ART

Advanced Redox Technology (ART) Lab

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Chapter 2. Stoichiometry and Bacterial Energetics

All the figures and tables in this material are from the reference below unless specified otherwise.
Reference: Bruce E. Rittmann and Perry L. McCarty, "Environmental Biotechnology: Principles and Applications", McGraw-Hill, 2001.

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2. Stoichiometry and Bacterial Energetics

√ **Mass balance:**

an important concept in the engineering design of biological treatment processes for environmental applications as well as many other physical/chemical processes

- **Determine the amount of chemicals that must be supplied to satisfy the energy, nutrient, and environmental needs of the microorganisms.**
- **The amounts of end products generated can be estimated.**
- **Chemicals** (for example)

- Oxygen (e- acceptor)
- N, P (nutrients)
- Lime, sulfuric acid (pH maintenance)

2. Stoichiometry and Bacterial Energetics

√ **Balanced chemical equations are based upon the concept of <u>stoichiometry</u>**

√ **The stoichiometry for microbial reactions is complicated because**

- **microbial reactions often involve oxidation and reduction of more than one species.**
- **microorganisms have dual roles**
  - as catalysts for the reaction
  - as products of the reaction
- **microorganisms carry out most chemical reactions**
  - in order to capture some of the energy released for cell synthesis and for maintaining cellular activity.

⇒ Reaction energetics as well as balancing for elements, electrons, charge should be considered.

2.1 An Example Stoichiometric Equation

√ **A balanced equations for biological oxidation of wastewater**

(Porges, Jasewicz, Hoover, 1956)

Wastewater	$C_8H_{12}O_3N_2 + 3O_2 \rightarrow C_5H_7O_2N + NH_3 + 3CO_2 + H_2O$						
	Casein		Bacterial cell				
Formula weight	184	96	113	17	132	18	
	$\sum = 280$		$\sum = 280$				

- **Some of the carbon in casein is fully oxidized to CO2 → (e-donner substrate)**
- **However, the rest of the carbon in casein is incorporated into the newly synthesized biomass → (carbon source)**
- **$C_5H_7O_2N$ represents bacterial cells. This is generally satisfactory for most practical purposes. However, phosphorus normally represents about 2% of the bacterial organic dry weight.**
- **If 1,000kg/d of casein were consumed, $0.02 \times \frac{113}{184} \times 1000kg\ casein = 12kg/d\ of\ p$ should be present in the wastewater.**
- **Complex protein-containing mixture in casein is represented through empirical $C_8H_{12}O_3N_2$**

2.1 An Example Stoichiometric Equation

√ How to set up the balanced equation for our own system

Three things are needed:

1. Empirical formula for cells

2. Framework for describing how the electron-donor substrate is partitioned between energy generation and synthesis

3. Means to relate the proportion of the electron-donor substrate that is used to synthesize new biomass to the energy gained from catabolism and the energy needed for anabolism.

2.2 Empirical Formula for Microbial Cells

√ How to calculate the empirical cell formula

$$C_nH_aO_bN_c$$

$$n = \%C/12T,\ a = \%H/T,\ b = \%O/16T, \text{ and } c = \%N/14T$$

where, $T = \%C/12 + \%H + \%O/16 + \%N/14$

- **The organic composition (%C, %H, %O, %N) can be determined by the elemental analysis of dried cells.**
- **The calculated cell formula is generally normalize to c = 1 for N**

√ Calculated chemical oxygen demand for cell oxidation

$$C_nH_aO_bN_c + \left(\frac{2n + 0.5a - 1.5c - b}{2}\right)O_2 \rightarrow nCO_2 + cNH_3 + \frac{a - 3c}{2}H_2O$$

$$\text{COD}'/\text{Weight} = \frac{(2n + 0.5a - 1.5c - b)16}{12n + a + 16b + 14c}$$

- **COD´/Weight: Calculated oxygen demand for full oxidation of the cellular carbon per unit weight of cells (g O_2 / g cell).**

2.2 Empirical Formula for Microbial Cells

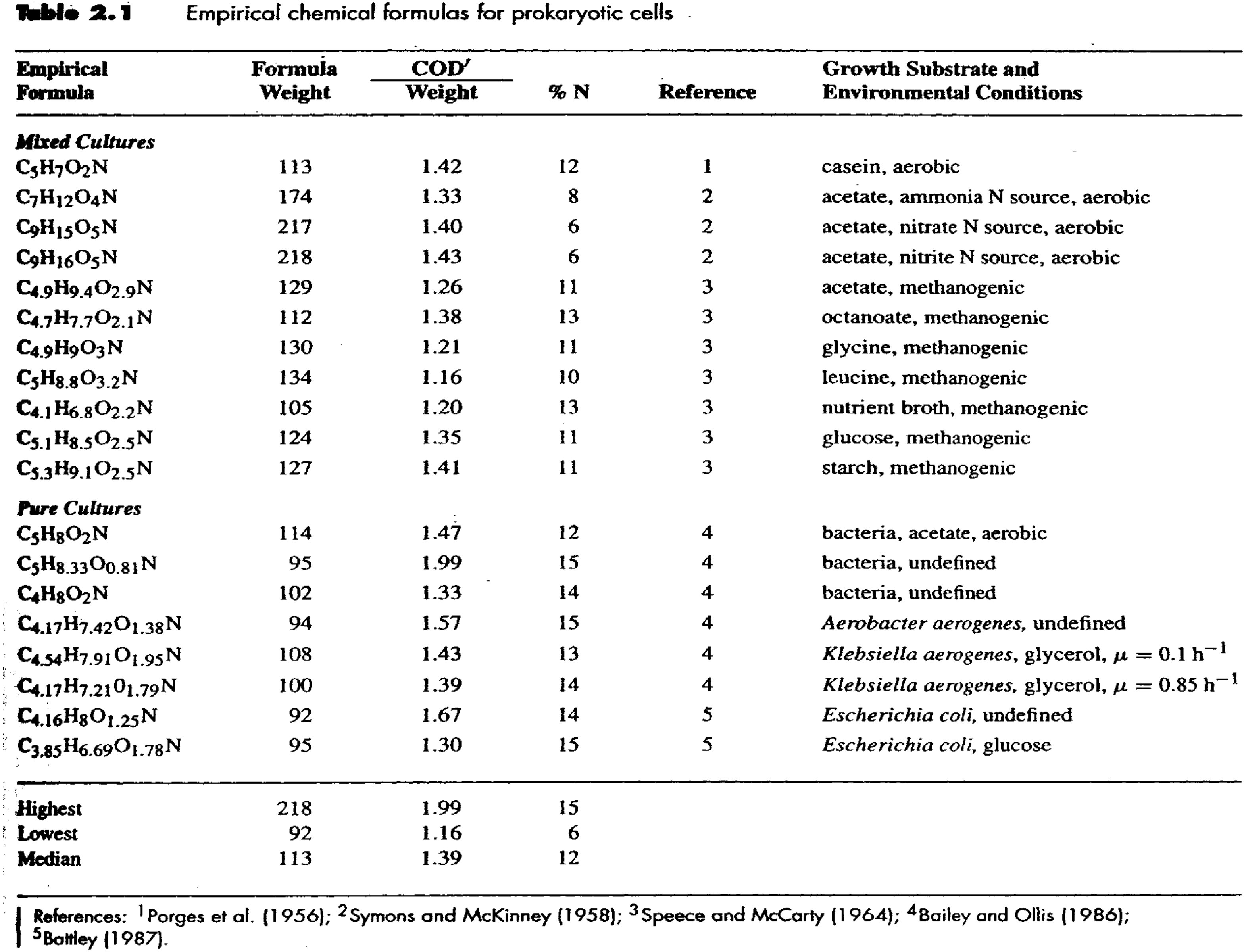


Table 2.1 Empirical chemical formulas for prokaryotic cells

Empirical Formula	Formula Weight	COD′ / Weight	% N	Reference	Growth Substrate and Environmental Conditions
Mixed Cultures					
$C_5H_7O_2N$	113	1.42	12	1	casein, aerobic
$C_7H_{12}O_4N$	174	1.33	8	2	acetate, ammonia N source, aerobic
$C_9H_{15}O_5N$	217	1.40	6	2	acetate, nitrate N source, aerobic
$C_9H_{16}O_5N$	218	1.43	6	2	acetate, nitrite N source, aerobic
$C_{4.9}H_{9.4}O_{2.9}N$	129	1.26	11	3	acetate, methanogenic
$C_{4.7}H_{7.7}O_{2.1}N$	112	1.38	13	3	octanoate, methanogenic
$C_{4.9}H_9O_3N$	130	1.21	11	3	glycine, methanogenic
$C_5H_{8.8}O_{3.2}N$	134	1.16	10	3	leucine, methanogenic
$C_{4.1}H_{6.8}O_{2.2}N$	105	1.20	13	3	nutrient broth, methanogenic
$C_{5.1}H_{8.5}O_{2.5}N$	124	1.35	11	3	glucose, methanogenic
$C_{5.3}H_{9.1}O_{2.5}N$	127	1.41	11	3	starch, methanogenic
Pure Cultures					
$C_5H_8O_2N$	114	1.47	12	4	bacteria, acetate, aerobic
$C_5H_{8.33}O_{0.81}N$	95	1.99	15	4	bacteria, undefined
$C_4H_8O_2N$	102	1.33	14	4	bacteria, undefined
$C_{4.17}H_{7.42}O_{1.38}N$	94	1.57	15	4	*Aerobacter aerogenes*, undefined
$C_{4.54}H_{7.91}O_{1.95}N$	108	1.43	13	4	*Klebsiella aerogenes*, glycerol, $\mu = 0.1\ h^{-1}$
$C_{4.17}H_{7.21}O_{1.79}N$	100	1.39	14	4	*Klebsiella aerogenes*, glycerol, $\mu = 0.85\ h^{-1}$
$C_{4.16}H_8O_{1.25}N$	92	1.67	14	5	*Escherichia coli*, undefined
$C_{3.85}H_{6.69}O_{1.78}N$	95	1.30	15	5	*Escherichia coli*, glucose
Highest	218	1.99	15		
Lowest	92	1.16	6		
Median	113	1.39	12		

References: [1]Porges et al. (1956); [2]Symons and McKinney (1958); [3]Speece and McCarty (1964); [4]Bailey and Ollis (1986); [5]Battley (1987).

