ferro-iron to act as an electron donor in anoxygenic photosynthesis.

Can you think of other redox pairs which could act as electron donors in anoxygenic photosynthesis ?







## Phototrophy with Arsenite



## Electron flow in oxygenic phototrophs







## Bio-energetic question: Fixation of dinitrogen oxide (N<sub>2</sub>O)

Would a process that fixes N<sub>2</sub>O instead of N<sub>2</sub> into NH<sub>4</sub><sup>+</sup> be energetically less or more favorable than the well known N<sub>2</sub> fixation process with nitrogenase?



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Microbial Ecol

## Weathering - How serpentinization can support life What happens at Totalp?





Prerequisite for life: Are these reactions energetically exergonic?



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Can small organic molecules be formed without the involvement of life processes? (chemical origin of first organic molecules)





Under what conditions can simple organics be produced by purely chemical synthesis from CO<sub>2</sub> and "high energy electrons" in hot & cold aqueous fluids?

e.g. synthesis of acetate:



 $\Delta$ Gr in kJoule/mol of Acetate produced from CO<sub>2</sub> and H<sub>2</sub> in hot and cold fluids 2 CO<sub>2</sub> + 4 H<sub>2</sub>  $\longrightarrow$  CH<sub>3</sub>COO<sup>-</sup> + H<sup>+</sup> + H<sub>2</sub>O



Acetate formation from  $CO_2$  and  $H_2$  is strongly exergonic above the µmolar activities of  $H_2$  in hot fluids, and above nanomolar activities in cold fluids

## $\Delta$ Gr for Acetate from CO<sub>2</sub> and H<sub>2</sub> with various electron donors



## $\Delta$ Gr for Acetate from CO<sub>2</sub> and H<sub>2</sub> in hot and cold fluids is not always exergonic



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#### $\Delta$ Gr for Acetate from CO<sub>2</sub> and H<sub>2</sub> is most exergonic with ferrous iron as e<sup>-</sup> donor



## How does the pH affect $\Delta$ Gr for Acetate from CO<sub>2</sub> and H<sub>2</sub>?



 $\Delta$ Gr is less affected by pH (8 vs. 6.5) at low and at high temperatures than by the kind of electron donor (H<sub>2</sub> vs. HS<sup>-</sup>) At low temperature (0°C)  $H_2$  is the preferred

electron donor over HS<sup>-</sup> at both pH values

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## Summary

Formation of organics, e.g. acetate from  $CO_2$  in fluids at 0°C is thermodynamically

feasible with

- Hydrogen oxidation
- Ferrous hydroxyde oxidation

not feasible with

- Anaerobic sulfur oxidation to sulfate
- · Methane oxidation

Formation of organics, e.g. acetate from  $CO_2$  in fluids at 100°C is thermodynamically

#### feasible with

- Hydrogen oxidation
- Hydrogensulfide oxidation
- Ferrous hydroxyde oxidation

#### not feasible with

- Anaerobic sulfur oxidation to sulfate
- Methane oxidation

barely feasible with

Hydrogensulfide oxidation

Hydrogen and ferrous iron are more effective as electron donors at low temperatures than at high temperatures Hydrogensulfide is more effective as an electron donor at high temperatures

Ferrous iron is the most effective electron donor at all temperatures and pH

Hydrogen yields slightly more free energy at higher pH at both temperatures Hydrogensulfide yields more free energy at lower pH at both temperatures

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## Under which conditions can the hydrogenation reactions proceed as described?

Reactions 1 to 4 represent consecutive reductions of CO<sub>2</sub>-carbon to CH<sub>4</sub>-carbon. CO<sub>2</sub>  $\rightarrow$  HCOOH  $\rightarrow$  CH<sub>2</sub>O  $\rightarrow$  CH<sub>3</sub>OH  $\rightarrow$  CH<sub>4</sub>

What is the free energy for each reaction step?

