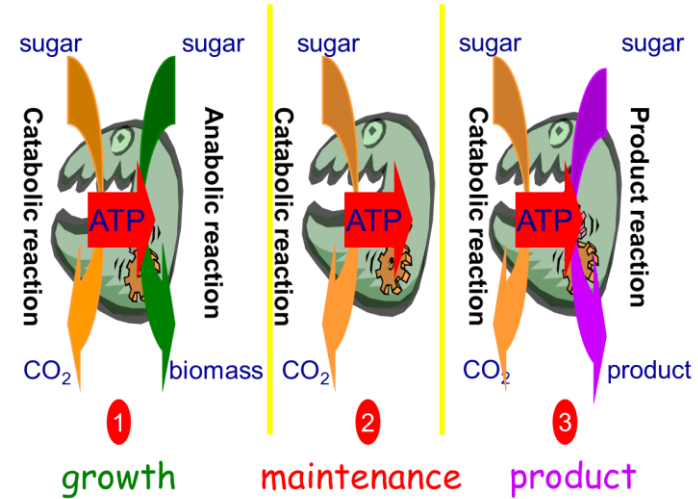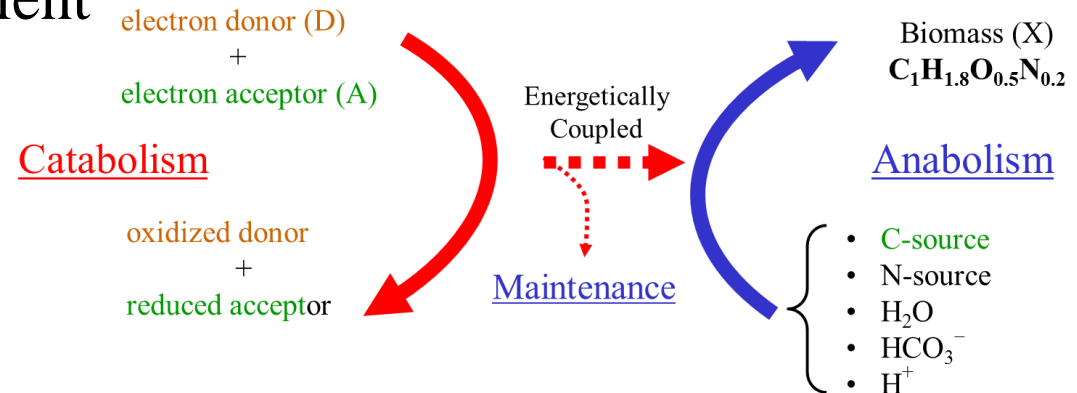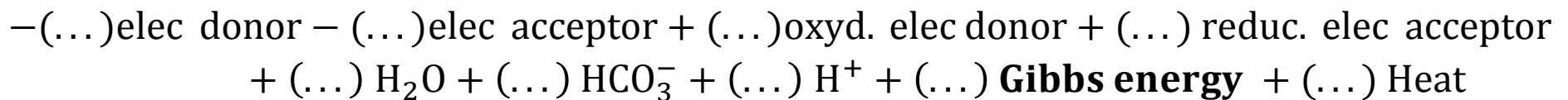# Thermodynamics of growth (To help us)

- Some thermodynamic fundamentals

- Thermodynamic analysis of anabolism and catabolism coupling

- Prediction of the **growth reaction stoichiometry**

- Prediction of maintenance coefficient $\mathbf{m_S}$

- Prediction of $\mathbf{\mu^{max}}$

- Prediction of growth Heat

Growth system definition

Catabolic reaction:

$$-(\ldots)\text{elec donor} - (\ldots)\text{elec acceptor} + (\ldots)\text{oxyd. elec donor} + (\ldots)\text{reduc. elec acceptor}$$
$$+ (\ldots)\, H_2O + (\ldots)\, HCO_3^- + (\ldots)\, H^+ + (\ldots)\, \textbf{Gibbs energy} + (\ldots)\, \text{Heat}$$

Thermodynamic fundamentals

From Energy of chemical bonds, the fraction available to do "work" is free energy:

ΔG: change in free Gibbs Energy = energy available to do "work"

$\Delta G = \Delta H - T\Delta S$

ΔH: change in Enthalpy

ΔS: change in Entropy

> Each compound represents Gibbs energy and Enthalpy

$\Delta G_f^o = \Delta G_f$ Gibbs energy of formation, under <u>standard</u> conditions [kJ.mole^{-1}]

$\Delta H_f^o = \Delta H_f$ Enthalpy of formation, under <u>standard</u> conditions [kJ.mole^{-1}]

(Available in Handbooks for many substances)

ΔG_f^o Standard conditions:

298 [K], 1 [bar] gaseous

or 1 [mole.L^{-1}] dissolved compound

$$\Delta G_f = \Delta G_f^o + RT \ln\left(\frac{C}{C^o}\right)$$

R = gas constant $8.314 10^{-3}$ [kJ.mole^{-1}.K^{-1}]

T = Absolute temperature in [K]

C = Concentration [mole.L^{-1}] (or for gas, partial pressure in [bar])

C^0 = reference Conc. [mole.L^{-1}] (or p^0=1 [bar])

Biochemical reference ΔG_f^{01} : pH = 7; 298 °K; 1 mole.L^{-1}; 1 atm

Standard Gibbs Energy and Enthalpy of formation ΔG_f^{01} at 298K, pH=7, 1bar, 1mole/L for relevant

Compound name	Composition	ΔG_f^{01} (kJ/mol)	ΔH_f (kJ/mol)
Biomass	$CH_{1.8}O_{0.5}N_{0.2}$	-67	-91
Water	H_2O	-237.18	-286
Bicarbonate	HCO_3^-	-586.85	-692
CO_2 (g)	CO_2	-394.359	-394.1
Ammonium	NH_4^+	-79.37	-133
Proton	H^+	-39.87	0
O_2 (g)	O_2	0	0
Oxalate^{2-}	$C_2O_4^{2-}$	-674.04	-824
Carbon monoxide	CO	-137.15	-111
Formate	CHO_2^-	-335	-410
Glyoxylate$^-$	$C_2O_3H^-$	-468.6	—
Tartrate^{2-}	$C_4H_4O_6^{2-}$	$-1,010$	—
Malonate^{2-}	$C_3H_2O_4^{2-}$	-700	—
Fumarate^{2-}	$C_4H_2O_4^{2-}$	-604.21	-777
Malate^{2-}	$C_4H_4O_5^{2-}$	-845.08	-843
Citrate^{3-}	$C_6H_5O_7^{3-}$	$-1,168.34$	$-1,515$
Pyruvate$^-$	$C_3H_3O_3^-$	-474.63	-596
Succinate^{2-}	$C_4H_4O_4^{2-}$	-690.23	-909
Gluconate$^-$	$C_6H_{11}O_7^-$	$-1,154$	—
Formaldehyde	CH_2O	-130.54	—
Acetate	$C_2H_3O_2^-$	-369.41	-486
Dihydroxyacetone	$C_3H_6O_3$	-445.18	—
Lactate	$C_3H_5O_3^-$	-517.18	-687
Glucose	$C_6H_{12}O_6$	-917.22	$-1,264$
Mannitol	$C_6H_{14}O_6$	-942.61	—
Glycerol	$C_3H_8O_3$	-488.52	-676
Propionate$^-$	$C_3H_5O_{2+}$	-361.08	—
Ethylene glycol	$C_2H_6O_2$	-330.50	—
Acetoine	$C_4H_8O_2$	-280	—
Butyrate	$C_4H_7O_2^-$	-352.63	-535

Compound name	Composition	ΔG_f^{01} (kJ/mol)	ΔH_f (kJ/mol)
Propanediol	$C_3H_8O_2$	-327	—
Butanediol	$C_4H_{10}O_2$	-322	—
Methanol	CH_4O	-175.39	-246
Ethanol	C_2H_5O	-181.75	-288
Propanol	C_3H_8O	-175.81	-331
n-Alkane	$C_{15}H_{32}$	$+60$	-439
Propane	C_3H_8	-24	-104
Ethane	C_2H_6	-32.89	-85
Methane	CH_4	-50.75	-75
H_2 (g)	H_2	0	0
N_2 (g)	N_2	0	0
Nitrite ion	NO_2^-	-37.2	-107
Nitrate ion	NO_3^-	-111.34	-173
Iron II	Fe^{2+}	-78.87	-87
Iron III	Fe^{3-}	-4.6	-4
Hydrogen sulfide (g)	H_2S	-33.56	-20
Sulfide ion	HS^-	$+12.05$	-17
Sulfate ion	SO_4^{2-}	-744.63	-909
Thiosulfate ion	$S_2O_3^{2-}$	-513.2	-608

Note: pH = 7, 1 atm, 1 mol/L, 298 K.

