Bacterial energetics

Today's class
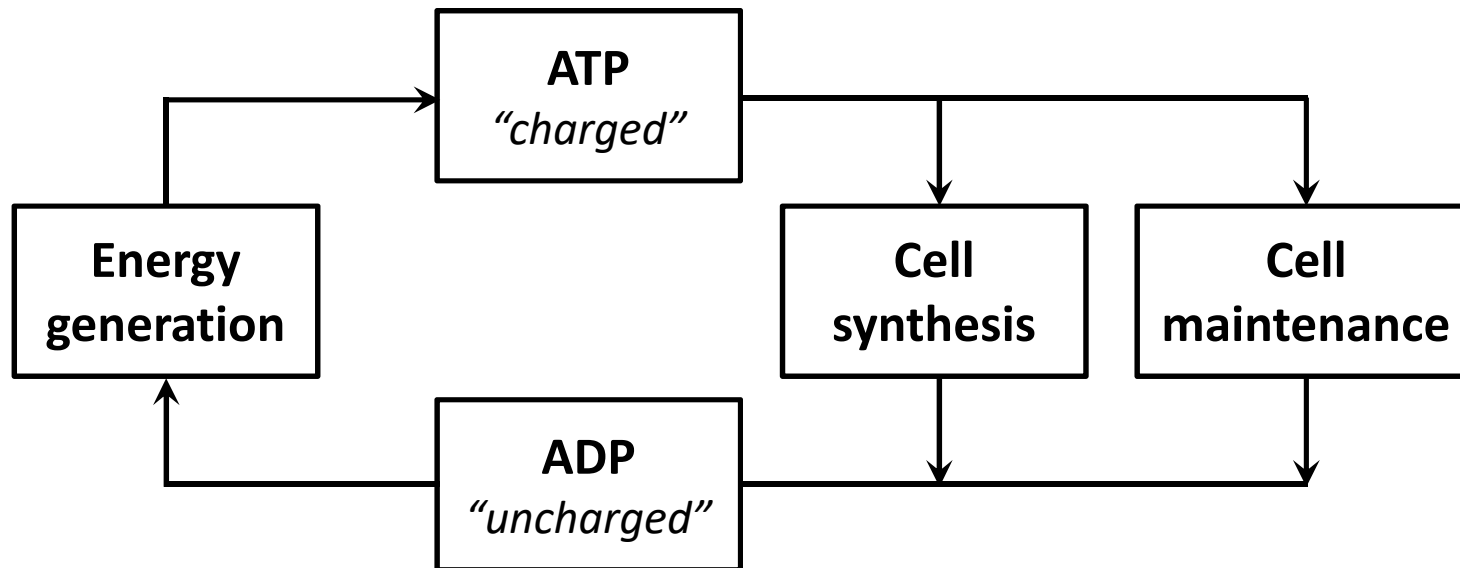
- Bacterial energetics – overview
- Gibbs free energy of reaction
- Correlating reaction energetics and yield coefficient

Bacterial energetics

- Microorganisms carry out redox reactions to obtain energy for growth and cell maintenance

Organic half-reactions and their Gibb's free energy at pH = 7.0 (1)