Re	action			∆Gr [kJ/mol]
1.	$CO_{2(aq)} + H_{2(aq)}$	$\rightarrow$	HCOOH <sub>(aq)</sub>	exergonic or endergonic?
2.	$\text{HCOOH}_{(aq)} + \text{H}_{2(aq)}$	$\rightarrow$	$CH_2O_{(aq)}$ + $H_2O_{(I)}$	exergonic or endergonic?
3.	$CH_2O_{(aq)}$ + $H_{2(aq)}$	→	$CH_3OH_{(aq)}$	exergonic or endergonic?
4.	$CH_3OH_{(aq)} + H_{2(aq)}$	$\rightarrow$	$CH_{4(aq)}$ + $H_2O_{(I)}$	exergonic or endergonic?
5.	$\Sigma$ CO <sub>2(aq)</sub> + 4 H <sub>2(aq)</sub>	$\rightarrow$	$CH_{4(aq)}$ + 2 $H_2O_{(l)}$	exergonic or endergonic?

#### Is the formation of acetic acid exergonic or endergonic? 6. $2 \text{ CO}_2 + 4 \text{ H}_2 \rightarrow \text{ CH}_3\text{COOH} + 2 \text{ H}_2\text{O}$







## Free energy of reactions 1 to 5 under standard ( $\Delta$ Gr<sup>°</sup>) and actual ( $\Delta$ Gr for 10<sup>-6</sup> $H_2$ ) for 2 temperatures (0°C and 100°C)





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## Cinder pool, Yellowstone National Park - A case study



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Microbial Ecolog

#### Three key questions for life in hydrothermal springs

How is reducing power generated in the subsurface? (precondition for life processes to occur)

Can small organic molecules be formed without the involvement of life processes? (chemical origin of first organic molecules)

How are organisms changing their habitat? (interacting geochemical cycles)



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## Boiling, acid sulfate-chloride Cinder pool (Norris basin, YNP). Is it a model to study processes of the sulfur cycle as they happen at "black smokers"?

Dissolved sulfur species:

- sulfide (H<sub>2</sub>S), 16 48 μM
- thiosulfate (S<sub>2</sub>O<sub>3</sub><sup>2-</sup>), 35 45 μM, formed during S<sup>o</sup> hydrolysis  $2 S^{\circ} + 3 H_2 O \longrightarrow S_2 O_3^{2-} + 2 H^+ + 2 H_2$
- polythionates (S<sub>v</sub>O<sub>6</sub><sup>2-</sup>), max. 8 μM
- sulfate (SO<sub>4</sub><sup>2-</sup>), 1150 1300 μM
- floating, black, hollow, sulfur spherules (?  $\sim 5 \text{ mm}$ ) on surface, surrounded by polythionates
- molten sulfur (S°) at 18 m depth

Chemica	l analysis of C	inder Pool, Yell	owstone Natic	nal Park (mg/l	) <sup>a</sup>				
Ca	Mg	Na	K	SO <sub>4</sub>	F	C1	SiO <sub>2</sub>	В	Li
5.9	0.024	373	57.6	96	6.24	601	433	9.60	4.70
S <sub>2</sub> O <sub>3</sub>	H <sub>2</sub> S	NO <sub>3</sub> <sup>-</sup>	NH4 <sup>+</sup>	A1	Fe <sub>tot</sub>	Fe(II)	Sr	Br	As <sub>tot</sub>
5.10	1.6	1.63	18	1.13	0.088	0.088	0.011	1.7	2.4

Table 1

<sup>a</sup> Sample collected 8/22/95, sample code 95WA110; pH = 4.22;  $T = 95^{\circ}$ C, Specific Conductance = 2320 microS cm<sup>-1</sup>.

Xu, Y. et al. 2000. Journal of Volcanology and Geothermal Research 97, 407-423.