2.2 Empirical Formula for Microbial Cells

√ **Example**

- **Calculate the empirical formula and COD´/Weight for the cells containing**

%C = 48.9%, %H = 5.2%, %O = 24.8%, %N =9 .46%

Solution:

T = 48.9/12+5.2+24.8/16+9.46/14 = 11.50,

n = 48.9/(12*11.5) = 0.354, a = 5.2/11.5 = 0.452

b = 24.8/(16*11.5) = 0.135, c = 9.46/(14*11.5) = 0.0588

$\Rightarrow C_{0.345}H_{0.452}O_{0.135}N_{0.0588}$

- **In order to normalize to c = 1 for N, divide by 0.0588, the following empirical formula for the cells is obtained :** $C_{6.0}H_{7.7}O_{2.3}N$
- **The COD' to organic weight ratio is then equal to**

(2*6.0 + 0.5*7.7 – 1.5 – 2.3) * 16 / (12x6 + 7.7 + 16*2.3 +14) = 1.48g COD'/g cells

- **1.48g of oxygen is needed for the complete oxidation of 1 g of cells**

2.3 Substrate Partitioning and Cellular Yield

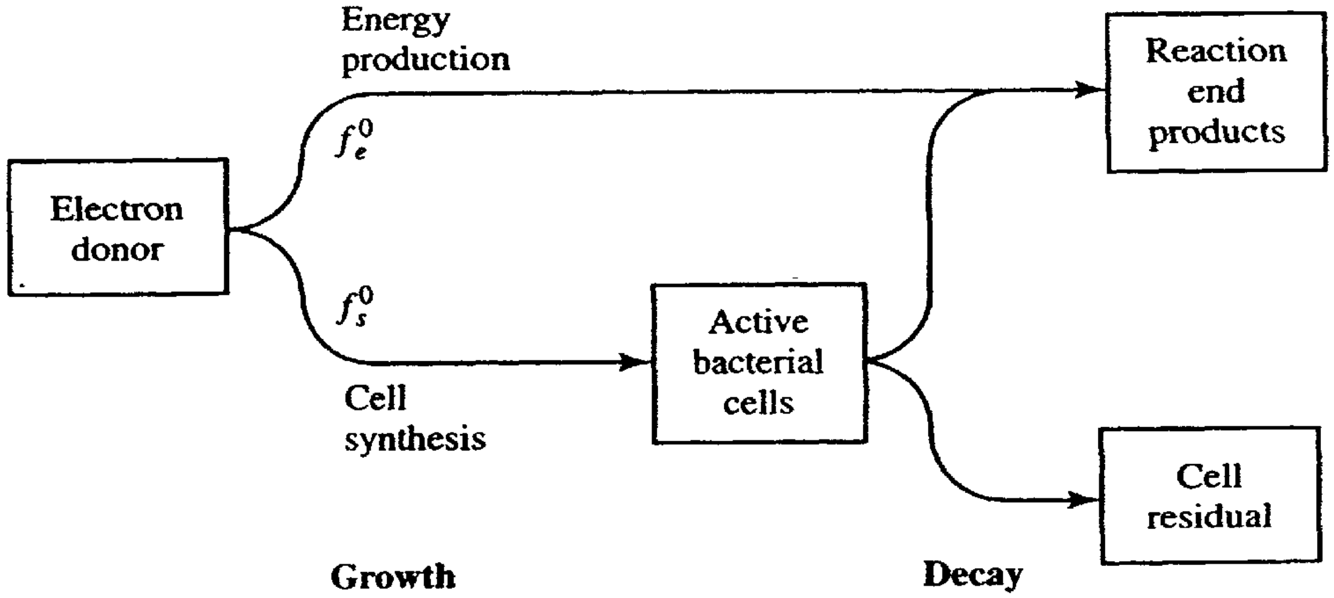


Figure 2.1 Utilization of electron donor for energy production and synthesis.

f_e^0 : portion of electrons of an e$^-$ donor substrate to provide energy

f_s^0 : portion of electrons of an e- donor substrate which are converted into microbial cells

$$f_e^0 + f_s^0 = 1$$

2.3 Substrate Partitioning and Cellular Yield

√ How to convert f_s^0 into mass units (g Cell produced / g COD' consumed)

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

- Y : true yield (g cell produced / g COD consumed)
- M_c : empirical formula weight of cells
- n_e : number of electron equivalents in an empirical mole of cells

- In redox relationship, 1 O transfer is equivalent to - 2 e^- transfer.

  ⇒ e^-_{eq} donor is equivalent to 8 g of COD.
- $n_e e^-_{eq}$ are required to produce one mole of cells

2.3 Substrate Partitioning and Cellular Yield

√ How to convert f_s^0 into mass units (g Cell produced / g COD' consumed)

$$Y = \frac{f_s^0 (M_c)}{(n_e)(8)}$$

- **Example**

Cells : $C_5H_7O_2N$, M_c : 113 g / mol cells, NH_4^+ : nitrogen source

$$\frac{1}{5}CO_2 + \frac{1}{20}HCO_3^- + \frac{1}{20}NH_4^+ + H^+ + e^- \rightarrow \frac{1}{20}C_5H_7O_2N + \frac{9}{20}H_2O$$

In the above equation, $n_e e^-_{eq} = 20$

$$Y = f_s^0 \frac{113}{20 \times 8} = 0.706\, f_s^0$$

2.3 Substrate Partitioning and Cellular Yield

The net growth rate of microbial cells:

$$\frac{dx_a}{dt} = Y\left(\frac{-ds}{dt}\right) - bx_a$$

$$Y_n = \frac{dx_a/dt}{-ds/dt} = Y - b\frac{x_a}{-ds/dt}$$

If $Y_n = 0$, *then* $Y - b\dfrac{x_a}{-ds/dt} = 0$, $\dfrac{-ds/dt}{x_a} = \dfrac{b}{Y} = m$

$(dx_a/dt = 0)$

$\dfrac{dx_a}{dt}$: net growth rate of active organism (M/L^{3}T)

$\dfrac{-ds}{dt}$: rate of disappearance of substrate (M/L^{3}T)

b : decay rate (T^{-1}) → decay due to endogenous respiration and predation

Y_n : net yield of microorganisms (M/M)

m : maintenance energy (M/MT)
= the substrate utilization rate per unit mass of organisms

2.3 Substrate Partitioning and Cellular Yield

$$Y_n = 0, then \; \frac{-ds/dt}{x_a} = \frac{b}{Y} = m$$

- If Y_n = 0, the substrate utilization rate is then just sufficient to maintain the cells.
- The substrate utilization rate per unit mass of organisms is termed as the maintenance energy.
- When the substrate utilization rate is less than m, the substrate available is insufficient to satisfy the total metabolic needs of the microorganisms.

This represents a form of starvation.