Reaction Number	Reduced Compounds	Half-reaction		$\Delta G^{0\prime}$ (kJ/e$^-$ eq)
O-1	Acetate	$\frac{1}{8}CO_2 + \frac{1}{8}HCO_3^- + H^+ + e^-$	$= \frac{1}{8}CH_3COO^- + \frac{3}{8}H_2O$	27.40
O-2	Alanine	$\frac{1}{6}CO_2 + \frac{1}{12}HCO_3^- + \frac{1}{12}NH_4^+ + \frac{11}{12}H^+ + e^-$	$= \frac{1}{12}CH_3CHNH_2COO^- + \frac{5}{12}H_2O$	31.37
O-3	Benzoate	$\frac{1}{5}CO_2 + \frac{1}{30}HCO_3^- + H^+ + e^-$	$= \frac{1}{30}C_6H_5COO^- + \frac{13}{30}H_2O$	27.34
O-4	Citrate	$\frac{1}{6}CO_2 + \frac{1}{6}HCO_3^- + H^+ + e^-$	$= \frac{1}{18}(COO^-)CH_2COH(COO^-)CH_2COO^- + \frac{4}{9}H_2O$	33.08
O-5	Ethanol	$\frac{1}{6}CO_2 + H^+ + e^-$	$= \frac{1}{12}CH_3CH_2OH + \frac{1}{4}H_2O$	31.18
O-6	Formate	$\frac{1}{2}HCO_3^- + H^+ + e^-$	$= \frac{1}{2}HCOO^- + \frac{1}{2}H_2O$	39.19
O-7	Glucose	$\frac{1}{4}CO_2 + H^+ + e^-$	$= \frac{1}{24}C_6H_{12}O_6 + \frac{1}{4}H_2O$	41.35
O-8	Glutamate	$\frac{1}{6}CO_2 + \frac{1}{9}HCO_3^- + \frac{1}{18}NH_4^+ + H^+ + e^-$	$= \frac{1}{18}COOHCH_2CH_2CHNH_2COO^- + \frac{4}{9}H_2O$	30.93
O-9	Glycerol	$\frac{3}{14}CO_2 + H^+ + e^-$	$= \frac{1}{14}CH_2OHCHOHCH_2OH + \frac{3}{14}H_2O$	38.88
O-10	Glycine	$\frac{1}{6}CO_2 + \frac{1}{6}HCO_3^- + \frac{1}{6}NH_4^+ + H^+ + e^-$	$= \frac{1}{6}CH_2NH_2COOH + \frac{1}{2}H_2O$	39.80

- low concentration of inhibiting compounds

Inorganic half-reactions and their Gibb's free energy at pH = 7.0

Reaction Number	Reduced-oxidized Compounds	Half-reaction		$\Delta G^{0\prime}$ (kJ/e⁻ eq)
I-1	Ammonium-nitrate	$\frac{1}{8}NO_3^- + \frac{5}{4}H^+ + e^-$	$= \frac{1}{8}NH_4^+ + \frac{3}{8}H_2O$	-35.11
I-2	Ammonium-nitrite	$\frac{1}{6}NO_2^- + \frac{4}{3}H^+ + e^-$	$= \frac{1}{6}NH_4^+ + \frac{1}{3}H_2O$	-32.93
I-3	Ammonium-Nitrogen	$\frac{1}{6}N_2 + \frac{4}{3}H^+ + e^-$	$= \frac{1}{3}NH_4^+$	26.70
I-4	Ferrous-Ferric	$Fe^{3+} + e^-$	$= Fe^{2+}$	-74.27
I-5	Hydrogen-H⁺	$H^+ + e^-$	$= \frac{1}{2}H_2$	39.87
I-6	Nitrite-Nitrate	$\frac{1}{2}NO_3^- + H^+ + e^-$	$= \frac{1}{2}NO_2^- + \frac{1}{2}H_2O$	-41.65
I-7	Nitrogen-Nitrate	$\frac{1}{5}NO_3^- + \frac{6}{5}H^+ + e^-$	$= \frac{1}{10}N_2 + \frac{3}{5}H_2O$	-72.20
I-8	Nitrogen-Nitrite	$\frac{1}{3}NO_2^- + \frac{4}{3}H^+ + e^-$	$= \frac{1}{6}N_2 + \frac{2}{3}H_2O$	-92.56
I-9	Sulfide-Sulfate	$\frac{1}{8}SO_4^{2-} + \frac{19}{16}H^+ + e^-$	$= \frac{1}{16}H_2S + \frac{1}{16}HS^- + \frac{1}{2}H_2O$	20.85
I-10	Sulfide-Sulfite	$\frac{1}{6}SO_3^{2-} + \frac{5}{4}H^+ + e^-$	$= \frac{1}{12}H_2S + \frac{1}{12}HS^- + \frac{1}{2}H_2O$	11.03
I-11	Sulfite-Sulfate	$\frac{1}{2}SO_4^{2-} + H^+ + e^-$	$= \frac{1}{2}SO_3^{2-} + \frac{1}{2}H_2O$	50.30
I-12	Sulfur-Sulfate	$\frac{1}{6}SO_4^{2-} + \frac{4}{3}H^+ + e^-$	$= \frac{1}{6}S + \frac{3}{2}H_2O$	19.15
I-13	Thiosulfate-Sulfate	$\frac{1}{4}SO_4^{2-} + \frac{5}{4}H^+ + e^-$	$= \frac{1}{8}S_2O_3^{2-} + \frac{5}{8}H_2O$	23.58
I-14	Water-Oxygen	$\frac{1}{4}O_2 + H^+ + e^-$	$= \frac{1}{2}H_2O$	-78.72

