P XXX/0/2 Introduction to thermodynamic modelling with Thermodyn

(for students who had no prior contact with Thermodyn)

This exercise is based on experiment 18 (Bio-III) from where the following explanations are taken. We will examine a few reactions which were derived in experiment 1 (Microbial Diversity in the Rumen, Bio-III) with regard to

- the likelyhood with which they can take place,
- the environmental conditions under which they can take place and
- the interactions with the host needed to make them happen in the rumen.

The reactions are treated as if they would obey ideal thermodynamic laws, that is they take place

- at a standard temperature of 298.15 K (25 ^{o}C),
- under ideally diluted conditions, such that activities of reactants equal concentrations.

The student will learn how to **formulate biochemical processes** as **stoichiometric equations**, define the **conditions** under which the processes might take place and analyze the outcome of **simulation runs**.

The following problem will be explained here in detail first. Then, additional problems will be offered which are to be investigated by the student independently.

Problem 1:
Under which boundary conditions is the degradation of glucose by *Ruminococcus flavefaciens* in axenic batch culture energetically feasible ?
Glucose fermentation by *R.flavefaciens* to acetate, formate and succinate can be described by the stoichiometrically balanced equation

$$100\ C_6H_{12}O_6 + 48\ HCO_3^- \rightarrow 107\ CH_3COO^- + 62\ HCOO^- + 93\ {}^-OOC(CH_2)_2COO^- + 59\ H_2 + 307\ H^+ + 34\ H_2O$$

The reaction is thermodynamically feasible as long as the Gibbs free energy of the reaction is < 0. This depends on the actual activities of the substrates and products and on the standard free energy of the conversion reaction.

Explanations

We will recall the background first.
From Physical Chemistry we remember that the reaction

$$aA^{n-} + bB^{m-} \rightarrow cC^{p-} + dD^{q-} + (cp+dq-an-bm)H^+$$

will proceed in the direction as written, if $\Delta Gr < 0$. The free energy (ΔGr) is defined as

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

with Q being the ratio of the algebraic product of the activities (concentrations) of the reaction products, divided by the algebraic product of the activities of the reaction substrates; stoichiometric factors become exponents.
R is the gas constant $= 8.31451 \cdot 10^{-3}$ [kJ· mol^{-1}·K^{-1}] (concentration basis)
T is the thermodynamic temperature in [Kelvin]
ΔGr^o is calculated from the free energies of formation according to

$$\Delta Gr^o = \sum_j v_j Gf^o_{P_j} - \sum_i v_i Gf^o_{S_i}$$

(for the meaning of terms see Abbreviations below)

$$Q = \frac{\left[C^{p-}\right]^{c} \cdot \left[D^{q-}\right]^{d} \cdot \left[H^{+}\right]^{(p+q-n-m)}}{\left[A^{n-}\right]^{a} \cdot \left[B^{m-}\right]^{b}}$$

The temperature correction follows from

$$\Delta Gr^{o}_{T_{act}} = \Delta Gr^{o}_{T_{ref}} \cdot \frac{T_{act}}{T_{ref}} + \Delta Hr^{o}_{T_{ref}} \cdot \frac{T_{ref} - T_{act}}{T_{ref}}$$

Abbreviations :

Gf^{o}	standard free energy of formation [kJ/mol]
ΔGr^{o}	change of Gibbs free energy of reaction at standard conditions $= -R \cdot T \cdot \ln K^{o}$
ΔGr	change of Gibbs free energy of reaction under actual conditions
Hf^{o}	standard enthalpy of formation
ΔHr^{o}	enthalpy change of reaction at standard conditions
K^{o}	thermodynamic equilibrium coefficient
Q	ratio of actual activity products of reactants
R	gas constant $= 8.31451 \cdot 10^{-3}$ [$kJ \cdot mol^{-1} \cdot K^{-1}$] (concentration basis)
T	thermodynamic temperature in [Kelvin]
T_{ref}, T_{act}	reference and actual temperature, respectively
P_j, S_i	product and substrate of species j and i, respectively
v	stoichiometric factor

For our example

$$Q = \frac{\left[CH_3COO^{-}\right]^{107} \cdot \left[HCOO^{-}\right]^{62} \cdot \left[{}^{-}OOC(CH_2)_2COO^{-}\right]^{93} \cdot \left[H_2\right]^{59} \cdot \left[H^{+}\right]^{307} \cdot 1}{\left[C_6H_{12}O_6\right]^{100} \cdot \left[HCO_3^{-}\right]^{48}}$$

- activity of water in aqueous solutions is by convention 1;
- Proton concentration follows from pH; $\left[H^{+}\right] = 10^{-pH}$
- $C_6H_{12}O_6$ is α - D - glucose
- The concentrations (activities) of the other reactants are defined as boundary conditions.