2.3 Substrate Partitioning and Cellular Yield

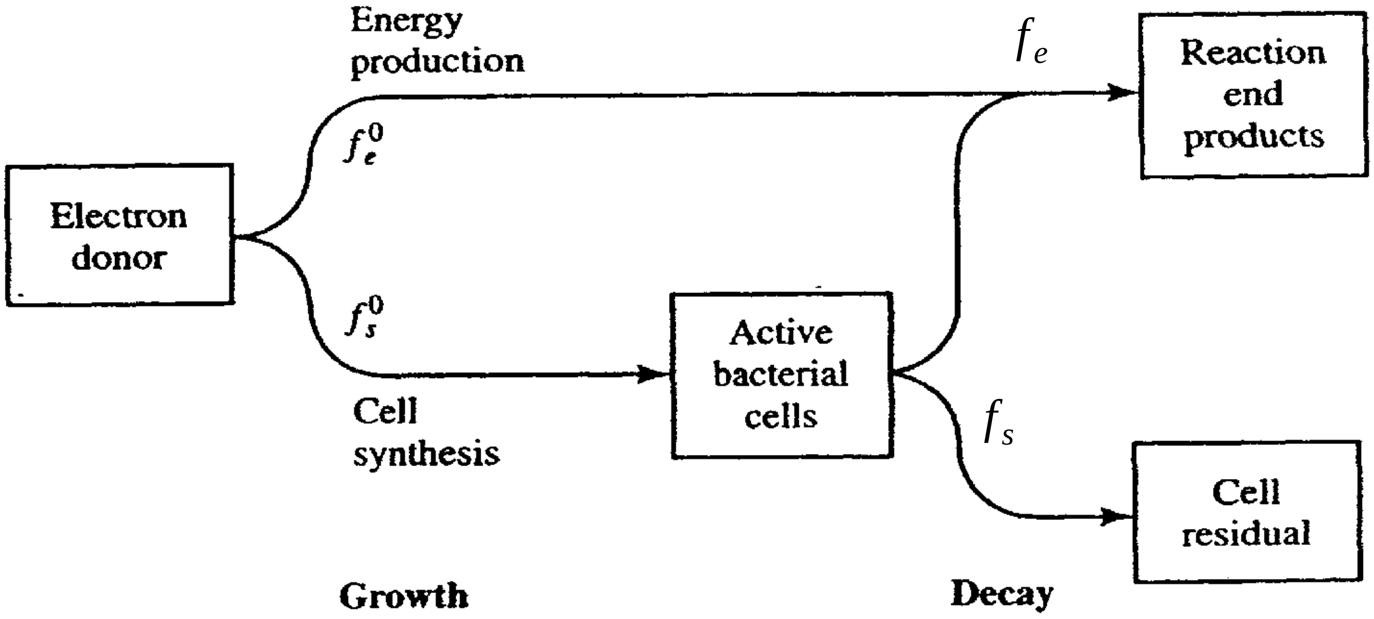


Figure 2.1 Utilization of electron donor for energy production and synthesis.

- Y_n is less than Y, because some of the electrons originally present in the substrate must be consumed for energy of maintenance.

- When considering net yield, the portion of e^- used for synthesis is f_s rather than f_s^0 : the portion of e- for energy production is f_e rather than f_e^0

$$f_e^0 + f_s^0 = 1 \qquad f_e + f_s = 1 \qquad f_s < f_s^0 \qquad f_e > f_e^0$$

2.4 Energy Reactions

√ Oxidation – Reduction reactions

provide microorganisms' energy for growth and maintenance

- **Electron donor**

  - Chemoorganotrophs : organic matter (most common)
  - Chemolithotrophs : inorganic compound (NH_3, S_2^- (H_2S))

- **Electron acceptor**

  - Oxygen under aerobic conditions
  - Nitrate, sulfate, carbon dioxide under anaerobic conditions

- **Fermentation : organic matters work as both the electron donor and acceptor.**

2.4 Energy Reactions

	Free Energy kJ / mol glucose
Aeorbic oxidation $C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + H_2O$	$-2{,}880$
Denitrification $5C_6H_{12}O_6 + 24NO_3^- + 24H^+ \rightarrow 30CO_2 + 42H_2O + 12N_2$	$-2{,}720$
Sulfate reduction $2C_6H_{12}O_6 + 6SO_4^{2-} + 9H^+ \rightarrow 12CO_2 + 12H_2O + 3H_2S + 3HS^-$	-492
Methanogenesis $C_6H_{12}O_6 \rightarrow 3CO_2 + 3CH_4$	-428
Ethanol Fermentation $C_6H_{12}O_6 \rightarrow 2CO_2 + 2CH_3CH_2OH$	-244

- **Microorganisms would like to obtain as much energy as possible from a reaction**
- **Preference for electron acceptors : oxygen > nitrate > sulfate > carbon dioxide > organics (fermentation)**

- *E.coli* : use several different electron acceptor (facultative)

- *Methanogens* : use acetate or carbon dioxide (strict anaerobes)

2.4 Energy Reactions

- **Aerobic organisms need to send less electrons from their donor substrate to oxygen (the largest energy = -2,880 kJ) than anaerobic organisms in order to generate the energy required to synthesize a given amount of new biomass.**

- **Aerobic organisms : f_e^0 → small, f_s^0 → large**

- Y is larger than anaerobic microorganisms

- They grow faster than anaerobic microorganisms

2.4 Energy Reactions

√ How to construct the energy reaction

- Oxidation half–reaction for glucose

$$\frac{1}{24}C_6H_{12}O_6 + \frac{1}{4}H_2O \rightarrow \frac{1}{4}CO_2 + H^+ + e^-$$

- Reduction half-reaction for nitrate

$$\frac{1}{5}NO_3^- + \frac{6}{5}H^+ + e^- \rightarrow \frac{1}{10}N_2 + \frac{3}{5}H_2O$$

- Overall balanced reaction

$$\frac{1}{24}C_6H_{12}O_6 + \frac{1}{5}NO_3^- + \frac{1}{5}H^+ \rightarrow \frac{1}{4}CO_2 + \frac{7}{20}H_2O + \frac{1}{10}N_2$$

$$5\ C_6H_{12}O_6 + 24\ NO_3^- + 24\ H^+ \rightarrow 30\ CO_2 + 42\ H_2O + 12\ N_2 \qquad -2{,}720\ \text{kJ/mol (glucose)}$$

2.4 Energy Reactions

√ How to construct reduction half reaction

- **Only one element is allowed to have its oxidation state changed in a half-reaction.**

- Example : alanine (CH_3CHNH_2COOH)

Carbon (12 e^- equivalent → CH_3; 7 e^- , $CHNH_2$; 4 e^- , COOH; 1 e^-)

The other elements (H; +1, O; -2, N; -3) must retain their oxidation state the same on both sides of equation.

- **In half-reactions, for organic compounds, the oxidized form is always CO_2, or in some cases, HCO_3^- or CO_3^{2-} depending on solution environment like pH.**

2.4 Energy Reactions

√ How to construct reduction half reaction

- **Example: Amino acid alanine (CH_3CHNH_2COOH)**

- Product is CO_2

- step 1 : $CO_2 \rightarrow CH_3CHNH_2COOH$

Add other species (H_2O)

Electron on the left side

N in the reduced form (NH_3 or NH_4^+) like in alanine on the left side

- step 2 : $CO_2 + H_2O + NH_3 + e^- \rightarrow CH_3CHNH_2COOH$

Balance the reaction for the elements except O and H

- step 3 : $3CO_2 + H_2O + NH_3 + e^- \rightarrow CH_3CHNH_2COOH$

Balance the O through addition or subtraction of water.

- step 4 : $3CO_2 + NH_3 + e^- \rightarrow CH_3CHNH_2COOH + 4H_2O$

2.4 Energy Reactions

√ How to construct reduction half reaction

- **Example: Amino acid alanine (CH3CHNH2COOH)**

Balance the H by introducing H^+.

- step 5 : $3CO_2 + NH_3 + 12H^+ + e^- \rightarrow CH_3CHNH_2COOH + 4H_2O$

Balance the charge on the reaction by adding sufficient e^- to the left side.

- step 6 : $3CO_2 + NH_3 + 12H^+ + 12e^- \rightarrow CH_3CHNH_2COOH + 4H_2O$

Convert the eqn. to the electron-equivalent form by dividing by the coefficient on e^-.

- step 7 : $\frac{1}{4}CO_2 + \frac{1}{12}NH_3 + H^+ + e^- \rightarrow \frac{1}{12}CH_3CHNH_2COOH + \frac{1}{3}H_2O$