Energetics and growth: thoughts

- Bacterial cells grow more rapidly when:
  - More energy is obtained by oxidation of (an e^- equivalent of) an e^- donor
  - More energy is obtained by reduction of (an e^- equivalent of) an e^- acceptor
  - Conditions are favorable for rapid utilization of substrates
    - abundance of e^- donor, e^- acceptor, nutrients, etc.

    - low concentration of inhibiting compounds

Energetics and growth: math

$$Y_n = Y - b \frac{X_a}{-dS/dt}$$

- $Y_n \uparrow$ when $Y \uparrow$: favorable e$^-$ donor and acceptor
- $Y_n \uparrow$ when $X_a << -dS/dt$: favorable conditions for substrate utilization

We will discuss about the $-dS/dt$ term in forthcoming lectures!

Gibbs free energy: standard conditions

- "Standard" free energy
  - ΔG^0, free energy at 25°C, 1 atm, and unit activity for any chemicals involved
  - $\Delta G^{0\prime}$, standard free energy adjusted to pH=7

ex) $\frac{1}{4}O_2 + H^+ + e^- = \frac{1}{2}H_2O$
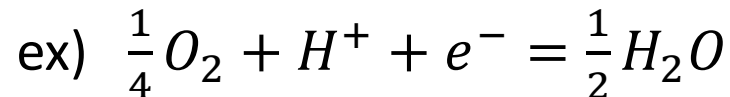
ΔG^0 is for $\quad \{O_2\} = P_{O_2} = 1\ atm$

$\{H^+\} = 1$ (pH = 0)

$\{H_2O\} = 1$

$\Delta G^{0\prime}$ is for $\quad \{O_2\} = P_{O_2} = 1\ atm$

pH=7 $\quad (\{H^+\} = 10^{-7})$

$\{H_2O\} = 1$

$\boxed{\{H_2O\} = 1 \text{ applies to any dilute aqueous solutions}}$

Free energy of formation, ΔG_f

- Gibbs free energy that accompanies the formation of 1 mole of the substance from its component elements

Gibbs free energy change of reaction

- Free energy change of reaction, ΔG_r

$$\sum(product\ \Delta G_f) - \sum(reactant\ \Delta G_f)$$

For a generic reaction written as

$$0 = \sum_{i=1}^{n} v_{ir} A_i$$

v_{ir} = stoichiometric coefficient, (-) for reactants, (+) for products
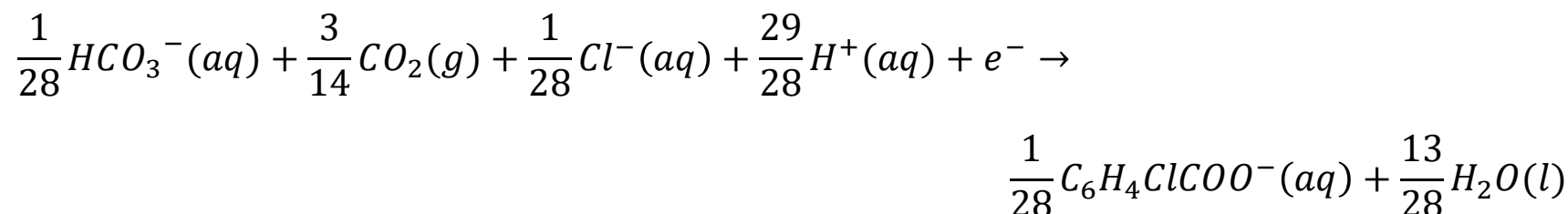
A_i = reaction constituent, reactants or products