Gibbs Energy and Heat of reactions

For a given REACTION: $\quad \alpha A + \beta B \rightarrow \gamma C + \delta D_{(gas)}$

$\quad\quad\quad\quad$ or $\quad\quad - \alpha A - \beta B + \gamma C + \delta D_{(gas)}$

Gibbs Free Energy of a reaction:

$$\Delta G_R^0 = \delta . \Delta G_{fD}^0 + \gamma . \Delta G_{fC}^0 - \alpha . \Delta G_{fA}^0 - \beta . \Delta G_{fB}^0$$

2nd Law: $\Delta G_R < 0$

$\Delta G_R^0 =$ Standard Gibbs Free Energy of a reaction when all reactants are at 1 mole/L, 1atm

$\Delta G_R^{01} =$ Standard Gibbs Free Energy when all reactants are at 1 mole/L, 1atm and pH 7.

$$\Delta G_R = \Delta G_R^0 + RT \ln \frac{\left(\dfrac{C}{1M}\right)^\gamma . \left(\dfrac{D}{1bar}\right)^\delta}{\left(\dfrac{A}{1M}\right)^\alpha . \left(\dfrac{B}{1M}\right)^\beta}$$

Heat of the reaction:

$\Delta H_R > 0$ or $\Delta H_R < 0$

$$\Delta H_R^0 = \delta \Delta H_{fD}^0 + \gamma \Delta H_{fC}^0 - \alpha \Delta H_{fA}^0 - \beta \Delta H_{fB}^0$$

Diversity of microbial growth system

Depending on growth biosystem, a huge variation is observed :
- Biomass yield on substrate

    Y_{SX}: 0.01 - 0.7 [C-moleX.moleS^{-1}]

- Maximum growth rate μ^{max} varies from : 0.001 to 1.5 [h^{-1}]
- Maintenance coefficients

    m_S = 0.01 - 1 [moleS.C-moleX^{-1}·h^{-1}]

This microbial bioprocess behavior diversity comes from huge environmental conditions, depending on:

C-source	e-donor	e-acceptor
Organic	Organic	Organic
Inorganic	Inorganic	Inorganic

Energy !

Why such variation? . . . .

Prof. J.J. Heijnen

Thermodynamics of growth
"The Energetic Anabolism/Catabolism coupling"

electron donor (D)
+
electron acceptor (A)

Biomass (X)
$C_1H_{1.8}O_{0.5}N_{0.2}$

Catabolism

Energetically
Coupled

Anabolism

oxidized donor
+
reduced acceptor

Maintenance

- C-source
- N-source
- H_2O
- HCO_3^-
- H^+
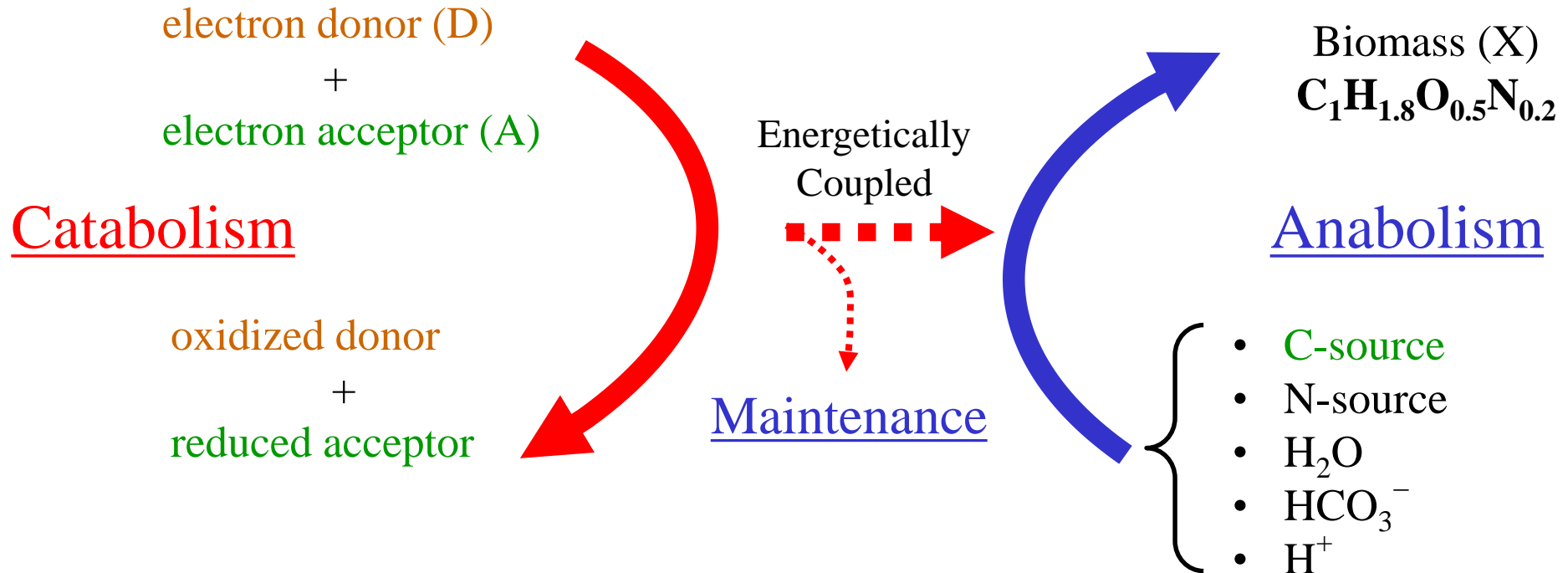
3 arrows = 3 Gibbs Energy flux due to Anabolism/Catabolism coupling:
1   Gibbs energy needed in the **anabolic reaction**
2   Gibbs energy needed for **maintenance**
3   Gibbs energy produced by **catabolic reaction**

Gibbs energy required for anabolic reaction
to produce 1 C-mole of biomass

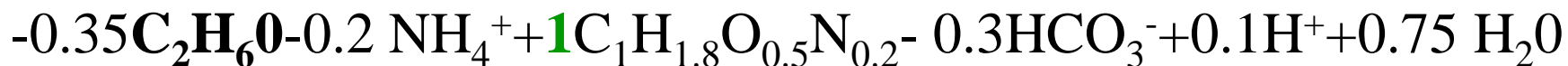All coefficients follow from conservation C , H, O, N and charge balances

(Electron acceptor is absent!)

$$\Delta G_R^0 = \delta . \Delta G_{fD}^0 + \gamma . \Delta G_{fC}^0 - \alpha . \Delta G_{fA}^0 - \beta . \Delta G_{fB}^0$$

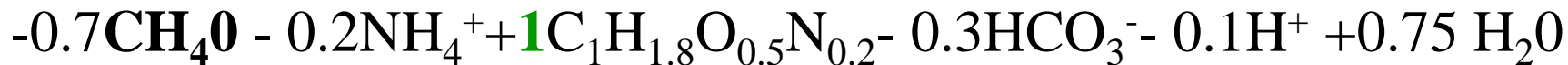Case of Organic C-source (using ΔG_f^{01} table)

$-0.175 C_6 H_{12} O_6 - 0.2 NH_4^+ + 1 C_1 H_{1.8} O_{0.5} N_{0.2} + 0.05\ HCO_3^- + 0.25\ H^+ + 0.4\ H20$

$$\Delta G_R^{01} = -24.8\ [kJ.C\_moleX^{-1}]$$

$-0.35 C_2 H_6 0 - 0.2\ NH_4^+ + 1 C_1 H_{1.8} O_{0.5} N_{0.2} - 0.3 HCO_3^- + 0.1 H^+ + 0.75\ H_2 0$

$$\Delta G_R^{01} = +14.63\ [kJ.C\_moleX^{-1}]$$

$-0.7 CH_4 0 - 0.2 NH_4^+ + 1 C_1 H_{1.8} O_{0.5} N_{0.2} - 0.3 HCO_3^- - 0.1\ H^+ + 0.75\ H_2 0$
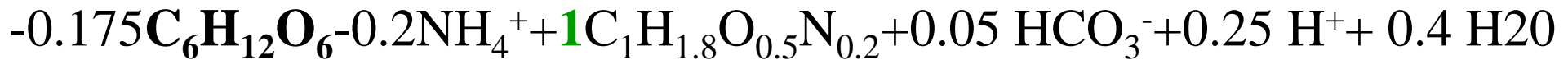
$$\Delta G_R^{01} = -9.2\ [kJ.C\_moleX^{-1}]$$