The energies of formation (Gf^{o}) are :

Compound	Formula	$Gf^{o}\left[kJ/mole\right]$
a - D - Glucose	$CH_2OH(CHOH)_4CHO$	- 917.2
Formate	$HCOO^{-}$	- 351.0
Acetate	H_3CCOO^{-}	- 369.4
Succinate	${}^{-}OOC(CH_2)_2COO^{-}$	- 690.2
Bicarbonate	HCO_3^{-}	- 586.9
Hydrogen	H_2	+ 17.55
Proton	H^{+}	0
Water	H_2O	- 237.2
Methane	CH_4	- 34.4

Calculating ΔGr for the actual conditions at the beginning of the batch culture experiment using Thermodyn©

The Excel spreadsheet program Thermodyn© allows one to calculate free reaction energies for a number of microbially mediated chemical ractions. Thermodyn© is ment to be used as a learning tool to make applying thermodynamic laws in microbiology more understandable to the student. Comparing free reaction energies which are calculated for real conditions (activities, concentrations, pH, temperatures) make thermodynamics

in many cases a more useful concept to understand processes in nature than if one has to rely on values calculated for standard state conditions solely. In addition, the graphs will aid in quickly getting an idea on how changes will influence the outcome of a reaction.

HOW TO PROCEED WHEN USING THERMODYN©

1. Define process of interest: e.g. Mixed acid glucose fermentation by *Ruminococcus flavefaciens*
1. Write process as a stoichiometrically balanced equation: 1 $C_6H_{12}O_6$ + 0,48 HCO_3^- → 1,07 CH_3COO^- + 0,62 $HCOO^-$ + 0,93 $^-OOC(CH_2)_2COO^-$ + 0,59 H_2 + 3,07 H^+ + 0,34 H_2O
   (The stoichiometric factors are reduced to 1 glucose. Please note that all numbers are written with commas; this is necessary if the preferences in defining the cells of your spread sheet are set the same way)
1. Define **boundary conditions**, variable and range of applicability:
   Choose the conditions for the beginning and the end of the reaction in the batch culture as follows: (all concentrations in mole/l)

Reactant	**beginning**	**end of experiment**
Glucose	0,020	0,0002
Bicarbonate	0,030	0,020
Acetate	10^{-7}	0,020
Formate	10^{-7}	0,060
Succinate	$0.5*10^{-6}$	0,020
pH *	6.9	6.3
Hydrogen (dissolved)	variable 10^{-2} to 10^{-10}	variable 10^{-2} to 10^{-10}
Temperature	25°C [298.15K]	25°C [298.15K]

* remember: $[H^+] = 10^{-pH}$

1. Enter reaction number, stoichiometric coefficient, formula, state, and activity into the corresponding spreadsheet columns (s stands for substrate, p for product): 1 is the reaction under beginning conditions, 2 under end conditions. Reactants may be entered as text (an in the 1st table) or as chemical formulas (an in the 2nd table)

Beginning of batch culture experiment

Reaction No.	(S,P)	Stoich. Coeff.	Enter formula	State	Special remarks	Activity	Variable
1	s	1	a-D-Glucose	aq		2,00E-02	
1	s	0,48	bicarbonate	aq		3,00E-02	
1	p	1,07	acetate	aq		1,00E-07	
1	p	0,62	formate	aq		2,00E-07	
1	p	0,93	succinate	aq		5,00E-07	
1	p	0,59	H2	aq			v
1	p	3,07	Proton	aq		1,26E-07	
1	p	0,34	water	l		1,00E+00	