ΔG_r *can be written as*

$$\Delta G_r = \sum_{i=1}^{n} v_{ir} \Delta G_f$$
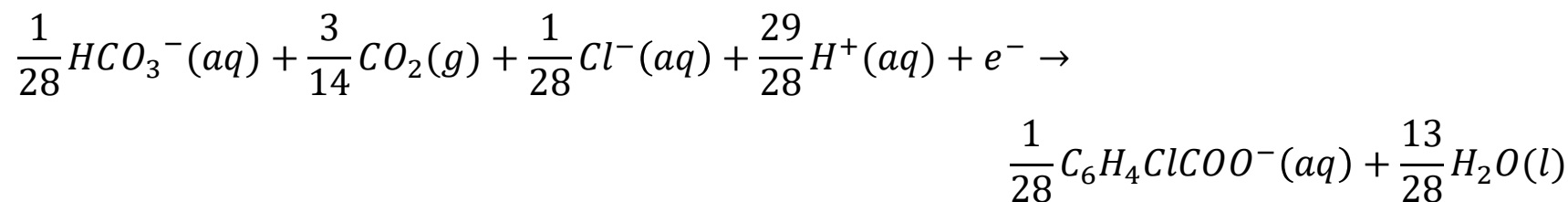
Reaction free energy

Q1: Calculate the standard free energy adjusted pH 7 for the half reaction of 2-chlorobenzoate formation as given below.

$$\frac{1}{28} HCO_3^-(aq) + \frac{3}{14} CO_2(g) + \frac{1}{28} Cl^-(aq) + \frac{29}{28} H^+(aq) + e^- \rightarrow$$

$$\frac{1}{28} C_6H_4ClCOO^-(aq) + \frac{13}{28} H_2O(l)$$

Use the following values of free energy of formation (in textbook Appendix A):

Species	$HCO_3^-(aq)$	$CO_2(g)$	$Cl^-(aq)$	$H^+(aq, 10^{-7})$	$C_6H_4ClCOO^-(aq)$	$H_2O(l)$
$\Delta G_f^{0'}$ @ 25°C (kJ/mole)	-586.85	-394.36	-31.35	-39.87	-237.9	-237.18

$$\frac{1}{28}HCO_3^-(aq) + \frac{3}{14}CO_2(g) + \frac{1}{28}Cl^-(aq) + \frac{29}{28}H^+(aq) + e^- \rightarrow$$

$$\frac{1}{28}C_6H_4ClCOO^-(aq) + \frac{13}{28}H_2O(l)$$

Species	HCO_3^-(aq)	CO_2(g)	Cl^-(aq)	H^+(aq, 10^{-7})	$C_6H_4ClCOO^-$(aq)	H_2O(l)
$\Delta G_f^{0\prime}$ @ 25ºC (kJ/mole)	-586.85	-394.36	-31.35	-39.87	-237.9	-237.18

$$\Delta G_r = \sum_{i=1}^{n} v_{ir}\Delta G_f$$

v_{ir} = *stoichiometric coefficient, (-) for reactants, (+) for products*

$$\Delta G_r^{0\prime} = (-)\left\{\frac{1}{28} \times (-586.85) + \frac{3}{14} \times (-394.36) + \frac{1}{28} \times (-31.35) + \frac{29}{28} \times (-39.87)\right\}$$

$$+ \left\{\frac{1}{28} \times (-237.9) + \frac{13}{28} \times (-237.18)\right\}$$

$$= \mathbf{29.26\ kJ/e^-\ eq}$$

Reaction free energy

Q2: Calculate the standard free energy adjusted pH 7 for overall energy reaction with ethanol as an e⁻ donor and oxygen as an e⁻ acceptor. Use the half reactions listed in Table 2.2 and 2.3.