In case of Inorganic C-source

$-1\ CO_2 - 0.2\ NH_4^+ + 1 C_1 H_{1.8} O_{0.5} N_{0.2} - 4 H^+ - 4.2 Fe^{2+} + 4.2 Fe^{3+} + 1.5 H20$
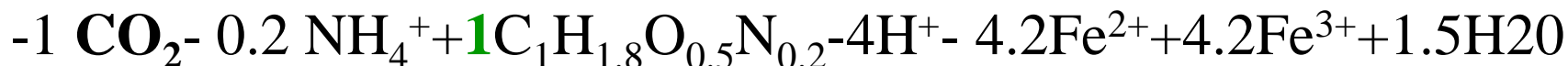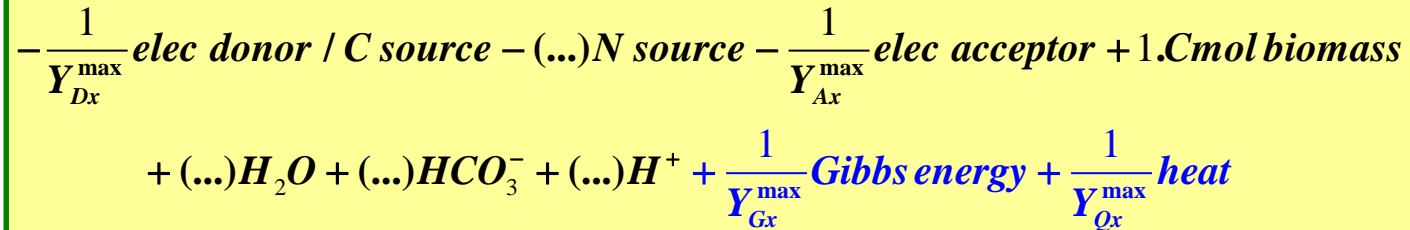
$$\underline{\Delta G_R^{01} = +341.56}\ [kJ.C\_moleX^{-1}]$$

Note: Electron from Fe^{2+}/Fe^{3+} donor has not enough energy to reduce CO_2 to biomass X. The reversed electron transfer (RET) mechanism is needed.

Gibbs energy required for GROWTH reaction
to produce 1 C-mole of biomass

Free energy required **anabolic** reaction:
- Organic C-source $\quad\quad\quad\quad\quad$ $\Delta G \approx 0$
- CO_2 (inorganic C-source) $\quad\quad$ $\Delta G \gg 0$

Overall Growth reaction:

$$-\frac{1}{Y_{Dx}^{max}}\,elec\ donor\ /\ C\ source - (...)N\ source - \frac{1}{Y_{Ax}^{max}}\,elec\ acceptor + 1.Cmol\ biomass$$

$$+ (...)H_2O + (...)HCO_3^- + (...)H^+ + \frac{1}{Y_{Gx}^{max}}\,Gibbs\ energy + \frac{1}{Y_{Qx}^{max}}\,heat$$

Is highly irreversible, because of the large Gibbs energy required, Y_{GX}^{max}:

$$1/Y_{Gx}^{max} = \textbf{200- 4000 kJ}\ \text{for production of 1 C-mole X}$$

Y_{GX}^{max} ? Issued from a data set for growth of different micro-organisms

The data set covers a wide range of **MEDIUM** :

- Mineral media, **C-source limited**, unknown product formation excluded …
- Wide variety of carbon sources:
  - **1 to 6 C atoms**
  - 0 – 8, γ_c **degree of reduction** [e_mole/C_mole])
- Different **electron acceptors**
- Different electron donors with and without reversed electron transport RET requirement

MICROORGANISMS : Many different microorganisms

METABOLISM :

- **Heterotrophic** and **autotrophic** growth
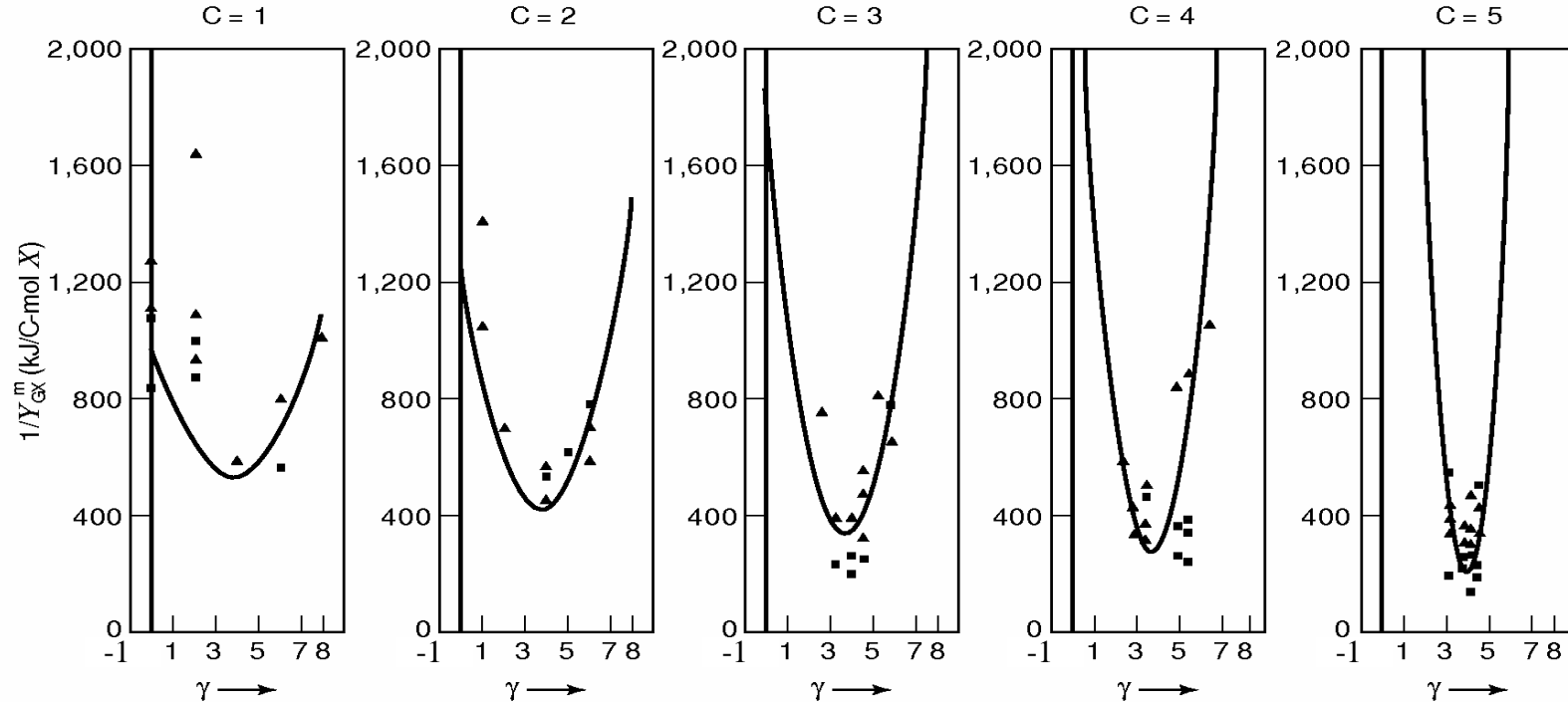- **Aerobic** / **denitrification** / **fermentation**