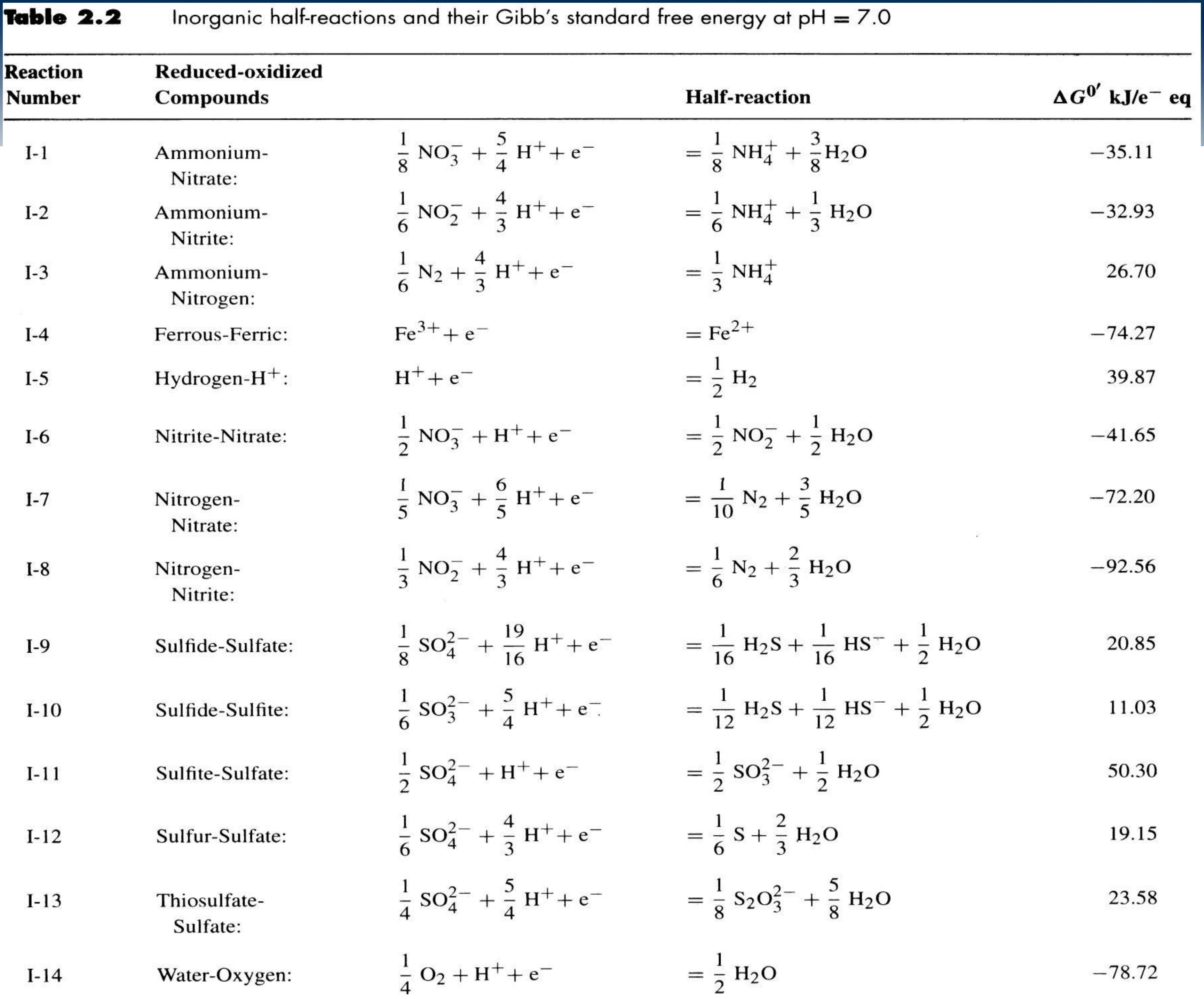


Table 2.2 Inorganic half-reactions and their Gibb's standard free energy at pH = 7.0

Reaction Number	Reduced-oxidized Compounds		Half-reaction	$\Delta G^{0'}$ 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:	$\frac{1}{6}SO_4^{2-} + \frac{4}{3}H^+ + e^-$	$= \frac{1}{6}S + \frac{2}{3}H_2O$	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

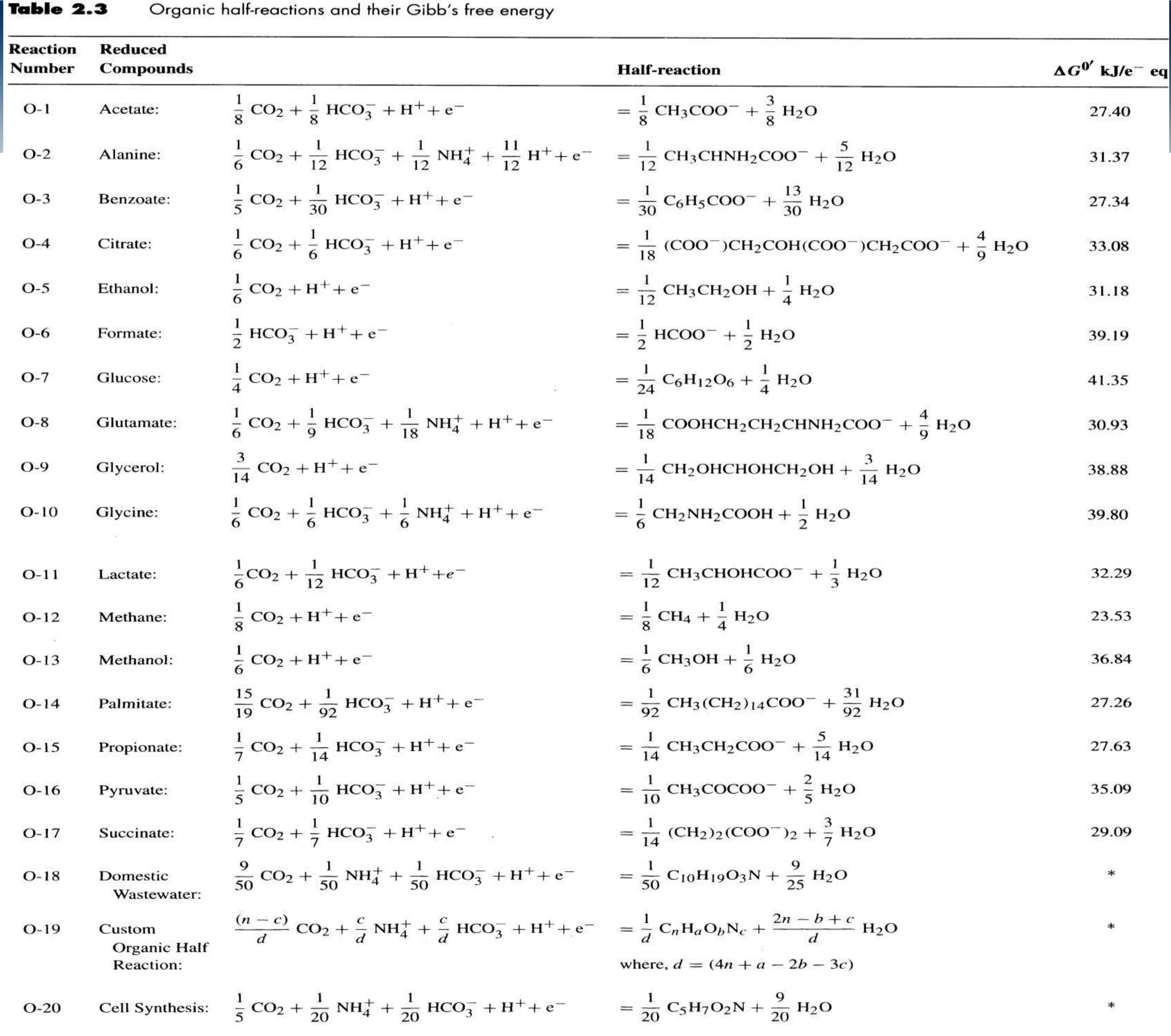


Table 2.3 Organic half-reactions and their Gibb's free energy

Reaction Number	Reduced Compounds		Half-reaction	$\Delta G^{0'}$ 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
O-11	Lactate:	$\frac{1}{6} CO_2 + \frac{1}{12} HCO_3^- + H^+ + e^-$	$= \frac{1}{12} CH_3CHOHCOO^- + \frac{1}{3} H_2O$	32.29
O-12	Methane:	$\frac{1}{8} CO_2 + H^+ + e^-$	$= \frac{1}{8} CH_4 + \frac{1}{4} H_2O$	23.53
O-13	Methanol:	$\frac{1}{6} CO_2 + H^+ + e^-$	$= \frac{1}{6} CH_3OH + \frac{1}{6} H_2O$	36.84
O-14	Palmitate:	$\frac{15}{19} CO_2 + \frac{1}{92} HCO_3^- + H^+ + e^-$	$= \frac{1}{92} CH_3(CH_2)_{14}COO^- + \frac{31}{92} H_2O$	27.26
O-15	Propionate:	$\frac{1}{7} CO_2 + \frac{1}{14} HCO_3^- + H^+ + e^-$	$= \frac{1}{14} CH_3CH_2COO^- + \frac{5}{14} H_2O$	27.63
O-16	Pyruvate:	$\frac{1}{5} CO_2 + \frac{1}{10} HCO_3^- + H^+ + e^-$	$= \frac{1}{10} CH_3COCOO^- + \frac{2}{5} H_2O$	35.09
O-17	Succinate:	$\frac{1}{7} CO_2 + \frac{1}{7} HCO_3^- + H^+ + e^-$	$= \frac{1}{14} (CH_2)_2(COO^-)_2 + \frac{3}{7} H_2O$	29.09
O-18	Domestic Wastewater:	$\frac{9}{50} CO_2 + \frac{1}{50} NH_4^+ + \frac{1}{50} HCO_3^- + H^+ + e^-$	$= \frac{1}{50} C_{10}H_{19}O_3N + \frac{9}{25} H_2O$	*
O-19	Custom Organic Half Reaction:	$\frac{(n-c)}{d} CO_2 + \frac{c}{d} NH_4^+ + \frac{c}{d} HCO_3^- + H^+ + e^-$	$= \frac{1}{d} C_nH_aO_bN_c + \frac{2n-b+c}{d} H_2O$ where, $d = (4n + a - 2b - 3c)$	*
O-20	Cell Synthesis:	$\frac{1}{5} CO_2 + \frac{1}{20} NH_4^+ + \frac{1}{20} HCO_3^- + H^+ + e^-$	$= \frac{1}{20} C_5H_7O_2N + \frac{9}{20} H_2O$	*

2.4 Energy Reactions

- **Different half-reaction forms are sometimes used for different reasons (e.g., pH-dependent speciation, hydrolysis)**

  - All are technically correct as long as the equations are written in a balanced form.