Microbial

Possible fates of sulfur compounds in Cinder pool and at its surface



## Sulfur oxidation to thiosulfate is thermodynamically not feasible









## Sulfur disproportionation is thermodynamically feasible at alkaline pH



# Thiosulfate and tetrathionate dismutation reactions are thermodynamically feasible



## Possible origins of thiosulfate 1

Aerobic  $H_2S$  oxidation by:

subsurface transport of  $O_2$  with the infiltration water: -, quantitatively relevant? above surface ingassing of  $O_2$  from the atmosphere: + but quickly further oxidized

Subsurface reactions between ingassed magmatic  $H_2S$  (-?) and ingassed magmatic  $SO_2$  (-?): but no evidence for  $SO_2$  in the subsurface water

SO<sub>2</sub> hydrolysis (disproportionation of SO<sub>2</sub>-S:

 $4 \text{ SO}_2 + 4 \text{ H}_2\text{O} \longrightarrow \text{HS}^- + 3 \text{ SO}_4^{2-} + 7 \text{ H}^+$ 

and further interaction of SO<sub>2</sub> with HS<sup>-</sup>

 $4 \text{ SO}_2 + 2 \text{ HS}^- + \text{H}_2\text{O} \longrightarrow 3 \text{ S}_2\text{O}_3^{2-} + 4\text{H}^+$ 

which can also produce elemental sulfur

 $SO_2 + 2 HS^- + 2 H^+ \longrightarrow 3 S^0 + 2 H_2O$ 



## Possible origins of thiosulfate 2 Hydrolysis (disproportionation) of elemental sulfur as a source for thiosulfate (requires opening of the $S_8$ ring at elevated temperatures)





## Cinder pool: proposed processes







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Can small organic molecules be formed without the involvement of life processes? (chemical origin of first organic molecules)  $\rightarrow$  Repetition from above

Question 2

How are organisms changing their habitat? (interacting geochemical cycles)



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## Under what conditions can simple organics be produced by purely chemical synthesis from CO<sub>2</sub> and "high energy electrons" in hydrothermal fluids?

e.g. synthesis of acetate:

2 CO <sub>2</sub> + 4 H <sub>2</sub>	$\longrightarrow$ CH <sub>3</sub> COO <sup>-</sup> + H <sup>+</sup> + H <sub>2</sub> O
2 CO <sub>2</sub> + 4 HS <sup>-</sup> + 3 H <sup>+</sup>	$\longrightarrow$ CH <sub>3</sub> COO <sup>-</sup> + 4 S <sup>o</sup> + 2 H <sub>2</sub> O
2 CO <sub>2</sub> + 1 <sup>1</sup> /3 S <sup>o</sup> + 3 <sup>1</sup> /3 H <sub>2</sub> O	$\longrightarrow$ CH <sub>3</sub> COO <sup>-</sup> + 1 <sup>1</sup> /3 SO <sub>4</sub> <sup>2-</sup> + 3 <sup>2</sup> /3 H <sup>+</sup>
2 CO <sub>2</sub> + S <sub>2</sub> O <sub>3</sub> <sup>2-</sup> + 3 H <sub>2</sub> O	$\longrightarrow$ CH <sub>3</sub> COO <sup>-</sup> + 2 SO <sub>4</sub> <sup>2-</sup> + 3H <sup>+</sup>
2 CO <sub>2</sub> + 4 S <sup>o</sup> + 4 H <sub>2</sub> O	$\longrightarrow$ CH <sub>3</sub> COO <sup>-</sup> + 2 S <sub>2</sub> O <sub>3</sub> <sup>2-</sup> + 5 H <sup>+</sup>
$CO_2 + CH_4$	$\longrightarrow$ CH <sub>3</sub> COO <sup>-</sup> + H <sup>+</sup>
2 CO <sub>2</sub> + 8 Fe(OH) <sup>+</sup> + 7 H <sup>+</sup>	$\longrightarrow$ CH <sub>3</sub> COO <sup>-</sup> + 8 Fe(OH) <sup>2+</sup> + 2 H <sub>2</sub> O

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Organics produced by purely chemical reactions in hydrothermal fluids (e.g. acetate formation from  $CO_2$  and  $H_2$  is strongly exergonic above the µmolar range of  $H_2$ )