Gibbs energy needed for <u>heterotrophic</u> growth
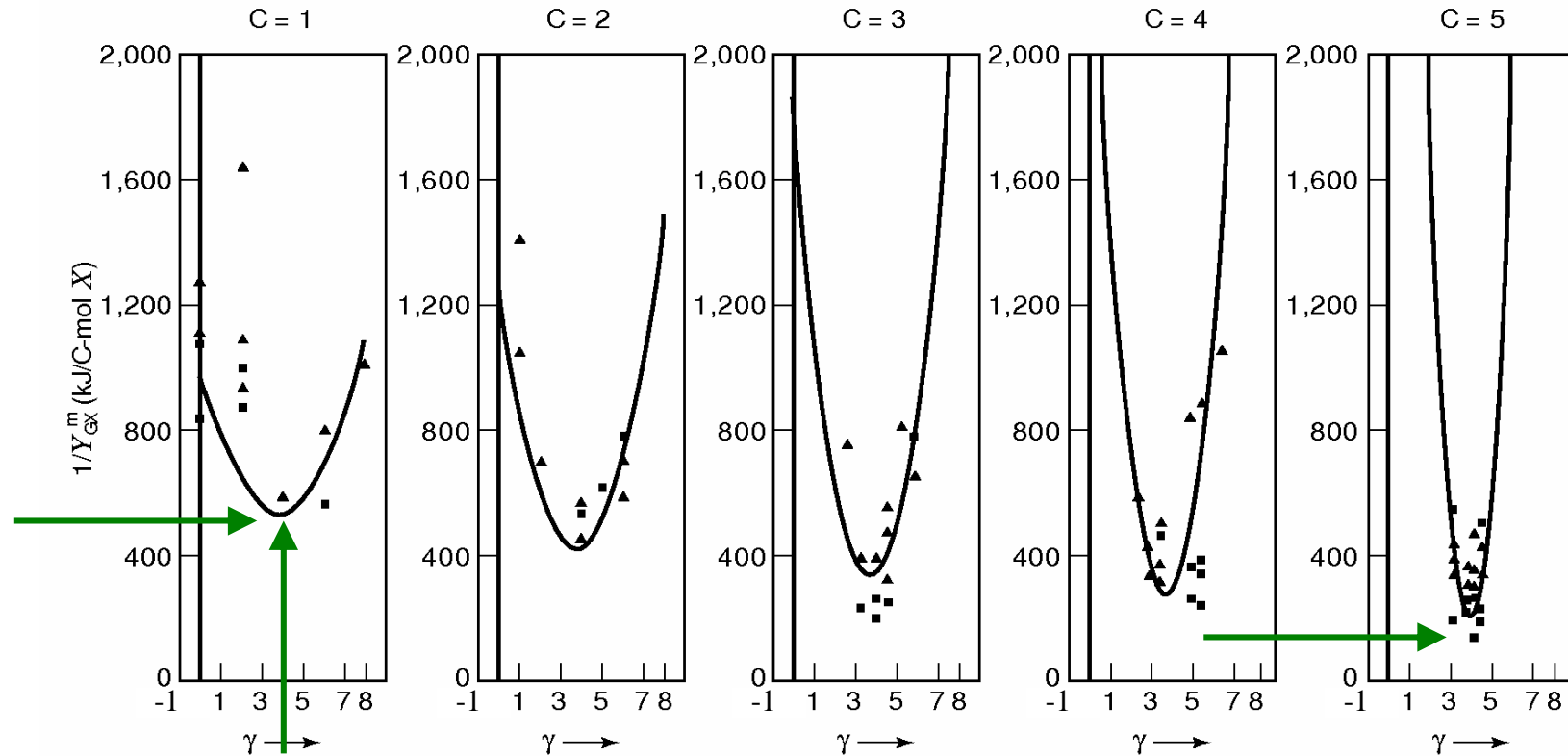
In **heterotrophic growth**, $1/Y_{Gx}^{max}$ depends only on C-source (organic):

- its **degree of reduction** (γ_c) [e mole/C_mole]   (energy needs increase with γ deviating from 4) ?
- its number of **C-atoms (C)**   (energy needs increase with smaller C content) ?

Gibbs energy needed for <u>heterotrophic</u> growth

In **heterotrophic growth, $1/Y_{gx}^{max}$** - its **degree of reduction** (γ_c) [e mole/C_mole]
[kJ.C_moleX^{-1}] depends on : - its number of **C-atoms (C)**

$$\frac{1}{Y_{GX}^{max}} = 200 + 18 * (6 - C)^{1.8} + exp\left\{\left[(3.8 - \gamma_c)^2\right]^{0.16}(3.6 + 0.4 * C)\right\}$$
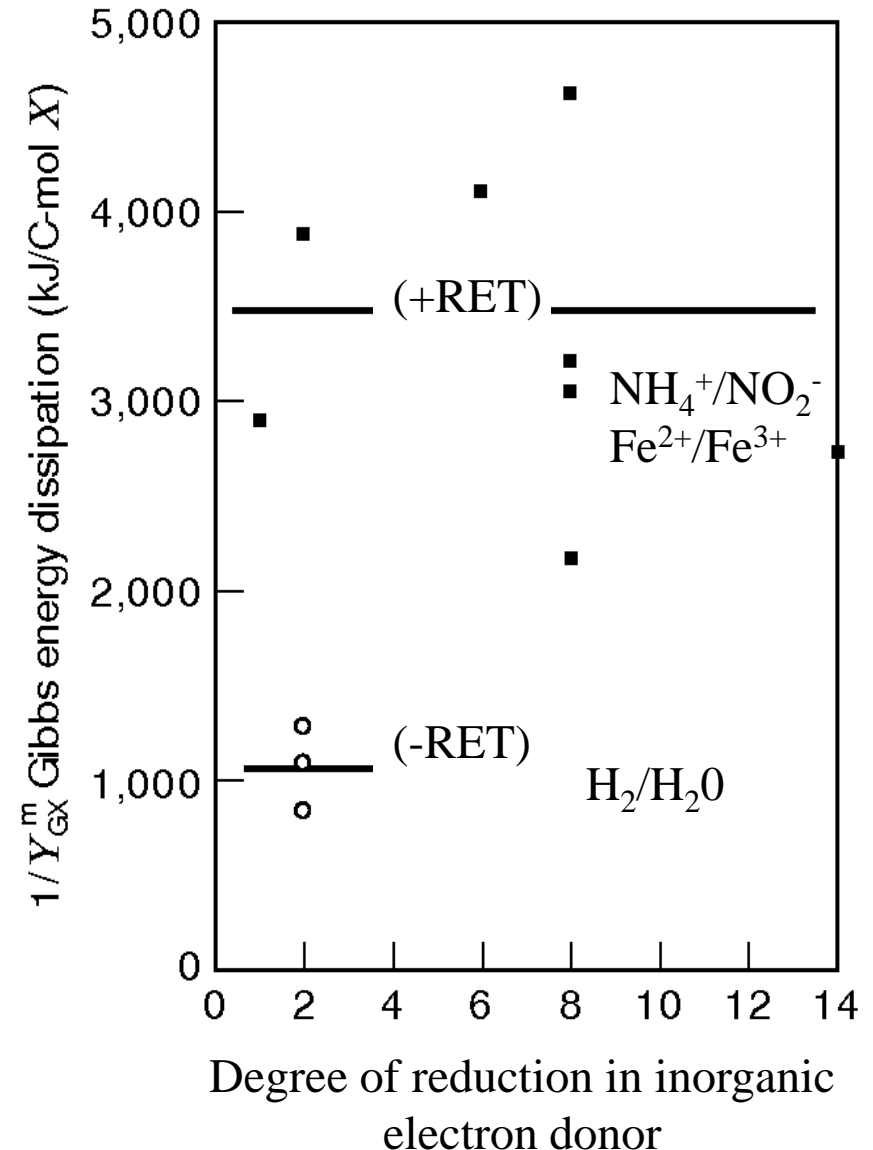
Gibbs energy needed for <u>autotrophic</u> growth

In autotrophic growth, with CO_2 as C-source, $1/Y_{Gx}^{max}$:

- **Depends on the nature of electron donor** (if it requires or not RET (Reverse Electron Transfer) mechanism
- **Does not depend on electron acceptor**

Explanation: more biochemical work has to be done with CO_2 as C-source, specially if RET is required:

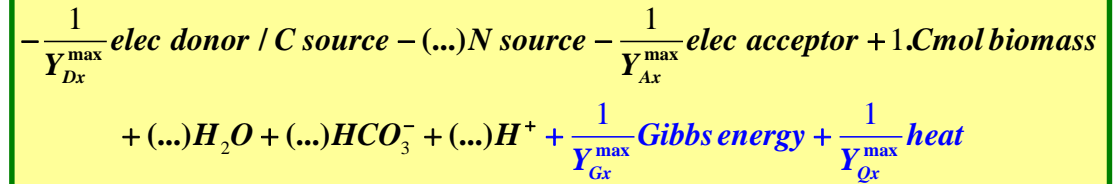$$\frac{1}{Y_{GX}^{max}} = 1000 \quad (\textit{Without RET})$$
$$= 3500 \quad (\textit{With RET})$$

Y-axis: $1/Y_{GX}^m$ Gibbs energy dissipation (kJ/C-mol X)

(+RET)

NH_4^+/NO_2^-
Fe^{2+}/Fe^{3+}

(-RET)

H_2/H_2O

Degree of reduction in inorganic electron donor

Gibbs energy needed for Growth of 1 C-mole biomass, $1/Y_{GX}^{max}$ [kJ.C-moleX^{-1}]

Energy required for Overal Growth reaction,

2 cases:

$$-\frac{1}{Y_{Dx}^{max}} elec\ donor\ /\ C\ source - (...)N\ source - \frac{1}{Y_{Ax}^{max}} elec\ acceptor + 1.Cmol\ biomass$$

$$+ (...)H_2O + (...)HCO_3^- + (...)H^+ + \frac{1}{Y_{Gx}^{max}} Gibbs\ energy + \frac{1}{Y_{Qx}^{max}} heat$$

Heterotrophic Growth on organic C-Source: → Correlation for $1/Y_{GX}^{max}$ [kJ.C-moleX^{-1}]
Which depends only on:

$$\frac{1}{Y_{GX}^{max}} = 200 + 18 * (6 - C)^{1.8} + exp\left\{\left[(3.8 - \gamma_c)^2\right]^{0.16} (3.6 + 0.4 * C)\right\}$$

- its degree of reduction (γ_c)
- its number of C-atoms (C)

Autotrophic growth on inorganic C-Source (CO_2) which depends on:

- Nature of electron donor (and the need of RET)
- Does not depend on electron acceptor

$$\frac{1}{Y_{GX}^{max}} = 1000\ (Without\ RET)$$
$$= 3500\ (With\ RET)$$

Gibbs energy needed for Maintenance of 1 C-mole biomass, m_G [kJ.C-moleX^{-1}.hr^{-1}]
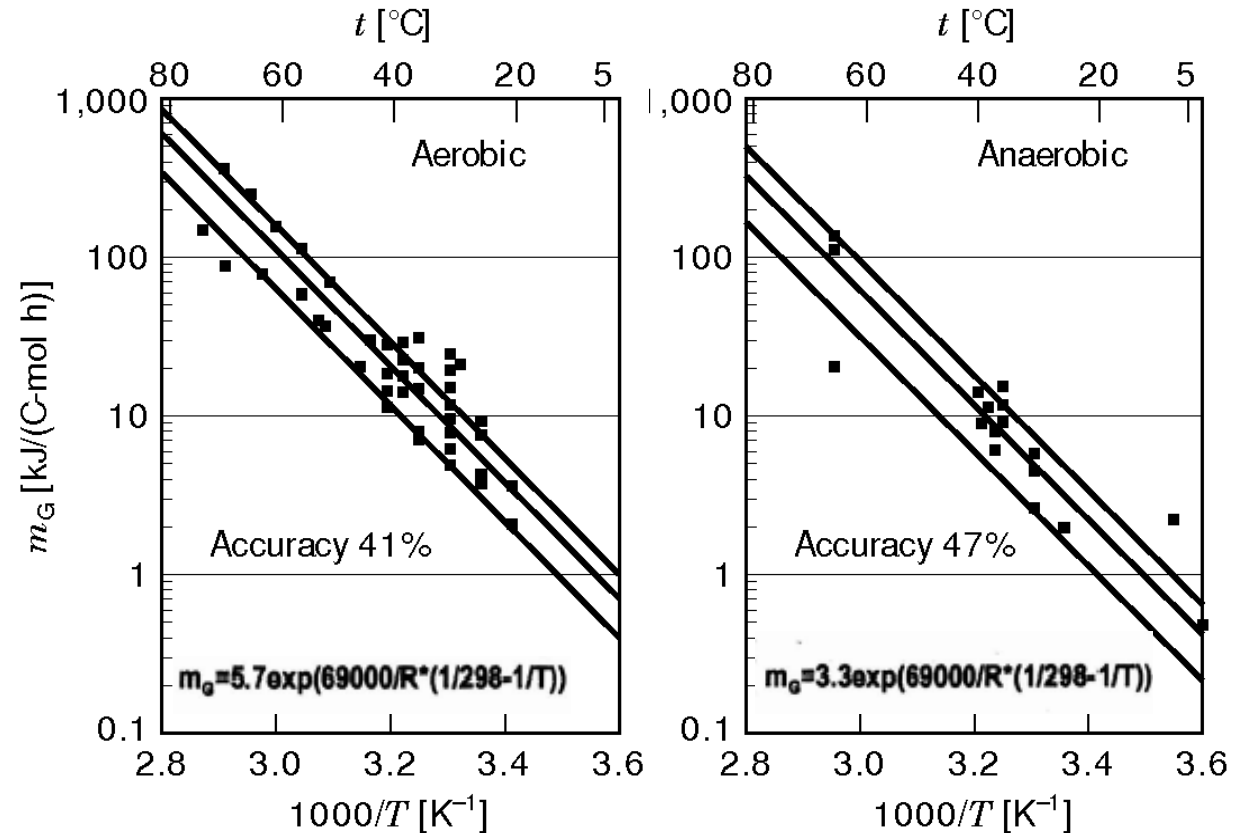
In micro-organisms, degradation processes occur with low rates. It concerns:

- Protein decay into amino acid
- Leaks of ions over cell membranes e.g. Na$^+$ influx
- And many other processes
- → Repair requires Gibbs energy from catabolic reaction for maintenance:

$$m_G = \frac{kJ\,Gibbs\,energy}{C\_moleX\,.hr}$$

→ m_G correlation

$$m_G = 4.5\,exp^{\left(\frac{-69000}{R}\left(\frac{1}{T}-\frac{1}{298}\right)\right)}$$

Aerobic

Accuracy 41%

$m_G = 5.7\,exp(69000/R*(1/298-1/T))$

Anaerobic

Accuracy 47%

$m_G = 3.3\,exp(69000/R*(1/298-1/T))$

R = 8.314 J/mole K, T = absolute T

- holds for many organisms
- holds for aerobic/anaerobic/denitrification growth
- **independently of C-source**
- **only Temperature-dependent**
- each 10 ^{o}C increase in temperature, m_G **doubles**

Summary Gibbs energy needed for growth and maintenance of 1 C-mole biomass, $1/Y_{GX}^{max}$, m_G

For $1/Y_{GX}^{max}$ [kJ.C-moleX^{-1}], 2 cases:

1. On organic C-source (**heterotrophic growth**), $\rightarrow 1/Y_{GX}^{max}$ correlation

$$\frac{1}{Y_{GX}^{max}} = 200 + 18 * (6 - C)^{1.8} + exp\left\{\left[(3.8 - \gamma_c)^2\right]^{0.16}(3.6 + 0.4 * C)\right\}$$

2. For **autotrophic growth** on CO_2 as C-Source

$$\frac{1}{Y_{GX}^{max}} = 1000 \quad (\textit{Without RET})$$
$$= 3500 \quad (\textit{With RET})$$

For m_G [kJ.C-moleX^{-1}.hr^{-1}] $\rightarrow$ m_G correlation:

$$m_G = 4.5\, exp^{\left(\frac{-69000}{R}\left(\frac{1}{T} - \frac{1}{298}\right)\right)}$$

Required Gibbs energy comes from catabolic reaction: ΔG_{Cat} [kJ.C-moleS^{-1}] :
Thus:

$$m_S = \frac{m_G}{-\Delta G_{cat}}$$

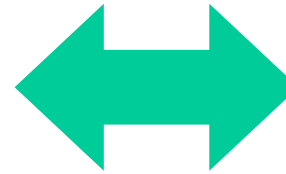$\rightarrow$ These are the 2 Gibbs Energy correlations!
$\rightarrow$ **Prediction of growth stoichiometry**

Prediction of growth stoichiometry

The growth stoichiometry prediction is based on :

1. **Energetic coupling** Anabolism - Maintenance / Catabolism

| Gibbs energy needed for Maintenance and Growth	⟷	Gibbs energy provided by catabolism

2. **Gibbs energy balance**

To calculate stoichiometric yields of the global growth equation, a new 6th balance is now available:

- 5 conservative C, H, O, N, Charges balances

- + **Gibbs energy balance**… using $1/Y_{GX}^{max}$ **correlation**
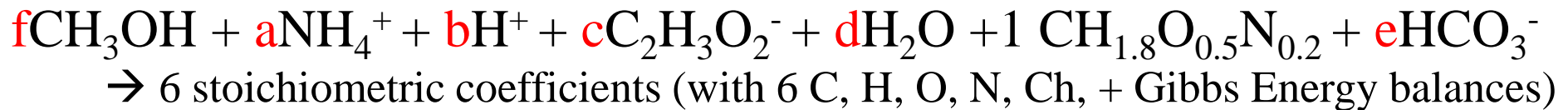 Required information: N-source, C-source, e-donor, e-acceptor

Ex: Assuming microorganism grows
anaerobically on methanol as C-source
and electron donor, with NH_4^+
as the N-source with Acetate production.

$$f CH_3OH + a NH_4^+ + b H^+ + c C_2H_3O_2^-$$
$$+ d H_2O + 1 CH_{1.8}O_{0.5}N_{0.2} + e HCO_3^- + 1/Y_{GX}^{max}$$
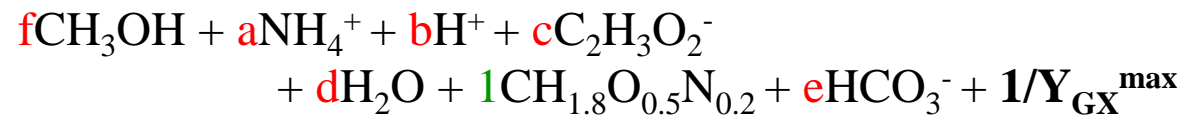
→ 6 balances for 6 stoichiometric coefficients !