  - Regardless of the form selected 1/12 mole of alanine gives one e- equivalent.

$$\frac{1}{4}CO_2 + \frac{1}{12}NH_3 + H^+ + e^- \rightarrow \frac{1}{12}CH_3CHNH_2COOH + \frac{1}{3}H_2O$$

$$\frac{1}{6}CO_2 + \frac{1}{12}NH_3 + \frac{1}{12}HCO_3^- + H^+ + e^- \rightarrow \frac{1}{12}CH_3CHNH_2COOH + \frac{5}{12}H_2O$$

$$\frac{1}{6}CO_2 + \frac{1}{12}NH_3 + \frac{1}{12}HCO_3^- + \frac{11}{12}H^+ + e^- \rightarrow \frac{1}{12}CH_3CHNH_2COOH + \frac{5}{12}H_2O$$

$$\frac{1}{12}NH_3 + \frac{1}{4}HCO_3^- + \frac{13}{12}H^+ + e^- \rightarrow \frac{1}{12}CH_3CHNH_2COOH + \frac{7}{12}H_2O$$

2.5 Overall Reactions for Biological Growth

√ How to write an overall reaction (Energy + Synthesis)

- **Bacterial growth involves two basic reactions:**

  - Energy production
  - Cellular synthesis

- **R_c : half-reaction for synthesis (cell formation), e-acceptor**
- **R_a : acceptor half-reaction**
- **R_d : donor half-reaction**
- **R_e : energy reaction**
- **R_s : synthesis reaction**

- **$R_e = R_a – R_d$**
- **$R_s = R_c – R_d$**

2.5 Overall Reactions for Biological Growth

- General equation for constructing stoichiometric equations for microbial synthesis and growth:

$R = f_e * R_e + f_s * R_s$

$= f_e * (R_a – R_d) + f_s * (R_c – R_d)$

$= f_e * R_a + f_s * R_c – R_d (f_e + f_s)$

$(f_s + f_e = 1.0)$

$R = f_e * R_a + f_s * R_c – R_d$

- R represents net consumption of reactants and production of products when the microorganisms consume one e-equivalent of electron donor (an electron-equivalent basis).
- R represents not energy balance, but mass balance (stoichiometry).

2.5 Overall Reactions for Biological Growth

√ How to write an overall reaction (Energy + Synthesis)

- **Example**

- Benzoate : e- donor, C-source

- Nitrate : e- acceptor

- Ammonium : N source for cell synthesis

- Assumption on a net yield (Y_n) basis: f_e (= 0.6) and f_s (= 0.4)

- **Energy reaction: $R_e = R_a - R_d$**

$$\begin{array}{ll}
R_a\text{: } 1/5\ NO_3^- + 6/5\ H^+ + e^- & \rightarrow 1/10\ N_2 + 3/5\ H_2O \\
-\ R_d\text{: } 1/30\ C_6H_5COO^- + 13/30\ H_2O & \rightarrow 1/5\ CO_2 + 1/30\ HCO_3^- + H^+ + e^- \\
\hline
R_e\text{: } 1/30\ C_6H_5COO^- + 1/5\ NO_3^- + 1/5\ H^+ & \rightarrow 1/5\ CO_2 + 1/10\ N_2 + 1/30\ HCO_3 + 1/6\ H_2O
\end{array}$$

- **Synthesis reaction: $R_s = R_c - R_d$**

$$\begin{array}{ll}
R_c\text{: } 1/5\ CO_2 + 1/20\ NH_4^+ + 1/20\ HCO_3^- + H^+ + e^- & \rightarrow 1/20\ C_5H_7O_2N + 9/20\ H_2O \\
-\ R_d\text{: } 1/30\ C_6H_5COO^- + 13/30\ H_2O & \rightarrow 1/5\ CO_2 + 1/30\ HCO_3^- + H^+ + e^- \\
\hline
R_s\text{: } 1/30\ C_6H_5COO^- + 1/20\ NH_4 + 1/60\ HCO_3^- & \rightarrow 1/20\ C_5H_7O_2N + 1/60\ H_2O
\end{array}$$