R_d: O-5　　　　$\Delta G_r{}^{0\prime} = 31.18 \; kJ/e^- \; eq$

R_a: I-14　　　　$\Delta G_r{}^{0\prime} = -78.72 \; kJ/e^- \; eq$

$R_e = R_a - R_d$　　　　　　　　　$\Delta G_r{}^{0\prime}(R_e) = \Delta G_r{}^{0\prime}(R_a) - \Delta G_r{}^{0\prime}(R_d)$

R_a:　$\dfrac{1}{4}O_2 + H^+ + e^- = \dfrac{1}{2}H_2O$　　　　　$= -78.72 - 31.18$

-R_d:　$\dfrac{1}{12}C_2H_5OH + \dfrac{1}{4}H_2O = \dfrac{1}{6}CO_2 + H^+ + e^-$　　　$= \boldsymbol{-109.90 \; kJ/e^- \; eq}$

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R_e:　$\dfrac{1}{12}C_2H_5OH + \dfrac{1}{4}O_2 = \dfrac{1}{6}CO_2 + \dfrac{1}{4}H_2O$

Reaction free energy: general conditions

- For nonstandard conditions,

$$\Delta G_r = {\Delta G_r}^0 + RT \sum_{i=1}^{n} v_{ir} \ln a_i$$

a_i = activity of constituent A_i

R = gas constant, $8.314 \times 10^{-3} \ kJ/mole - K$

T = absolute temperature, K

Caution:

- v_{ir} is negative for reactants and positive for products
- ${\Delta G_r}^0$ is for standard conditions -- pH=0

    From ${\Delta G_r}^{0'}$, we can calculate ${\Delta G_r}^0$ by:

    $${\Delta G_r}^0 = {\Delta G_r}^{0'} - RT v_{H+} \ln[10^{-7}]$$

Reaction free energy

Q3: Calculate the free energy of reaction for aerobic ethanol degradation at the following conditions: T = 20°C, pH = 5.0, $[C_2H_5OH] = 2 \times 10^{-3}$ M, $P_{CO2} = 3 \times 10^{-4}$ atm, $P_{O2} = 0.21$ atm.

From Q2,

$$\Delta G_r{}^{0'} = -109.90 \ kJ/e^- \ eq$$

$$R_e: \quad \frac{1}{12}C_2H_5OH + \frac{1}{4}O_2 = \frac{1}{6}CO_2 + \frac{1}{4}H_2O$$
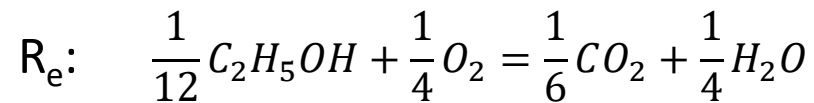
$$\Delta G_r{}^0 = \Delta G_r{}^{0'} - RT v_{H^+} ln[10^{-7}] = G_r{}^{0'} - 0 = G_r{}^{0'}$$

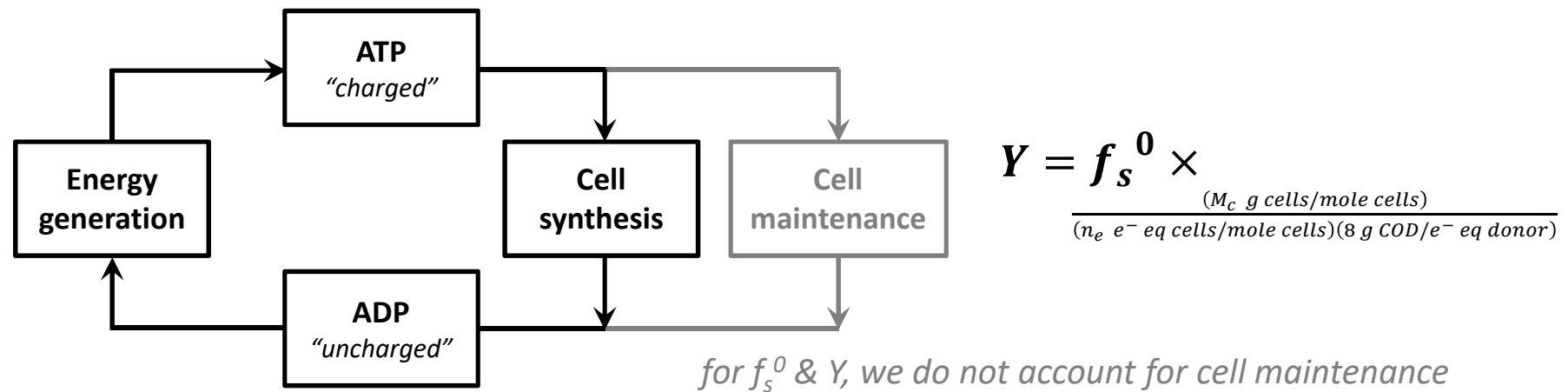$$\Delta G_r = \Delta G_r{}^0 + RT \sum_{i=1}^{n} v_{ir} ln a_i$$