Calculation of overall growth reaction stoichiometry: Assuming microorganism grows anaerobically on methanol as C-source and electron donor, with NH_4^+ as the N-source with acetate production.

$$fCH_3OH + aNH_4^+ + bH^+ + cC_2H_3O_2^- + dH_2O + 1\,CH_{1.8}O_{0.5}N_{0.2} + eHCO_3^-$$

→ 6 stoichiometric coefficients (with 6 C, H, O, N, Ch, + Gibbs Energy balances)

Information:	ΔG_f^{01} [kJ.mole^{-1}]	
N-source NH_4^+	CH_3OH	-175.39
C-source methanol (CH_4O, $\gamma = 6$, C=1)	NH_4^+	-79.37
e-donor CH_4O/ HCO_3^-	$C_2H_3O_2$	-369.41
e-acceptor HCO_3^-/ $C_2H_3O_2^-$ (acetate)	H_2O	-237.18
T = 25°C = 298K	$CH_{1.8}O_{0.5}N_{0.2}$	-67
	HCO_3^-	-586.85

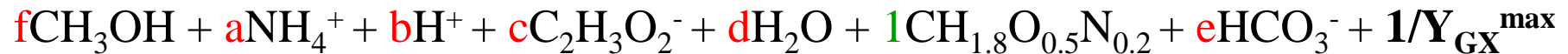# Growth stoichiometry and q_i, Y_{ij} prediction (2)

For solving stoichiometry of microbial growth:

Step 1. Calculate Gibbs energy required for growth of one C-mole of biomass using $1/Y_{GX}^{max}$ correlation with C=1, γ_C =6.

$$\rightarrow 1/Y_{GX}^{max} = 200 + 326 + 172$$
$$= \mathbf{698} \text{ [kJ.C-moleX}^{-1}]$$

$$\frac{1}{Y_{GX}^{max}} = 200 + 18 * (6 - C)^{1.8} + exp\left\{\left[(3.8 - \gamma_c)^2\right]^{0.16}(3.6 + 0.4 * C)\right\}$$

Step 2. Calculate the stoichiometric coefficients using the 5 conservation balances (C, H, O, N, charge) + <u>Gibbs energy balance</u>
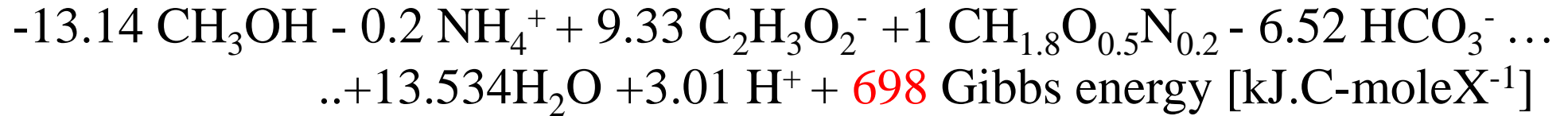
$$fCH_3OH + aNH_4^+ + bH^+ + cC_2H_3O_2^- + dH_2O + 1CH_{1.8}O_{0.5}N_{0.2} + eHCO_3^- + \mathbf{1/Y_{GX}^{max}}$$

C balance: $f+2c+1+e = 0$ O balance: $f+2c+d+0.5+3e = 0$ N balance: $a+0.2 = 0$
Charge balance: $a+ b- c- e = 0$ H balance: $4f+4a+b+3c+2d+1.8+e = 0$

Gibbs energy $(-175.39)f + (-79.37)a + (-39.87)b + (-369.41)c$
Balance: $+(-237.18)d + (-67)1 + (-586.85)e + \mathbf{698} = 0$

Growth stoichiometry and q_i, Y_{ij} prediction (2)

$$-13.14\ CH_3OH - 0.2\ NH_4^+ + 9.33\ C_2H_3O_2^- + 1\ CH_{1.8}O_{0.5}N_{0.2} - 6.52\ HCO_3^- \dots$$

$$\dots +13.534 H_2O + 3.01\ H^+ + \textcolor{red}{698}\ \text{Gibbs energy [kJ.C-moleX}^{-1}\text{]}$$

GLOBAL GROWTH REACTION

Electron donner couple:	CH3OH/HCO3-	C_Source:	CH3OH
Electron acceptor couple:	HCO3-CH3COO-	N_Source:	NH4+

Primary form:

$$a.CH_3OH + b.NH_4^+ + c.CH_3COO^- + 1.C_1H_{1.8}O_{0.5}N_{0.2} + d.HCO_3^+ + e.H_2O + f.H^+ + \frac{1}{Y_{GX}^{max}} = (0)$$

$$-\left(1.C_1H_{1.8}O_{0.5}N_{0.2} + \frac{1}{Y_{GX}^{max}}\right) = a.CH_3OH + b.NH_4^+ + c.CH_3COO^- + d.HCO_3^+ + e.H_2O + f.H^+$$

	Biomass Production	Gibbs Energy requirement		CH3OH	NH4+	CH3COO-	HCO3-	H20	H+	
	C1H1.8O(	1/YGXmax								
Stoichiom. Coef.:	1	1	-(Known)	-13.1395	-0.2	9.329601	-6.51973	13.53947	3.00987	Check
C Bal.:	1	0	-1	1	0	2	1	0	0	-1
H Bal.:	1.8	0	-1.8	4	4	3	1	2	1	-1.8
O Bal. :	0.5	0	-0.5	1	0	2	3	1	0	-0.5
N Bal :	0.2	0	-0.2	0	1	0	0	0	0	-0.2
Ch. Bal. :	0	0	0	0	1	-1	-1	0	1	1.33E-15
ΔGf01[kJ/mol]	-67.00	698.23	-631.231	-175.39	-79.37	-369.41	-586.85	-237.18	-39.87	-631.231

	C1H1.8O(	CH3OH	NH4+	CH3COO-	HCO3-	H20	H+	
Gamma:	4.2	6	0	8	0	0	0	
Stoichiom. Coef. :	1	-13.1395	-0.2	9.329601	-6.51973	13.53947	3.009867	Check
Gamma Bal.:	4.2	-78.8368	0	74.63681	0	0	0	-1.4E-14

$$-13.14.(6) - 0.2.NH_4^+ + 9.33.(8) + 1.(4.2) - 6.52.HCO_3^+ + 13.54.H_2O + 3.01.H^+ = (0)$$

Ultimate form:

$$-13.14.CH_3OH - 0.2.NH_4^+ + 9.33.CH_3COO^- + 1.C_1H_{1.8}O_{0.5}N_{0.2} - 6.52.HCO_3^+ + 13.54.H_2O + 3.01.H^+ + \frac{1}{Y_{GX}^{max}} = (0)$$

Step 3. From m_G correlation

At T=298°K, m_G= **4.5** [kJ.C-moleX^{-1}.h^{-1}]

$$m_G = 4.5\, \text{exp}^{\left(\frac{-69000}{R}\left(\frac{1}{T}-\frac{1}{298}\right)\right)}$$

Step 4. Calculate ΔG_{cat}^{01} of catabolic reaction

Catabolic reaction:

-1 CH_4O - 0.5 HCO_3^- + 0.75 $C_2H_3O2^-$ + 0.25 H^+ + 1 H_2O

ΔG_{cat}^{01} = -1*(-175.39)-0.5*(-586.85)+…+1*(-237.18)

= **- 55.54** [kJ.moleS^{-1}]

ΔG_f^{01} [kJ.mole^{-1}]	
CH_3OH	-175.39
NH_4^+	-79.37
$C_2H_3O_2$	-369.41
H_2O	-237.18
$CH_{1.8}O_{0.5}N_{0.2}$	-67
HCO_3^-	-586.85

Step 5. Calculate maintenance rates, m_S and other m_i

m_s = 4.5 / 55.54 = 0.081 [moleCH_4O.C-moleX^{-1}.h^{-1}]

m_{CO2}= 0.081*0.5 = 0.0405 [moleHCO_3^-.C-moleX^{-1}.h^{-1}]

m_{Ace} = 0.081*0.75 = 0.0608 [moleAce.C-moleX^{-1}.h^{-1}]

m_w = 0.081*1 = 0.081 [moleH_2O.C-moleX^{-1}.h^{-1}]

m_{H+} = 0.081*0.25 = 0.0202[moleH^+C-moleX^{-1}.h^{-1}]

$$m_S = \frac{m_G}{-\Delta G_{cat}}$$

Step 6. Set-up linear expressions of specific rates with Herbert-Pirt Eq. (without non-catabolic product)

Global growth reaction (**at μ rate**):

$$-q_S = \frac{1}{Y_{SX}^{max}} \mu + m_S$$

-13.14 CH_3OH - 0.2NH_4^+ + 9.33 $C_2H_3O_2^-$ +1 $CH_{1.8}O_{0.5}N_{0.2}$ - 6.52 HCO_3^- …

+13.53 4H_2O + 3.01 H^+ + 698 Gibbs energy [kJ.C-moleX^{-1}]
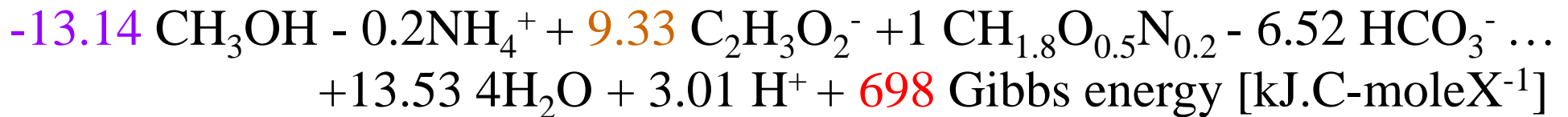
Catabolic reaction (**at rate m_S**)