2.5 Overall Reactions for Biological Growth

- **Electron donor half-reaction (R_d)**

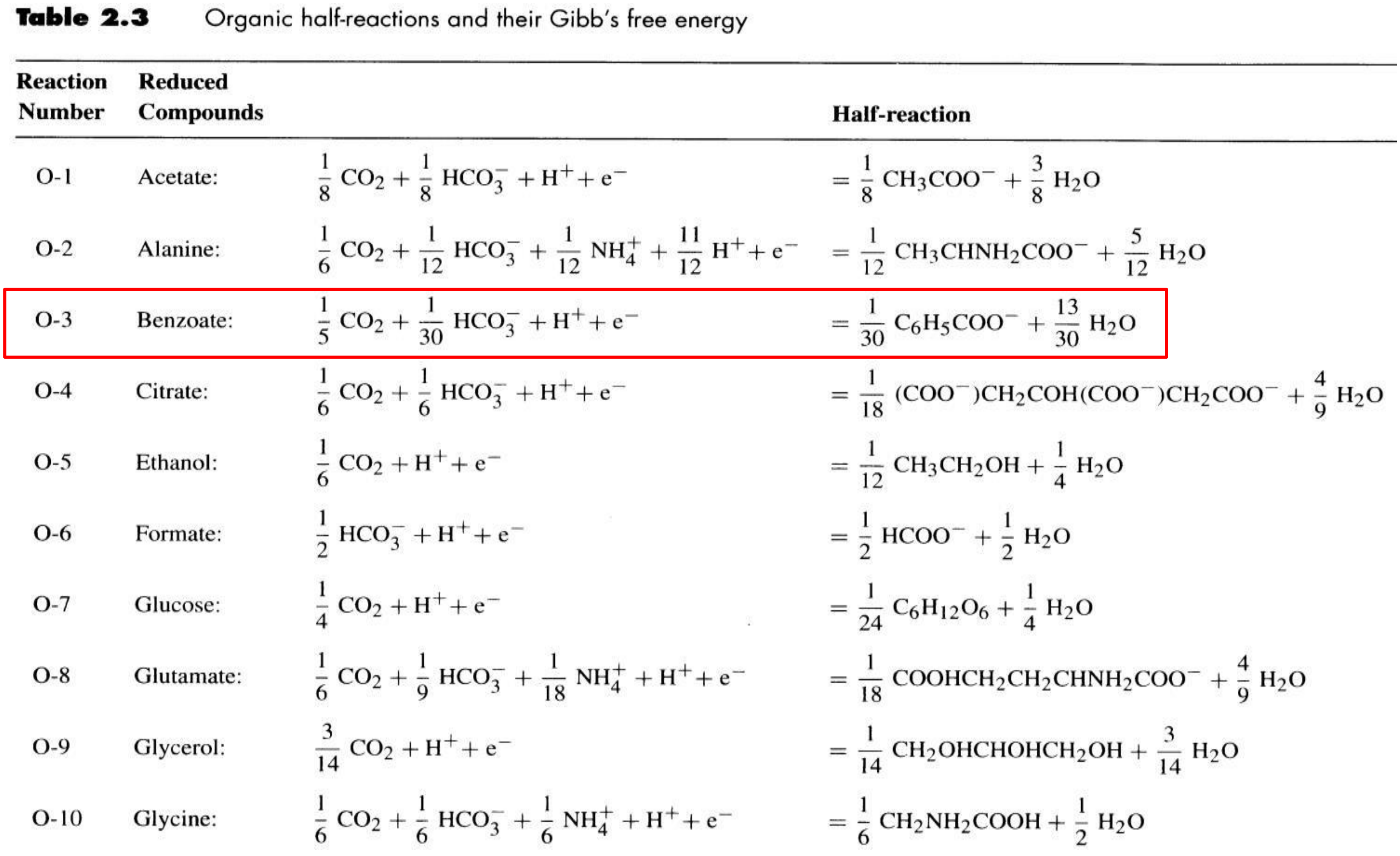


Table 2.3 Organic half-reactions and their Gibb's free energy

Reaction Number	Reduced Compounds	Half-reaction
O-1	Acetate:	$\frac{1}{8}CO_2 + \frac{1}{8}HCO_3^- + H^+ + e^- = \frac{1}{8}CH_3COO^- + \frac{3}{8}H_2O$
O-2	Alanine:	$\frac{1}{6}CO_2 + \frac{1}{12}HCO_3^- + \frac{1}{12}NH_4^+ + \frac{11}{12}H^+ + e^- = \frac{1}{12}CH_3CHNH_2COO^- + \frac{5}{12}H_2O$
O-3	Benzoate:	$\frac{1}{5}CO_2 + \frac{1}{30}HCO_3^- + H^+ + e^- = \frac{1}{30}C_6H_5COO^- + \frac{13}{30}H_2O$
O-4	Citrate:	$\frac{1}{6}CO_2 + \frac{1}{6}HCO_3^- + H^+ + e^- = \frac{1}{18}(COO^-)CH_2COH(COO^-)CH_2COO^- + \frac{4}{9}H_2O$
O-5	Ethanol:	$\frac{1}{6}CO_2 + H^+ + e^- = \frac{1}{12}CH_3CH_2OH + \frac{1}{4}H_2O$
O-6	Formate:	$\frac{1}{2}HCO_3^- + H^+ + e^- = \frac{1}{2}HCOO^- + \frac{1}{2}H_2O$
O-7	Glucose:	$\frac{1}{4}CO_2 + H^+ + e^- = \frac{1}{24}C_6H_{12}O_6 + \frac{1}{4}H_2O$
O-8	Glutamate:	$\frac{1}{6}CO_2 + \frac{1}{9}HCO_3^- + \frac{1}{18}NH_4^+ + H^+ + e^- = \frac{1}{18}COOHCH_2CH_2CHNH_2COO^- + \frac{4}{9}H_2O$
O-9	Glycerol:	$\frac{3}{14}CO_2 + H^+ + e^- = \frac{1}{14}CH_2OHCHOHCH_2OH + \frac{3}{14}H_2O$
O-10	Glycine:	$\frac{1}{6}CO_2 + \frac{1}{6}HCO_3^- + \frac{1}{6}NH_4^+ + H^+ + e^- = \frac{1}{6}CH_2NH_2COOH + \frac{1}{2}H_2O$

2.5 Overall Reactions for Biological Growth

- **Cell-formation (R_c) and electron acceptor half-reactions (R_a)**

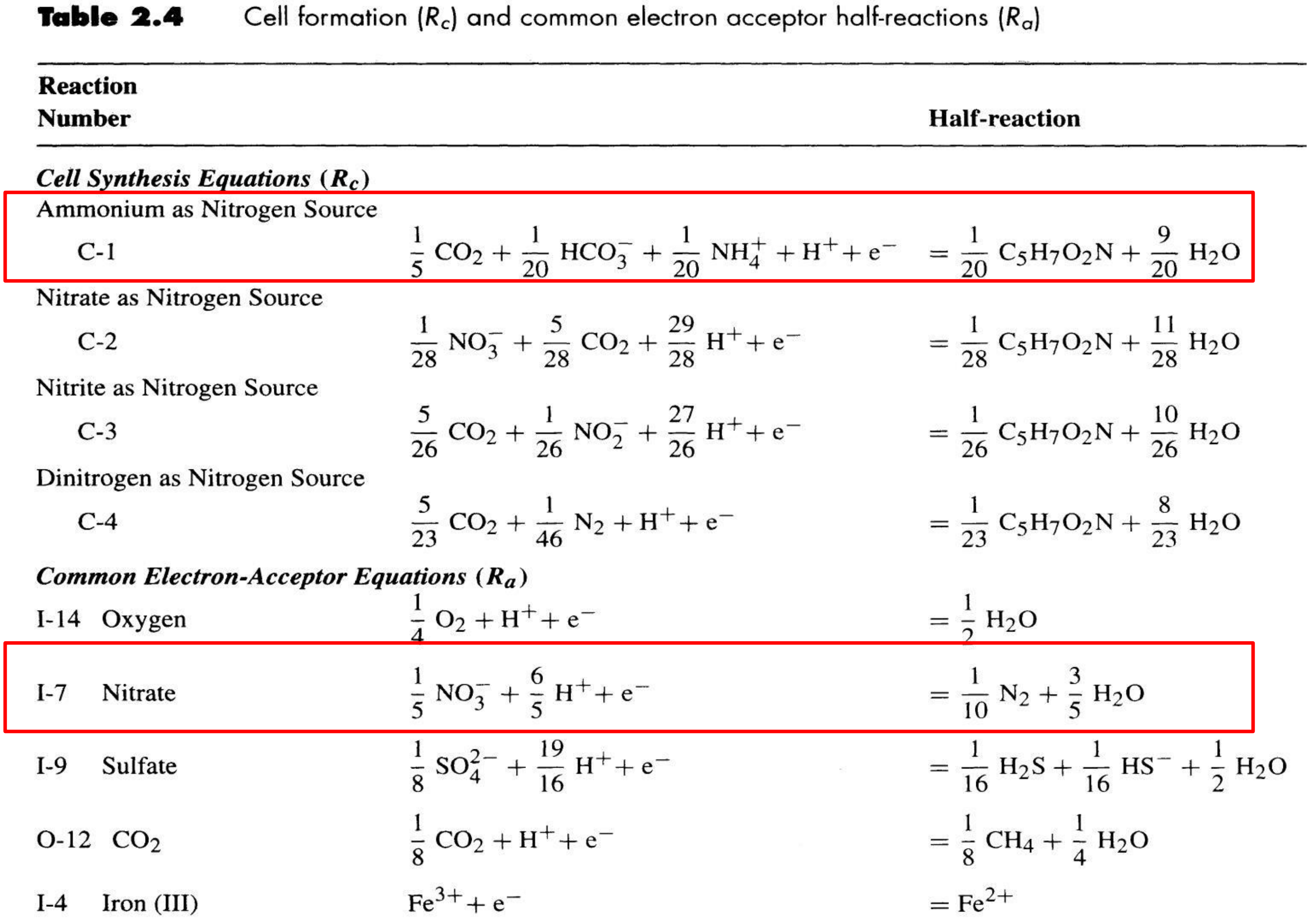


Table 2.4 Cell formation (R_c) and common electron acceptor half-reactions (R_a)

Reaction Number		Half-reaction
Cell Synthesis Equations (***R_c***)		
Ammonium as Nitrogen Source		
C-1	$\frac{1}{5}CO_2 + \frac{1}{20}HCO_3^- + \frac{1}{20}NH_4^+ + H^+ + e^-$	$= \frac{1}{20}C_5H_7O_2N + \frac{9}{20}H_2O$
Nitrate as Nitrogen Source		
C-2	$\frac{1}{28}NO_3^- + \frac{5}{28}CO_2 + \frac{29}{28}H^+ + e^-$	$= \frac{1}{28}C_5H_7O_2N + \frac{11}{28}H_2O$
Nitrite as Nitrogen Source		
C-3	$\frac{5}{26}CO_2 + \frac{1}{26}NO_2^- + \frac{27}{26}H^+ + e^-$	$= \frac{1}{26}C_5H_7O_2N + \frac{10}{26}H_2O$
Dinitrogen as Nitrogen Source		
C-4	$\frac{5}{23}CO_2 + \frac{1}{46}N_2 + H^+ + e^-$	$= \frac{1}{23}C_5H_7O_2N + \frac{8}{23}H_2O$
Common Electron-Acceptor Equations (***R_a***)		
I-14 Oxygen	$\frac{1}{4}O_2 + H^+ + e^-$	$= \frac{1}{2}H_2O$
I-7 Nitrate	$\frac{1}{5}NO_3^- + \frac{6}{5}H^+ + e^-$	$= \frac{1}{10}N_2 + \frac{3}{5}H_2O$
I-9 Sulfate	$\frac{1}{8}SO_4^{2-} + \frac{19}{16}H^+ + e^-$	$= \frac{1}{16}H_2S + \frac{1}{16}HS^- + \frac{1}{2}H_2O$
O-12 CO_2	$\frac{1}{8}CO_2 + H^+ + e^-$	$= \frac{1}{8}CH_4 + \frac{1}{4}H_2O$
I-4 Iron (III)	$Fe^{3+} + e^-$	$= Fe^{2+}$

2.5 Overall Reactions for Biological Growth

- **Obtain the overall reaction (R) including energy and synthesis using portions of electrons, f_e (= 0.6) and f_s (= 0.4)**

$R = f_e * R_e + f_s * R_s$

f_e*R_e:

$$0.02\ C_6H_5COO^- + 0.12\ NO_3^- + 0.12\ H^+ \rightarrow 0.12\ CO_2 + 0.06\ N_2 + 0.002\ HCO_3^- + 0.1 H_2O$$

f_s*R_s: $0.0133\ C_6H_5COO^- + 0.02\ NH_4^+ + 0.0067\ HCO_3^- \rightarrow 0.02\ C_5H_7O_2N + 0.0067\ H_2O$

R : $0.0333\ C_6H_5COO^- + 0.12\ NO_3^- + 0.02\ NH_4^+ + 0.12\ H^+$

$\rightarrow 0.02\ C_5H_7O_2N + 0.12\ CO_2 + 0.06\ N_2 + 0.0133\ HCO_3^- + 0.1067\ H_2O$

- **R represents the overall reaction for net synthesis of bacteria that are using benzoate as an e-donor, nitrate as an e-acceptor, and ammonium as nitrogen source.**

2.5 Overall Reactions for Biological Growth

- **Example**

- Benzoate : e-donor and C source (Rd = O-3 from Table 2.3)

- Nitrate : e-acceptor and N source (Rc, Ra = C-2, I-7 from Table 2.4)