#### Organics produced by purely chemical reactions in hydrothermal fluids (e.g. acetate formation from CO<sub>2</sub> and HS<sup>-</sup> is less exergonic than from H<sub>2</sub>)



#### Organics by purely chemical reactions in hydrothermal fluids (e.g. acetate formation from CO<sub>2</sub> and S<sup>o</sup> or S<sub>2</sub>O<sub>3</sub><sup>2-</sup> is mostly endergonic)



## Organics produced by purely chemical reactions in hydrothermal fluids (e.g. acetate formation from CO<sub>2</sub> and CH<sub>4</sub> or S<sub>2</sub>O<sub>3</sub><sup>2-</sup> is only slightly exergonic)



#### Organics produced by purely chemical reactions in hydrothermal fluids (more alkaline pH makes acetate formation from CO<sub>2</sub> and CH<sub>4</sub> or $S_2O_3^{2-}$ or S<sup>o</sup> more exergonic)



#### Organics produced by purely chemical reactions in hydrothermal fluids (e.g. acetate formation from CO<sub>2</sub> and ferrous hydroxide is strongly exergonic)



## Ferrous hydroxide is by far the best reductant for CO<sub>2</sub> conversion to acetate





### Acetate formation with thiosulfate is mostly endergonic. Can coupling it with thiosulfate dismutation make the reaction exergonic?



#### Thiosulfate dismutation can thermodynamically promote acetate formation



## Sulfur disproportionation cannot support acetate formation, but aerobic sulfur oxidation can



## Summary

Formation of organics, e.g. acetate from  $CO_2$ , in hydrothermal fluids at 100°C is thermodynamically feasible with

- Hydrogen oxidation
- Hydrogensulfide oxidation
- Ferrous hydroxyde oxidation
- Thiosulfate dismutation (coupled reactions)
- Aerobic sulfur oxidation (coupled reactions)

It is not feasible with

- Anaerobic sulfur oxidation to sulfate or thiosulfate
- Sulfur disproportionation

It is barely feasible with

- Thiosulfate to sulfate oxidation
- Methane oxidation

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## How Life could have originated

Panspermia?

Hydrothermal Vents?

Serpentinization?

Methane Coupling

Nutrient Soup? (Urey, Miller)

Others?





Microbial Ed

## Methane coupling: Production of C-C bonds from CH<sub>4</sub>

Could C-C- bonds, like those in Ethane  $(CH_3-CH_3)$  or Ethylene  $(CH_2=CH_2)$  have been produced from  $CH_4$  originally?

Propose pathways for

 $CH_4 + CH_4 \rightarrow CH_3-CH_3 + H_2$  $CH_4 + CH_4 \rightarrow CH_2=CH_2 + 2 H_2$ 

 $CH_4$  exhibits high C–H bond strength (434 kJ/mol), negligible electron affinity, large ionization energy, and low polarizability.

Thus, to achieve direct conversion of  $CH_4$ , one needs to cleave the first C–H bond while suppressing further catalytic dehydrogenation and this avoiding both  $CO_2$  generation and coke deposition.

It is reported to occur with the aid of a catalyst, e.g. Fe/SiO<sub>2</sub> (Xiaoguang Guo et all. 2014, *et al.* Direct, Nonoxidative Conversion of Methane to Ethylene, Aromatics and Hydrogen, Science, 344, 616-619)

Could it occur biologically?

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## Ethane and Ethene from Methane is feasible at very low Activities of H<sub>2</sub>

# Methane coupling: Production of C-C bonds from CO or $\rm CO_2$ stemming from $\rm CH_4$ oxidation

Could it occur via re-synthesis reactions?