-1 CH_4O - 0.5 HCO_3^- + 0.75 $C_2H_3O2^-$ + 0.25 H^+ + 1 H_2O

Thus:

If m_s = 4.5 / 55.54 = 0.081 [moleCH$_4$O.C-moleX^{-1}.h^{-1}]

-q$_{meOH}$ = 13.14 μ + 0.081 [moleCH$_4$O.C-moleX^{-1}.h^{-1}]

If m_{Ace} = 0.081*0.75 = 0.0608 [moleAce.C-moleX^{-1}.h^{-1}]

q$_{Acetate}$ = 9.33 μ + 0.0608 [moleAcetate.C-moleX^{-1}.h^{-1}]

q_i = 1/Y_{iX}^{max} μ + m_i [moleC$_i$.C-moleX^{-1}.h^{-1}]

Step 6. Linear expressions of specific rates q_i with Herbert-Pirt Eq.
(without non-catabolic product) [moleC$_i$.C-moleX^{-1}.h^{-1}]

$q_i \quad = \quad 1/Y_{iX}^{max} \mu + m_i$

$-q_{meOH} = 13.14 \mu + 0.081$

$q_{Acetate} = 9.33 \mu + 0.0608$

$q_{H2O} = 13.53 \mu + 0.081$

$-q_{NH4+} = 0.2 \mu$

$-q_{HCO3-} = 6.52 \mu + 0.0405$
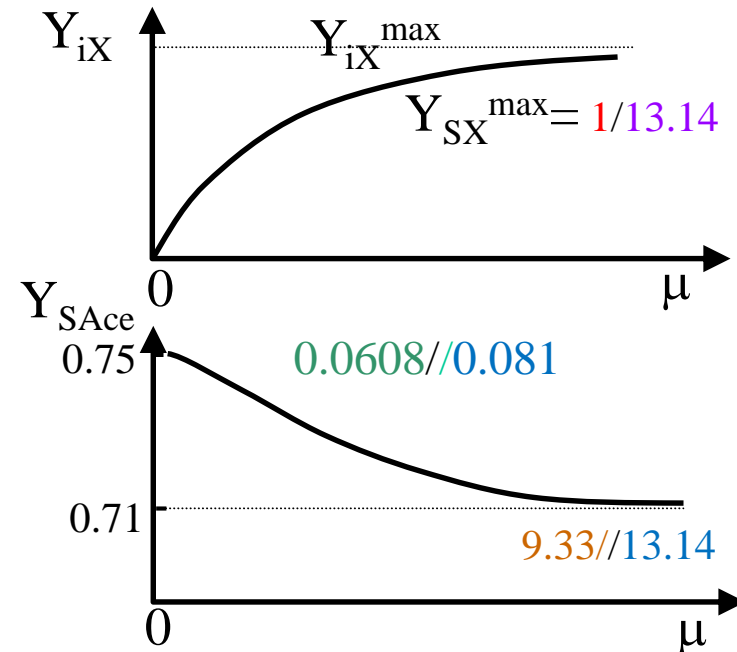
$q_{H+} = 3.01 \mu + 0.0202$

Step 7. Calculate **yields Y_{ij}**

Biomass yield on methanol

$$Y_{SX}(\mu) = \left| \frac{\mu}{q_s} \right| = \frac{\mu}{13.14\mu + 0.081}$$

Acetate yield on methanol

$$Y_{Sace}(\mu) = \left| \frac{q_{ace}}{q_s} \right| = \frac{9.33\mu + 0.061}{13.14\mu + 0.081}$$

Y_{iX} Y_{iX}^{max}

$Y_{SX}^{max} = 1/13.14$

Y_{SAce}

0.75   0.0608//0.081

0.71   9.33//13.14

➔ **All q_i and Y_{ij} are given and depend on <u>only ONE unknown</u>: μ rate!**

Predictive quality of thermodynamic method

The two **Gibbs energy correlations**
$1/Y_{GX}^{max}$, m_G

$$m_G = 4.5 \exp\left(\frac{-69000}{R}\left(\frac{1}{T} - \frac{1}{298}\right)\right)$$

$$\frac{1}{Y_{GX}^{max}} = 200 + 18 * (6 - C)^{1.8} + \exp\left\{\left[(3.8 - \gamma_c)^2\right]^{0.16} (3.6 + 0.4 * C)\right\}$$

Allows to estimate growth yields Y_{ij} and q_i for arbitrary growth system, knowing:

C_source, N_source,

e-donor, e-acceptor,

T and **growth rate** μ

Average relative error of 13% shows pretty good predictive quality!

Strong deviation were only found for particular microbial metabolism (rare or special catabolism or anabolism)
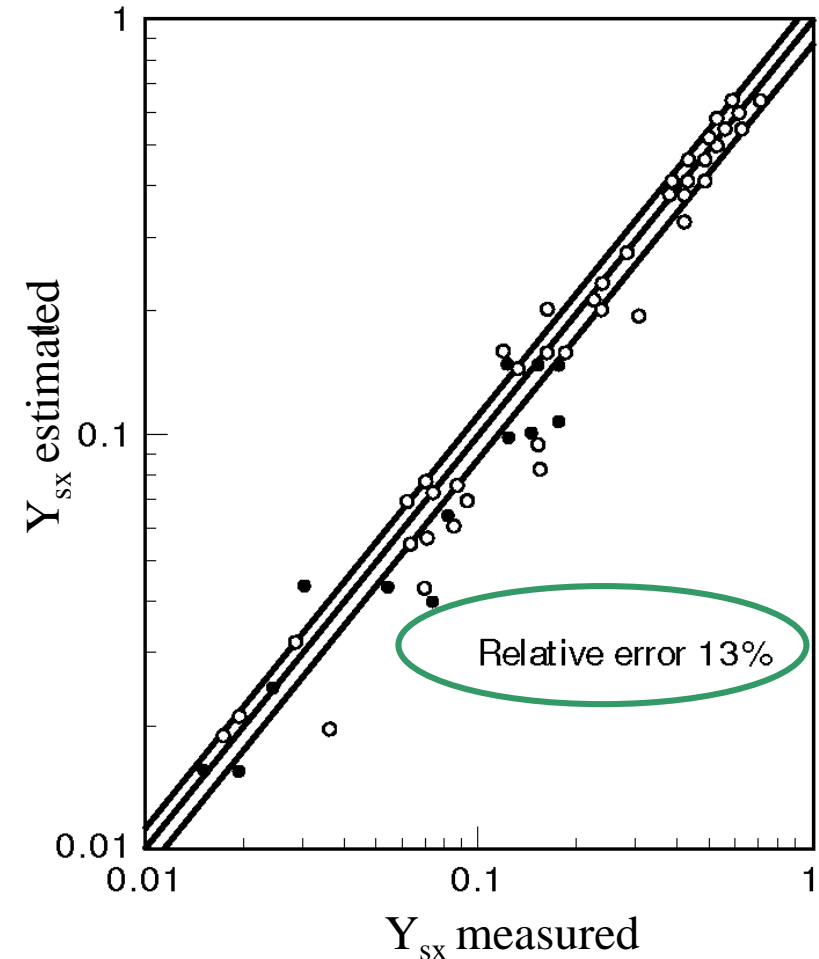
→ μ kinetic is still required !!!

Figure 7. Comparison of measured and predicted biomass yield Y_{sx} (solid circles, fermentative; open circles, aerobic growth systems).

"μ^{max} or q_S^{max}" prediction for μ kinetic (1)

For different microorganisms, using different C-sources, e-donors, e-acceptors: $\mu^{max} = 0.001$ to 1 h^{-1}

Recall: From Herbert-Pirt Eq. $\boxed{-q_S = \dfrac{1}{Y_{SX}^{max}}\mu + m_s}$ Hyperbolic Eq. $\boxed{q_S = q_S^{max}\dfrac{C_S}{K_S + C_S}}$

Thus $\boxed{\boldsymbol{\mu} = \left[\dfrac{(-\boldsymbol{q}_s^{max})\,\boldsymbol{C}_s}{\boldsymbol{K}_s + \boldsymbol{C}_s} - (\boldsymbol{m}_s)\right].\boldsymbol{Y}_{SX}^{max}}$ or $\boxed{\mu = \mu^{max}\,\dfrac{\boldsymbol{C}_s - \boldsymbol{C}_s^{min}}{\boldsymbol{C}_s + \boldsymbol{K}_s} \; ; \; \boldsymbol{C}_s^{min} = \boldsymbol{m}_s\boldsymbol{K}_s\boldsymbol{Y}_{sx}^{max}\,/\,\mu^{max}}$

$$\boxed{-k_d = -m_S.Y_{SX}^{max}}$$

And μ^{max} is linked to q_S^{max} and q_G^{max}…

$$\boxed{\boldsymbol{\mu}^{max}} = (\mathbf{q}_S^{max} - \mathbf{m}_S)\mathbf{Y}_{SX}^{max} \qquad \boxed{\boldsymbol{\mu}^{max}} = (\mathbf{q}_G^{max} - \mathbf{m}_G)\mathbf{Y}_{GX}^{max}$$

$\rightarrow$??? What determines μ^{max} or q_G^{max} ???