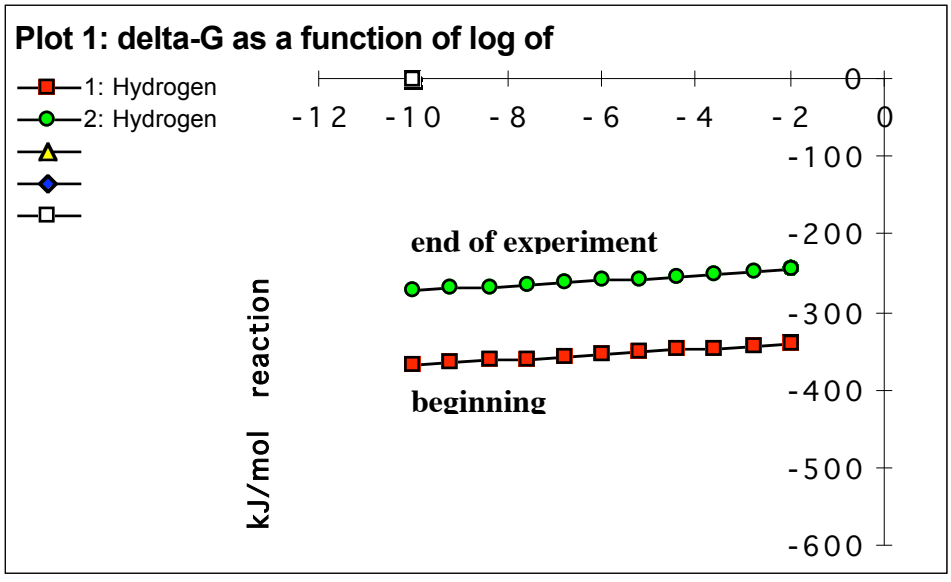
End of batch culture experiment

Reaction No.	(S,P)	Stoich. Coeff.	Enter formula	State	Special remarks	Activity	Variable
2	s	1	CH2OH(CHOH)4CHO	aq		2,00E-04	
2	s	0,48	HCO3-	aq		2,00E-02	
2	p	1,07	CH3COO-	aq		2,00E-02	
2	p	0,62	HCOO-	aq		6,00E-03	
2	p	0,93	(CH2)2(COO-)2	aq		2,00E-02	
2	p	0,59	H2	aq			v
2	p	3,07	H+	aq		5,01E-07	
2	p	0,34	H2O	l		1,00E+00	

5. Define temperature, boundary for variable and graphing options

Plot 1		
Temp. (K)	298,15	
Min. variable:	1,00E-10	
Max. variable:	1,00E-02	
Log plot?:	x	
Show react.1	x	
Show react.2	x	
Show react.3		
Show react.4		
Show react.5		

6. Run calculation and adjust scaling of graph coordinates if necessary.



red squares: conditions at the beginning of the batch culture experiment
green cicles: conditions at the end of the batch culture experiment

6. Interprete graphs and vary conditions.

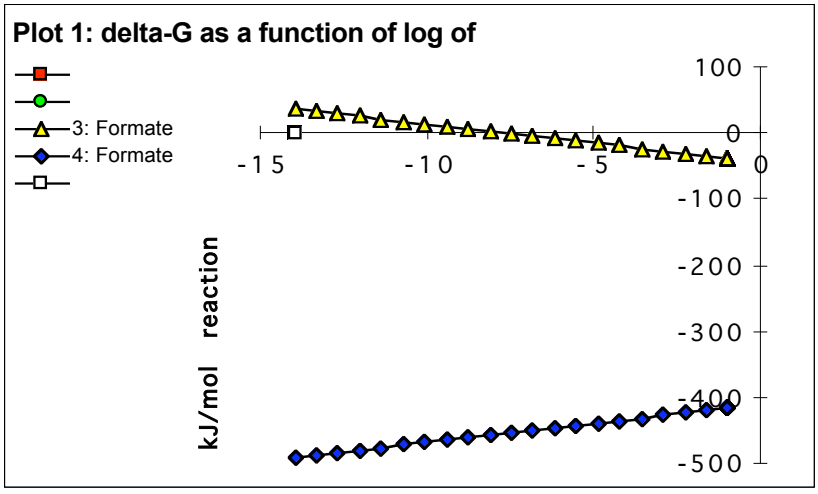
Problem 2: Proceed stepwise as outlined above to reconstruct the stoichiometric equation and the reaction conditions from the data given in the table below.

Comparison between glucose fermentation by *Ruminococcus flavefaciens* (reaction 4) and methane formation from formate by *Methanobrevibacter ruminantium* (reaction 3)

Reaction No.	(S,P)	Stoich. Coeff.	Enter formula[1]	State	Special remarks	Activity	Variable
4	s	1,61	CH2OH(CHOH)4CHO	aq		2,00E-04	
4	s	0,77	HCO3-	aq		2,00E-02	
4	p	1,73	CH3COO-	aq		2,00E-02	
4	p	1	HCOO-	aq			v
4	p	1,5	(CH2)2(COO-)2	aq		2,00E-02	
4	p	0,95	H2	aq		1,00E-07	
4	p	4,95	H+	aq		5,01E-07	
4	p	0,55	H2O	l		1,00E+00	
3	s	1	Formate	aq			v
3	s	0,25	Water	l		1,00E+00	
3	s	0,25	Proton	aq		5,01E-07	
3	p	0,25	Methane	aq		1,00E-04	
3	p	0,75	Bicarbonate	aq		2,00E-02	