- $f_s = 0.55$, $f_e = 0.45$

$f_e{*}R_a$: $0.09\ NO_3^- + 0.54\ H^+ + \underline{0.45\ e^-} \rightarrow 0.045\ N_2 + 0.27\ H_2O$

$f_s{*}R_c$: $0.0196\ NO_3^- + 0.0982 CO_2 + 0.5696\ H^+ + \underline{0.55\ e^-} \rightarrow 0.0196 C_5H_7O_2N + 0.2161\ H_2O$

\- R_d: $0.0333\ C_6H_5COO^- + 0.4333\ H_2O \rightarrow 0.2\ CO_2 + 0.0333\ HCO_3^- + H^+ + \underline{e^-}$

$$\mathbf{R = f_e * R_a + f_s * R_c - R_d}$$

R: $0.0333\ C_6H_5COO^- + \underline{0.1096\ NO_3^-} + 0.1096\ H^+ \rightarrow$

$\underline{0.0196\ C_5H_7O_2N} + \underline{0.045\ N_2} + 0.0333\ HCO_3^- + 0.1018\ CO_2 + 0.0528\ H_2O$

- **1 e^- = 0.45 e^- + 0.55 e^-**

- **0.09 mole nitrate is converted to nitrogen gas**

  0.0196 mole nitrate is converted into organic nitrogen of the cells

2.5 Overall Reactions for Biological Growth

√ Ex. 2.4 Nitrification Stoichiometry

- **Ammonium to nitrate, Aerobic condition, Inorganic carbon for cell synthesis, f_s=0.1, NH_4^+-N = 22 mg/L, Wastewater 1000 m^3,**

  Calculate oxygen consumption, biomass production, and concentration of NO_3^--N.

- NH_4^+: e^- donor, N source
- O_2: e^- acceptor
- CO_2: C source
- $f_s=0.1$, $f_e=0.9$

f_e*R_a: $0.225O_2 + 0.9\ H^+ + 0.9\ e^- \rightarrow 0.45\ H_2O$ (Table 2.4)

f_s*R_c: $0.02\ CO_2 + 0.005\ NH_4^+ + 0.005\ HCO_3^- + 0.1\ H^+ + 0.1\ e^-$

$\rightarrow 0.005\ C_5H_7O_2N + 0.045\ H_2O$ (Table 2.4)

-R_d: $0.125\ NH_4^+ + 0.375\ H_2O \rightarrow 0.125\ NO_3^- + 1.25\ H^+ + e^-$ (I-1 in Table 2.2)

$R = f_e * R_a + f_s * R_c - R_d$

R: $0.13\ NH_4^+ + 0.225O_2 + 0.02\ CO_2 + 0.005\ HCO_3$

$\rightarrow 0.005\ C_5H_7O_2N + 0.125\ NO_3^- + 0.25\ H^+ + 0.12\ H_2O$

2.5 Overall Reactions for Biological Growth

- **R: $0.13\ NH_4^+ + 0.225O_2 + 0.02\ CO_2 + 0.005\ HCO_3^- \rightarrow$**

$0.005\ C_5H_7O_2N + 0.125\ NO_3^- + 0.25\ H^+ + 0.12\ H_2O$

∴ 0.13 (14) = 1.82 g NH_4^+-N, 0.225 (32) = 7.2 g O_2,

0.005 (113) = 0.565 g cells, 0.125 (14) = 1.75 g NO_3^--N

- **Total NH_4^+-N in a wastewater of 1,000 m^3**

= (22 mg/l)(1000 m^3)(10^3 l/m^3)(1 kg/10^6 mg) = 22 kg

- **Then,**

- Oxygen consumption = 22 kg(7.2 g/1.82 g) = 87 kg

- Cell dry weight produced = 22 kg(0.565 g/1.82 g) = 6.83 kg

- Effluent NO_3- N con. = 22 mg/l(1.75 g/ 1.82 g) = 21 mg/l

2.5.1 Fermentation Reactions

√ **Fermentation**

an organic compound serves as both an e-donor and an e-acceptor

- **Knowledge of all reactants and products in a given case is all that is required to construct a balanced equation for the reaction, and we don't need to know the intermediates along the path, as long as they do not persist.**

  (It is because no matter how complicated are the pathways by which chemical A is transformed into chemical B, the laws of energy and mass conservation must be obeyed)

- **Simple fermentation: only one reduced product**

  (e.g., ethanol from glucose)

- **Mixed fermentation: more than one product or reactant**

2.5.1 Fermentation Reactions

√ Ex. 2.6 Simple Fermentation Stoichiometry

- **Glucose to ethanol fermentation, write the overall biological reaction.**

- Donor: glucose, Product: ethanol

- CO_2: e^- acceptor

- f_s=0.22, f_e= 1-0.22 = 0.78

$0.78*R_a$: $0.13\ CO_2 + 0.78\ H^+ + 0.78\ e^- \rightarrow 0.065\ CH_3CH_2OH + 0.195\ H_2O$ (o-5 in Table 2.3)

$0.22*R_c$: $0.044\ CO_2 + 0.011 NH_4^+ + 0.011\ HCO_3^- + 0.22\ H^+ + 0.22\ e^-$

$\rightarrow 0.011\ C_5H_7O_2N + 0.099\ H_2O$ (C-1 in Table 2.4)

$-R_d$: $0.0417\ C_6H_{12}O_6 + 0.25\ H_2O \rightarrow 0.25\ CO_2 + H^+ + e^-$ (o-7 in Table 2.3)

$$\mathbf{R = f_e * R_a + f_s * R_c - R_d}$$

R: $0.0417\ C_6H_{12}O_6 + 0.011 NH_4^+ + 0.011\ HCO_3^-$

$\rightarrow 0.011\ C_5H_7O_2N + 0.065\ CH_3CH_2OH + 0.076\ CO_2 + 0.044\ H_2O$

- **Every equivalent of glucose (one e^-) fermented, 0.065 mole ethanol is formed.**

2.5.1 Fermentation Reactions

√ Mixed Fermentation

more than one product and/or one reactant

- **More than one product (mixed e- acceptors)**

$$R_a = \sum_{i=1}^{n} e_{ai} R_{ai} \text{ , where} \qquad e_{ai} = \frac{equiv_{ai}}{\sum_{j=1}^{n} equiv_{aj}} \quad \text{and} \quad \sum_{i=1}^{n} e_{ai} = 1$$

- **More than a single reactant (mixed e- donors)**

$$R_d = \sum_{i=1}^{n} e_{di} R_{di} \text{ , where} \qquad e_{di} = \frac{equiv_{di}}{\sum_{j=1}^{n} equiv_{dj}} \quad \text{and} \quad \sum_{i=1}^{n} e_{di} = 1$$

R_a (R_d) : a half reaction for e-acceptors (e-donors)

e_{ai} (e_{di}): fraction of the reduced (oxidized) end product produced by the reaction R_{ai} (R_{di})

$equiv_{ai}$ $(equiv_{di})$: equivalents of *ai* (*di*) produced

2.5.1 Fermentation Reactions

√ Ex. 2.7 Citrate Fermentation (multiple products)

- **Bacteroides sp. converts 1 mol citrate into 1 mol formate, 2 mol acetate, and 1 mol bicarbonate. Write R_e.**
- **Energy reactions from Table 2.3**

$e_{formate}$ = 2/(2+16) = 0.111

$e_{acetate}$ = 16/(2+16) = 0.889

- **# of equivalents**

 - formate: reduced end product, 2 e^- for one mole formate (o-6)

 - acetate: reduced end product, 16 e^- for two moles acetate (o-1)

 - bicarbonate: not considered because it's oxidized end product, like CO_2

- **$R_a = e_{formate}R_{a,formate} + e_{acetate}R_{a,acetate}$**

$0.111 {*} R_{a,formate}$: $0.0555\ HCO_3^- + 0.111\ H^+ + 0.111\ e^- \rightarrow 0.0555\ HCOO^- + 0.0555\ H_2O$

$0.889 {*} R_{a,acetate}$: $0.111\ CO_2 + 0.111\ HCO_3^- + 0.889\ H^+ + 0.889\ e^- \rightarrow 0.111\ CH_3COO^- + 0.333\ H_2O$

R_a: $0.111\ CO_2 + 0.166\ HCO_3^- + H^+ + e^- \rightarrow 0.0555\ HCOO^- + 0.111\ CH_3COO^- + 0.388\ H_2O$