"μ^{max} or q_S^{max}" prediction for μ kinetic (2)

Hypothesis: "Universal Microbial Machinery is somewhat limited"

Rate of energy production is limited to a maximum because of a maximal electron processing capacity by the electron transport chain in biomass cells, at 3 moles of electrons per C-moleX per hour (298°K)

Maximum Electron Transfer Capacity of the biomass: [e-mole.CmoleX^{-1}.h^{-1}]

$$METC = 3 \cdot \exp\left[\frac{-69000}{R}\left(\frac{1}{T} - \frac{1}{298}\right)\right]$$

METC is used for catabolism for Gibbs energy production required for growth and maintenance. The amount of electrons depends on the donor couple, γ_D, mole of electrons to be donated :

$$\gamma_D = \gamma_{Red} - \gamma_{Oxy}$$

Examples

$C_6H_{12}O_6$ / HCO_3^- $\gamma_D = 24 - 0 = 24$ $C_6H_{12}O_6$ / $C_2H_4O_2$ $\gamma_D = 24 - 8 = 16$

H_2S/S^0 $\gamma_D = 8 - 6 = 2$ NH_4^+/NO_2^- $\gamma_D = 0 - (-6) = 6$

"μ^{max} or q_S^{max}" prediction for μ kinetic (3)

1. Max. Electron Transfer Capacity [e-mole.CmoleX^{-1}.h^{-1}]

$$METC = 3 \cdot \exp\left[\frac{-69000}{R}\left(\frac{1}{T} - \frac{1}{298}\right)\right]$$

2. Amount of transferred e-mole γ_D [e-mole.moleS^{-1}]

$$\gamma_D = \gamma_{Red} - \gamma_{Oxy}$$

3. Gibbs energy of catabolic reaction Δg_{cat} [kJ.moleS^{-1}]

→ Maximum specific Gibbs Energy production q_G^{max} [kJ.C_moleX^{-1}.h^{-1}]

$$q_G^{max} = \frac{3 \cdot (-\Delta G_{cat})}{\gamma_D} \exp\left(\frac{-69000}{R}\left(\frac{1}{T} - \frac{1}{298}\right)\right)$$

The specific energy rate for growth production is $q_G^{max} - m_G$. From stoichiometry of global growth reaction: $Y_{SX}^{max} = Y_{GX}^{max} / Y_{GS}^{max}$, then:

$$\boxed{\mu^{max}} = (q_G^{max} - m_G)Y_{GX}^{max}$$

$$\boxed{\mu^{max}} = (q_S^{max} - m_S)Y_{SX}^{max}$$

With $Y_{SX}^{max} = Y_{GX}^{max} / Y_{GS}^{max}$

$\left(q_G^{max} - m_G\right) \cdot Y_{GX}^{max} = \left(q_G^{max} - m_G\right) \cdot Y_{SX}^{max} \cdot Y_{GS}^{max}$

$= \left(q_G^{max} \cdot Y_{GS}^{max} - m_G \cdot Y_{GS}^{max}\right) \cdot Y_{SX}^{max}$

$= \left(q_S^{max} - m_S\right) \cdot Y_{GX}^{max} = \mu^{max}$

"μ^{max} or q_S^{max}" prediction for μ kinetic (4)

By knowing Y_{GX}^{max} and m_G

$$\frac{1}{Y_{GX}^{max}} = 200 + 18*(6-C)^{1.8} + exp\left\{\left[(3.8-\gamma_c)^2\right]^{0.16}(3.6+0.4*C)\right\}$$

$$m_G = 4.5\,exp^{\left(\frac{-69000}{R}\left(\frac{1}{T}-\frac{1}{298}\right)\right)}$$

With: $\boxed{\mu^{max}} = (q_G^{max} - m_G).Y_{Gx}^{max}$

With Maximum Gibbs Energy Capacity :

$$\left[\frac{e-mole}{CmoleX.h}\right].\left[\frac{kJ}{moleS}\right] \Big/ \left[\frac{e-mole}{moleS}\right] = \left[\frac{kJ}{CmoleX.h}\right]$$

$$q_G^{max} = \frac{3.(-\Delta G_{cat})}{\gamma_D}\,exp\left(\frac{-69000}{R}\left(\frac{1}{T}-\frac{1}{298}\right)\right)$$

→Predicted maximal specific growth rate μ^{max} is:

$$\mu^{max} = \left(\frac{3.(-\Delta G_{cat})}{\gamma_D} - 4.5\right).Y_{GX}^{max}\,exp^{\left(\frac{-69000}{R}\left(\frac{1}{T}-\frac{1}{298}\right)\right)}$$

Predicted μ^{max} (25 °C) [h^{-1}]

Glucose	Glc ,O_2 → CO_2, H_2O	1.5	Glc → ethanol, CO_2	0.10
Acetate	Acetate, O_2 → CO_2, H_2O	0.7	Acetate → CH_4, CO_2	0.015
CO_2 (+RET)	$NH_4^+ + O_2$ → NO_2^-	0.04		

Heat aspects in aerobic biosystems

1) <u>Heating value of sludge</u> (organic matter oxidation)

1 e-mole transferred (organic $\rightarrow O_2$) = 115 kJ

As 1 e-mole $\equiv$ 8 gram COD $\equiv$ 1 γ unit

Thus 1 g COD = 115/8 = **14.4 kJ**

$\rightarrow$ 1 g biomass org. matter. = 1.366 g COD = 19.7 kJ

2) <u>Heat production</u> (aerobic bioprocess) $\Delta T = f (BOD) = f (Y_{COD})$

1 g consumed O_2 (**BOD**) $\equiv$ **14.4 kJ** heat produced

Reactor Temperature depends on Y_{COD} of the growth

$\Delta T = 1.71 \Delta COD$ ($Y_{COD} = 0.50$) or $\Delta T = 0.86 \Delta COD$ ($Y_{COD} = 0.75$)

Where ΔCOD = Removed COD in g/L of wastewater

$\rightarrow$ For a given substrate consumption, the worst efficient substrate for the growth, will spoil the greatest energy into heat…;-(!

Calorimetry $Q = m.C.(\Delta T)$ (with C = Specific heat capacity of water : 4.18 [$J.g^{-1}.K^{-1}$], 1L= 1000g of water)

$\Delta T = Q/m.C = [BOD] \times 14.4 \times 10^3/(m.C) = [(1-Y_{COD}). \Delta COD].14.4 \times 10^3 /(m.C)$

$= [(1-0.75)). \Delta COD] \times 14.4 \times 10^3 /(1000 \times 4.18) = 0.86. \Delta COD$

Heat Generation by Microbial Growth

Total rate of heat evolution in a **batch fermentation** is given [kJ.hr^{-1}] by

$$Q = V_l . C_x . \frac{1}{Y_{HX}} . \mu$$

$1/Y_{HX}$ coming from global growth equation
V_l is the liquid volume, C_X is biomass concentration

$$Q = V_l . C_x . \frac{1}{Y_{HX}} . \mu = . \frac{1}{Y_{HX}} . \left[(q_x . C_x) . V_l \right] = R_H$$

In aerobic fermentations, the rate of **metabolic heat production** Q [kJ.hr^{-1}] can be roughly correlated to the **oxygen uptake rate R$_{O2}$**:

$$Q = 0.12 \times R_{O_2}$$

With: Q [kcal.hr^{-1}], and R$_{O2}$ [mMole O$_2$.hr^{-1}]

→ If temperature increases above 70°C the bioprocess stops, even for thermophile growth. **Cooling becomes necessary!**
Not the case in WWTP (dilution and low organic load), but in industrial fermentation processes (Ex: Single Cell Protein processes (Yeast))

Heat Generation by Microbial Growth

Cooling: Metabolic heat released during a fermentation can be removed by circulating cooling water through a cooling coil within the fermenter, or a cooling jacket surrounding the fermenter.

→ Temperature control may become a critical limitation on reactor design (particularly for scaling up)

→ Ability to estimate heat removal is essential to proper reactor design

Cooling Coils

Water-Jacketed Fermenter