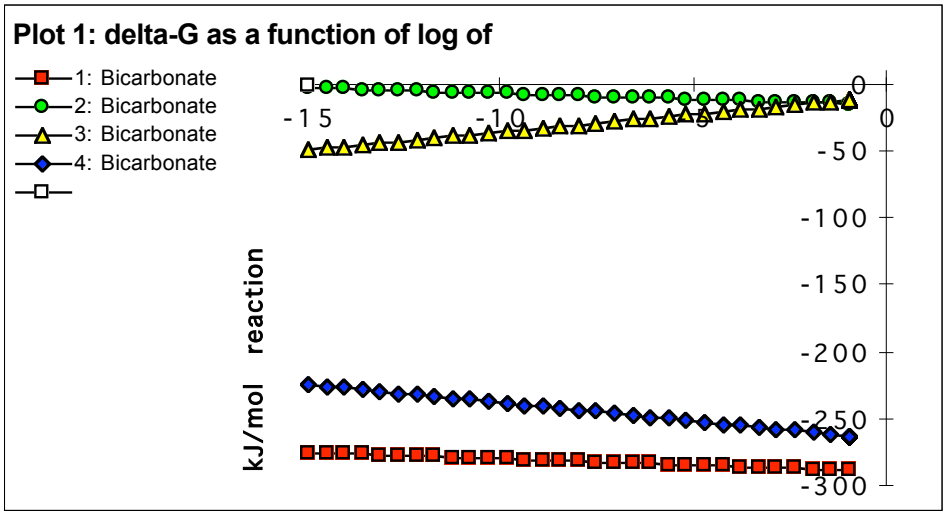
[1] reactants may be entered in words or as chemical formula

The result of the comparison is shown in the figure below as a function of the formate concentration.



Problem 3: Glucose fermentation by *R. flavefaciens* (reaction 4) and methane formation from formate (reaction 3) and from hydrogen (reaction 2) by *M. ruminantium* presented as a function of variable bicarbonate concentration, and the overall reaction (1) if formate and hydrogen are efficiently removed by the methanogen.

Reaction No.	(S,P)	Stoich. Coeff.	Enter formula	State	Special remarks	Activity	Variable	Compound	Formula
4	s	1	CH2OH(CHOH)4CHO	aq		2,00E-04		a-D-Glucose	CH2OH(CHOH)4CHO
4	s	0,48	HCO3-	aq			v	Bicarbonate	HCO3-
4	p	1,07	CH3COO-	aq		2,00E-02		Acetate	CH3COO-
4	p	0,62	HCOO-	aq		2,00E-04		Formate	HCOO-
4	p	0,93	(CH2)2(COO-)2	aq		2,00E-02		Succinate	(CH2)2(COO-)2
4	p	0,59	H2	aq		1,00E-05		Hydrogen	H2
4	p	3,07	H+	aq		5,01E-07		Proton	H+
4	p	0,34	H2O	l		1,00E+00		Water	H2O
3	s	0,62	HCOO-	aq		2,00E-04		Formate	HCOO-
3	s	0,155	H2O	l		1,00E+00		Water	H2O
3	s	0,155	H+	aq		5,01E-07		Proton	H+
3	p	0,155	CH4	aq		1,00E-04		Methane	CH4
3	p	0,465	HCO3-	aq			v	Bicarbonate	HCO3-
2	s	0,59	H2	aq		1,00E-05		Hydrogen	H2
2	s	0,1475	HCO3-	aq			v	Bicarbonate	HCO3-
2	s	0,1475	H+	aq		5,01E-07		Proton	H+
2	p	0,1475	CH4	aq		1,00E-04		Methane	CH4
2	p	0,4425	H2O	l		1,00E+00		Water	H2O
1	s	1	CH2OH(CHOH)4CHO	aq		2,00E-04		a-D-Glucose	CH2OH(CHOH)4CHO
1	s	0,1625	HCO3-	aq			v	Bicarbonate	HCO3-
1	p	1,07	CH3COO-	aq		2,00E-02		Acetate	CH3COO-
1	p	0,93	(CH2)2(COO-)2	aq		2,00E-02		Succinate	(CH2)2(COO-)2
1	p	0,3025	CH4	aq		1,00E-04		Methane	CH4
1	p	2,7675	H+	aq		5,01E-07		Proton	H+
1	p	0,6275	H2O	l		1,00E+00		Water	H2O



Further problems

4. *Ruminococcus flavefaciens* in axenic culture

a) In problem 1 which was presented above, we determined the free energy of the reaction varying the H_2 concentration. Define ΔGr for the same reaction at a constant H_2 concentration varying one of the other reactants.
b) How would a pH change in the rumen affect the energetic performance of *R.flavefaciens*?

5. *Ruminococcus flavefaciens* and *Methanobrevibacter ruminantium* in axenic co-culture

a) Which methanogenic reaction is thermodynamically more favourable for *Methanobrevibacter ruminantium* the hydrogenotrophic one or the formatotrophic one?
b) *In vitro*, the two organsims live in a syntrophic co-culture. Which are, thermodynamically speaking, the most successful conditions for the syntrophic interaction ?
c) What kind of insights into the complex rumen ecosystem can we derive from the theoretical analyses of individual processes ?