Synthesis of Ethane or Ethylene from CO and H<sub>2</sub>:

 $2 \text{ CO} + 5 \text{ H}_2 \rightarrow \text{ CH}_3\text{-}\text{CH}_3 + 2 \text{ H}_2\text{O}$ 

 $2 \text{ CO} + 4 \text{ H}_2 \rightarrow \text{ CH}_2 = \text{CH}_2 + 2 \text{ H}_2\text{O}$ 

Synthesis of Ethane or Ethylene from CO<sub>2</sub> and H<sub>2</sub>:

 $2 \text{ CO}_2 + 7 \text{ H}_2 \rightarrow \text{ CH}_3 - \text{CH}_3 + 4 \text{ H}_2\text{O}$ 

 $2 \text{ CO}_2 + 6 \text{ H}_2 \rightarrow \text{ CH}_2 = \text{CH}_2 + 4 \text{ H}_2\text{O}$ 

Are these reactions thermodynamically feasible?

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# Methane coupling: Production of C-C bonds from CO or $CO_2$ stemming from $CH_4$ oxidation - Energetic Feasibility

Reaction No.	(S,P)	Stoich. Coeff.	E	inter form	ula	State	Special remarks	Conc.	Activity correction		Activity	Variahla
1	s	2	со			aq		1.00E-04	1.00E+00		1.00E-04	Ε
1	s	5	H2			aq			1.00E+00	٢.	0.00E+00	N
1	р	1	C2H6			aq		1.00E-09	1.00E+00		1.00E-09	
1	р	2	H2O			I		1.00E+00	1.00E+00		1.00E+00	
										٢.	0.00E+00	
2	s	2	CO			aq		1.00E-04	1.00E+00		1.00E-04	
2	s	4	H2			aq			1.00E+00	٢.	0.00E+00	ľ
2	р	1	C2H4			aq		1.00E-09	1.00E+00		1.00E-09	
2	р	2	H2O			I		1.00E+00	1.00E+00		1.00E+00	
~	-	~	000						4.005.00	٢.	0.00E+00	
3	s	2				aq		1.00E-04	1.00E+00		1.00E-04	١.
ა 2	s	1				aq			1.00E+00		1.00E+00	ľ
2	p	1				aq		1.00E-09	1.00E+00		1.00E-09	
5	Ρ	4	1120			'		1.002+00	1.002+00	,	0.00E+00	
٨	c	2	CO2			20		1 00E-04	1.00E+00		1.00E+00	
4	s	6	H2			aq		1.002-04	1.00E+00	•	0.00E+00	١.
4	n	1	C2H4			aq		1 00E-09	1.00E+00		1.00E-09	11
4	р р	4	H2O			I I		1 00E+00	1.00E+00		1.00E+00	
Diet	4											
Tem Min. Max	<u> </u> p_1 vari . var	(K) able: iable:	1	298.15 1.00E-15 .00E+00	25	5 °C						
				pK calc	ulator			рН са	lculator			
Log	plot	?:	X	pK(T1)	pK298)	∆Gr°298 kJ/mol	3 ∆Hr°298 kJ/mol	рН	H(+)conc.			
5	Show	react.	1 <b>x</b>	-59.62	-59.62	-340.31	-410.96	0	1.00E+00			
5	Show	react.	2 x	-39.27	-39.27	-224.18	-276.62	0	1.00E+00			
5	Show	react.	3 x	-55.69	-55.69	-317.91	-389.78	0	1.00E+00			
5	Show	react.	4 x	-35.35	-35.35	-201.78	-255.44	0	1.00E+00			
S	Show	react.	5	0.00	0.00	0.00	0.00	0	1.00E+00			





# Methane coupling: Production of C-C bonds from CO or $CO_2$ stemming from $CH_4$ oxidation is energetically feasible even at low $H_2$ Activities



## Methane coupling: Production of H<sub>2</sub> and CO from CH<sub>4</sub>

Production of H<sub>2</sub>, CO and CO<sub>2</sub> through oxidation of CH<sub>4</sub>