$$= -109.90 \ kJ/e^- \ eq + (8.314 \times 10^{-3} \ kJ/mole - K) \times (293 \ K)$$

$$\times \left\{ -\frac{1}{12}ln(2 \times 10^{-3}) - \frac{1}{4}ln(0.21) + \frac{1}{6}ln(3 \times 10^{-4}) + \frac{1}{4}ln(1) \right\}$$

$$= \boldsymbol{-110.98 \ kJ/e^- \ eq}$$

$a_{H_2O} = 1$ *for aqueous solutions; you may omit the water term*

Deriving f_s^0 or Y by thermodynamic principles

$$Y = f_s^0 \times \frac{(M_c \ g \ cells/mole \ cells)}{(n_e \ e^- \ eq \ cells/mole \ cells)(8 \ g \ COD/e^- \ eq \ donor)}$$

for f_s^0 & Y, we do not account for cell maintenance

- To determine f_s^0 or Y we consider the energy balance for cell synthesis
  - energy is needed (ATP→ADP) to synthesize cells [1]
  - energy reaction (R_e) supplies the required energy (ADP→ATP) [2]
  - At steady state,

    energy required for [1] = energy supplied by [2]

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Two steps of cell synthesis, the efficiency

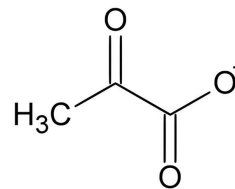- We conceptualize the cell synthesis mechanism into two steps

C-source

Organics (heterotrophs)
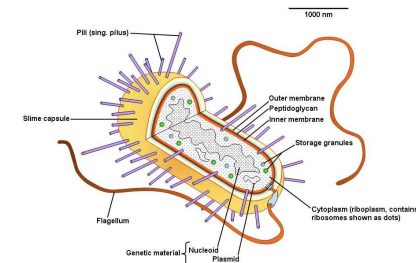
CO_2/HCO_3^- (autotrophs)

⇨

Building block

e.g., pyruvate (CH_3COCOO^-)

⇨

Cells

1) Carbon source is metabolized to form a building block
2) The building block is synthesized to a cell

- We should consider that bacteria are not 100% efficient engines -- each step involves energy loss!

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ΔG_p (C-source → pyruvate), ΔG_{pc} (pyruvate → cells)

- Energy required to convert carbon source to pyruvate, ΔG_p (heterotrophic bacteria, ammonia as N source):

$$\Delta G_p = 35.09 - \Delta G_c^{0\prime}$$

(in kJ/e⁻ eq)

35.09 = reaction free energy for formation of pyruvate from CO_2

$\Delta G_c^{0\prime}$ = reaction free energy for formation of carbon source from CO_2

- Energy required to convert pyruvate to cells, ΔG_{pc} = 18.8 kJ/e⁻ eq

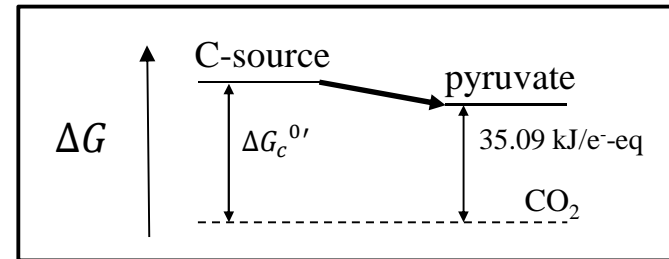(estimated value for a cell formula of $C_5H_7O_2N$ using NH_4^+ as a N source)

ΔG_S (C-source → cells)