 $\begin{array}{ll} \mathsf{CH}_4 + \mathsf{H}_2\mathsf{O} \ \ \overrightarrow{>}\ \mathsf{CO} + 3\ \mathsf{H}_2 \\ \mathsf{CO} \ \ + \ \mathsf{H}_2\mathsf{O} \ \ \overrightarrow{>}\ \mathsf{CO}_2 + \mathsf{H}_2 \end{array} \qquad \begin{array}{ll} \text{In industrial synthesis, this process is called the steam reforming reaction of hydrogen from natural gas (1<sup>st</sup> Syngas production reaction). \\ \text{In order to produce more hydrogen, more steam is added to the water gas and the hydrogen is then separated from the CO_2. \end{array}$ 

Overall reaction for the production of  $H_2$ :

 $CH_4 + 2 H_2O \rightarrow CO_2 + 4 H_2$ 

Are these reactions thermodynamically feasible to be carried out by microorganisms?



Microbial Ed

#### Methane coupling: Production of $H_2$ , CO and CO<sub>2</sub> from CH<sub>4</sub> Are the reactions thermodynamically feasible to be carried out by microorganisms?

Reac No.	(S,P)	Stoich Coeff.		Enter form	ula	State	Special remarks	Conc.	Activity correction	Activity	Variabl
1	s	1	CH4			aq		1.00E-02	1.00E+00	1.00E-02	
1	S	1	H2O					1.00E+00	1.00E+00	1.00E+00	
1	p	1	<u>со</u> цо			aq		1.00E-06	1.00E+00	1.00E-06	
	ρ	3	пΖ			aq			1.00E+00	0.00E+00	•
2	s	1	co			aq		1.00E-06	1.00E+00	1.00E-06	
2	s	1	H2O			1		1.00E+00	1.00E+00	1.00E+00	
2	р	1	CO2			aq		1.00E-04	1.00E+00	1.00E-04	
2	p	1	H2			aq			1.00E+00	0.00E+00	v
										0.00E+00	
3	s	1	CH4			aq		1.00E-02	1.00E+00	1.00E-02	
3	s	1	H2O			I		1.00E+00	1.00E+00	1.00E+00	
3	р	1	CO			aq		1.00E-03	1.00E+00	1.00E-03	
3	р	3	H2			aq			1.00E+00	0.00E+00	ν.
4		1	0			20		1.00E-03	1 00E+00	1.00E+00	
4	5 e	1	H2O			ay		1.00E+00	1.00E+00	1.00E+00	
4	p	1	CO2			aq		1.00E-04	1.00E+00	1.00E-04	
4	p	1	H2			aq			1.00E+00	0.00E+00	v
										0.00E+00	
5	s	1	CH4			aq		1.00E-02	1.00E+00	1.00E-02	
5	s	2	H2O			I		1.00E+00	1.00E+00	1.00E+00	
5	р	1	CO2			aq		1.00E-04	1.00E+00	1.00E-04	
- 5	р	4	H2	000 15		aq			1.00E+00	0.00E+00	V
Temp	_1 (P	<) 		298.15	25	-0					
Max y	aria	ble:	1	1.00E-15							
max.	and	ioie.									
				pK calc	ulator			pH c	alculator		
Log p	lot?	:	X	рК(Т1)	pK298)	∆Gr°298 kJ/mol	∆Hr°298 kJ/mol	pН	H(+)con	с.	
Sh	i woi	react.1	x	35.89	35.89	204.87	241.35	0	1.00E+00	)	
Sh	I woi	react.2	x	-1.96	-1.96	-11.20	-10.59	0	1.00E+00	)	
Sh	low I	react.3	X	35.89	35.89	204.87	241.35	0	1.00E+00	)	
Sh	I woi	react.4	X	-1.96	-1.96	-11.20	-10.59	0	1.00E+00	)	
Sh	I woi	react.5	X	33.93	33.93	193.67	230.76	0	1.00E+00	)	

Microbial Ecology

Methane coupling: Production of  $H_2$ , CO and CO<sub>2</sub> from  $CH_4$ Are the reactions thermodynamically feasible to be carried out by microorganisms?



 $CH_4$  can act as a source of  $H_2$  and CO and CO can act as as source of  $H_2$  and  $CO_2$ , if the activity of  $H_2$  is kept low