- Energy required for cell synthesis from the carbon source, ΔG_S:
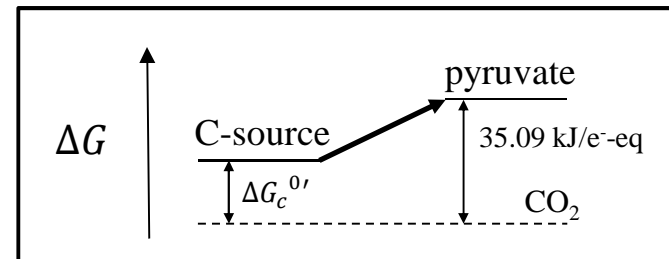
  if $\Delta G_p < 0$ (C-source is at higher energy state than pyruvate)

  $$\Delta G_S = \varepsilon \Delta G_p + \frac{\Delta G_{pc}}{\varepsilon}$$

  if $\Delta G_p > 0$ (C-source is at lower energy state than pyruvate)

  $$\Delta G_S = \frac{\Delta G_p}{\varepsilon} + \frac{\Delta G_{pc}}{\varepsilon}$$

  ε = energy transfer efficiency

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Energy balance of overall reaction

- The energy obtained by an energy reaction is used to synthesize cells

- Energy obtained = Energy used (at steady state)

- Keep in mind that bacteria cannot use 100% of the energy available by an energy reaction (energy transfer efficiency, ε)

- Therefore we write

$$A\varepsilon\Delta G_r + \Delta G_s = 0$$

A = e$^-$ equivalent of e$^-$ donor used for energy production per e$^-$ equivalent of cells formed

ΔG_r = reaction free energy for energy reaction = ΔG_a - ΔG_d (per e$^-$-eq)

ΔG_s = energy required for cell synthesis from the carbon source (per e$^-$-eq)

Deriving f_s^0 or Y by thermodynamic principles

- Solving for A:

$$A = -\frac{\Delta G_p / \varepsilon^n + \Delta G_{pc}/\varepsilon}{\varepsilon \Delta G_r}$$

  $n = -1$ for $\Delta G_p < 0$

  $+1$ for $\Delta G_p > 0$

- From A, we can calculate f_s^0 and f_e^0 as:

$$f_s^{\,0} = \frac{1}{1+A} \qquad f_e^{\,0} = 1 - f_s^{\,0} = \frac{A}{1+A}$$

- Energy transfer efficiency, ε
  - 55-70% under optimal conditions
  - Use 0.6 for ordinary cases

Deriving f_s^0 or Y by thermodynamic principles

Q4: Estimate f_s^0 and Y for aerobic oxidation of acetate assuming ε=0.4 and 0.6 at standard conditions except for a pH of 7.0. Ammonia is available for cell synthesis.

From O-1, $\Delta G_c^{0'} = 27.40 \; kJ/e^- \; eq$

$\Delta G_p = 35.09 - 27.40 = 7.69 \; kJ/e^- \; eq$

$\Delta G_r = \Delta G_a - \Delta G_d \; = -78.72 - 27.40 = -106.12 \; kJ/e^- \; eq$

$\Delta G_d = 27.40 \; kJ/e^- \; eq, \quad \Delta G_a = -78.72 \; kJ/e^- \; eq$ (from I-14)

$\Delta G_p > 0 \; \rightarrow \; n = +1$

$$A = - \frac{\dfrac{\Delta G_p}{\varepsilon^n} + \dfrac{\Delta G_{pc}}{\varepsilon}}{\varepsilon \Delta G_r} = - \frac{\dfrac{7.69}{\varepsilon^{+1}} + \dfrac{18.8}{\varepsilon}}{-106.12\varepsilon}$$

$\epsilon = 0.4 : A = 1.56$

$\epsilon = 0.6 : A = 0.69$

$$f_s^{\,0} = \frac{1}{1+A}$$

$$Y = f_s^{\,0} \frac{M_c}{n_e \cdot (8 \; g \; COD/e^- \; eq)}$$

$f_s^{\,0} = 0.39, \; Y = 0.28 \; g \; cells/g \; COD$

$f_s^{\,0} = 0.59, \; Y = 0.42 \; g \; cells/g \; COD$