2.5.1 Fermentation Reactions

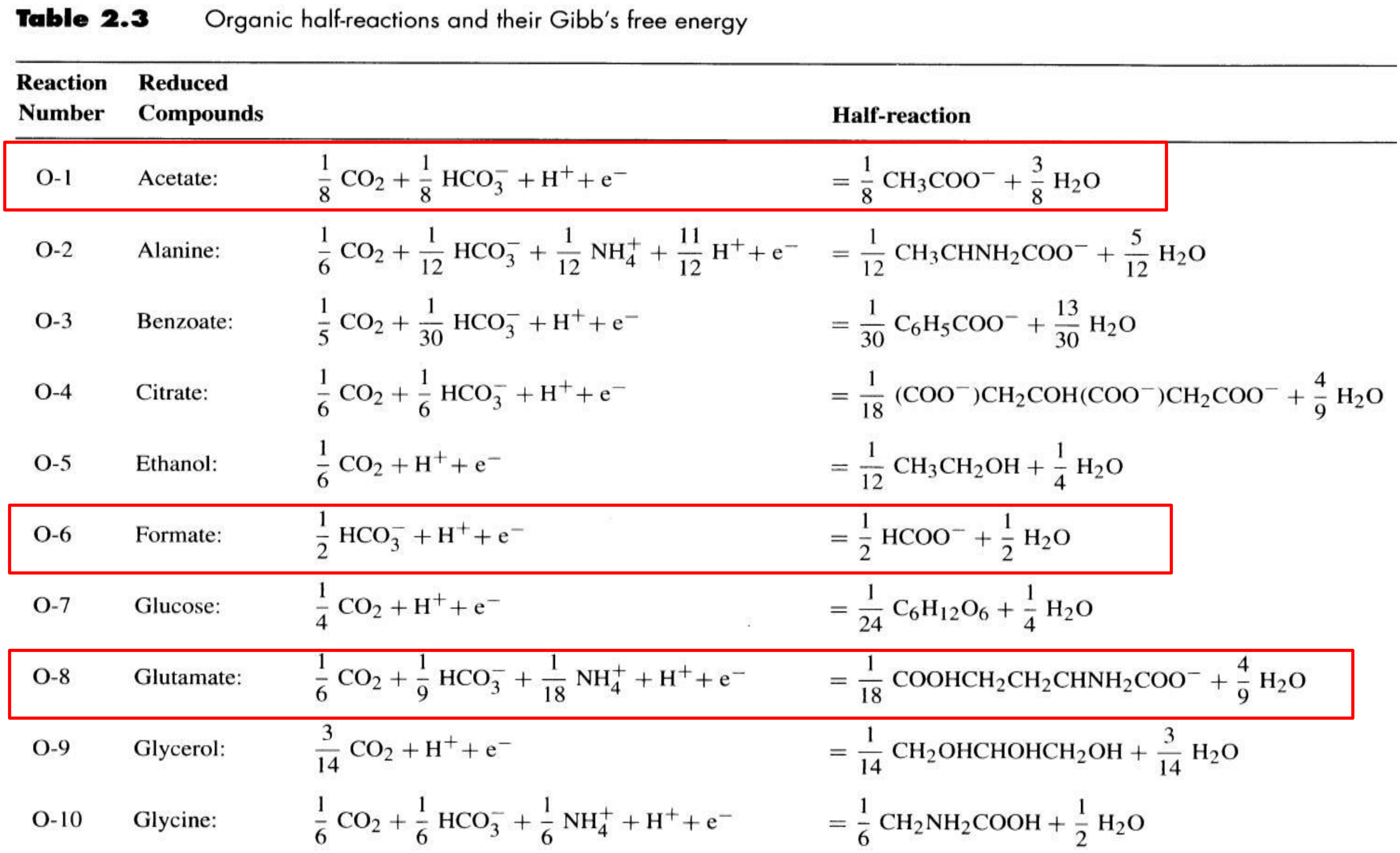


Table 2.3 Organic half-reactions and their Gibb's free energy

Reaction Number	Reduced Compounds		Half-reaction
O-1	Acetate:	$\frac{1}{8}\mathrm{CO_2} + \frac{1}{8}\mathrm{HCO_3^-} + \mathrm{H^+} + e^-$	$= \frac{1}{8}\mathrm{CH_3COO^-} + \frac{3}{8}\mathrm{H_2O}$
O-2	Alanine:	$\frac{1}{6}\mathrm{CO_2} + \frac{1}{12}\mathrm{HCO_3^-} + \frac{1}{12}\mathrm{NH_4^+} + \frac{11}{12}\mathrm{H^+} + e^-$	$= \frac{1}{12}\mathrm{CH_3CHNH_2COO^-} + \frac{5}{12}\mathrm{H_2O}$
O-3	Benzoate:	$\frac{1}{5}\mathrm{CO_2} + \frac{1}{30}\mathrm{HCO_3^-} + \mathrm{H^+} + e^-$	$= \frac{1}{30}\mathrm{C_6H_5COO^-} + \frac{13}{30}\mathrm{H_2O}$
O-4	Citrate:	$\frac{1}{6}\mathrm{CO_2} + \frac{1}{6}\mathrm{HCO_3^-} + \mathrm{H^+} + e^-$	$= \frac{1}{18}\mathrm{(COO^-)CH_2COH(COO^-)CH_2COO^-} + \frac{4}{9}\mathrm{H_2O}$
O-5	Ethanol:	$\frac{1}{6}\mathrm{CO_2} + \mathrm{H^+} + e^-$	$= \frac{1}{12}\mathrm{CH_3CH_2OH} + \frac{1}{4}\mathrm{H_2O}$
O-6	Formate:	$\frac{1}{2}\mathrm{HCO_3^-} + \mathrm{H^+} + e^-$	$= \frac{1}{2}\mathrm{HCOO^-} + \frac{1}{2}\mathrm{H_2O}$
O-7	Glucose:	$\frac{1}{4}\mathrm{CO_2} + \mathrm{H^+} + e^-$	$= \frac{1}{24}\mathrm{C_6H_{12}O_6} + \frac{1}{4}\mathrm{H_2O}$
O-8	Glutamate:	$\frac{1}{6}\mathrm{CO_2} + \frac{1}{9}\mathrm{HCO_3^-} + \frac{1}{18}\mathrm{NH_4^+} + \mathrm{H^+} + e^-$	$= \frac{1}{18}\mathrm{COOHCH_2CH_2CHNH_2COO^-} + \frac{4}{9}\mathrm{H_2O}$
O-9	Glycerol:	$\frac{3}{14}\mathrm{CO_2} + \mathrm{H^+} + e^-$	$= \frac{1}{14}\mathrm{CH_2OHCHOHCH_2OH} + \frac{3}{14}\mathrm{H_2O}$
O-10	Glycine:	$\frac{1}{6}\mathrm{CO_2} + \frac{1}{6}\mathrm{HCO_3^-} + \frac{1}{6}\mathrm{NH_4^+} + \mathrm{H^+} + e^-$	$= \frac{1}{6}\mathrm{CH_2NH_2COOH} + \frac{1}{2}\mathrm{H_2O}$

2.5.1 Fermentation Reactions

- **Overall reaction, $R_e = R_a - R_d$ (from table 2-3)**

R_a: $0.111\ CO_2 + 0.166\ HCO_3^- + H\ + e^- \rightarrow 0.0555\ HCOO^- + 0.111\ CH_3COO + 0.388\ H_2O$

$-R_d$: $0.0555\ (COO^-)CH_2COH(COO^-)CH_2COO^- + 0.444\ H_2O$

$\rightarrow 0.166\ CO_2 + 0.166\ HCO_3^- + H\ + e^-$

R_e: $0.0555\ (COO^-)CH_2COH(COO^-)CH_2COO^- + 0.056\ H_2O$

$\rightarrow 0.0555\ HCOO^- + 0.111\ CH_3COO^- + 0.056\ CO_2$

- **By normalization, this equation becomes :**

$$\boxed{\therefore\ 1\ (COO^-)CH_2COH(COO^-)CH_2COO^- + H_2O \rightarrow 1\ HCOO^- + 2\ CH_3COO^- + 1CO_2}$$

2.6 Energetics and Bacterial Growth

√ The purpose of this section is to explore relationships between reaction energetics and bacterial growth

- When cells grow rapidly in the presence of nonlimiting concentrations of all factors required for growth, cells make the maximum investment of energy for synthesis.

$$\frac{dx_a}{dt} = Y\left(\frac{-ds}{dt}\right) - bx_a$$

$$Y_n = \frac{dx_a / dt}{-ds / dt} = Y - b\frac{x_a}{-ds/dt}$$

- When substrate and all other required factors are unlimited in amount, -dS/dt will be at its maximum, and the net yield (Y_n) will approach the true yield (Y)

- However, when substrate is limited, the net yield decreases as a decrease in the rate of substrate utilization.

$$Y_n = 0,\ Y - b\frac{x_a}{-ds/dt} = 0, \qquad then \quad \frac{-dS / dt}{X_a} = \frac{b}{Y} = m$$

- The net yield becomes zero (X_a = const.) when the energy supplied through substrate utilization is just equal to m, the maintenance energy

2.6 Energetics and Bacterial Growth

- Battley(1987) provided us with relationships presented between energy production and cell yield (the equation below)

$$R = f_e R_a + f_s R_c - R_d$$

- This method is based on electron equivalents and differentiates between the energy portion of an overall biological reaction and the synthesis portion.

(MaCatry, 1971, 1975; Christensen and McCarty 1975)

- A practical advantage of the approach : electron equivalents are easily related to measurements of widespread utility in environmental engineering practice

- BOD (biochemical oxygen demand), COD` (calculated oxygen demand), COD (chemical oxygen demand)

- One equivalent of electron donor = 8 g O_2

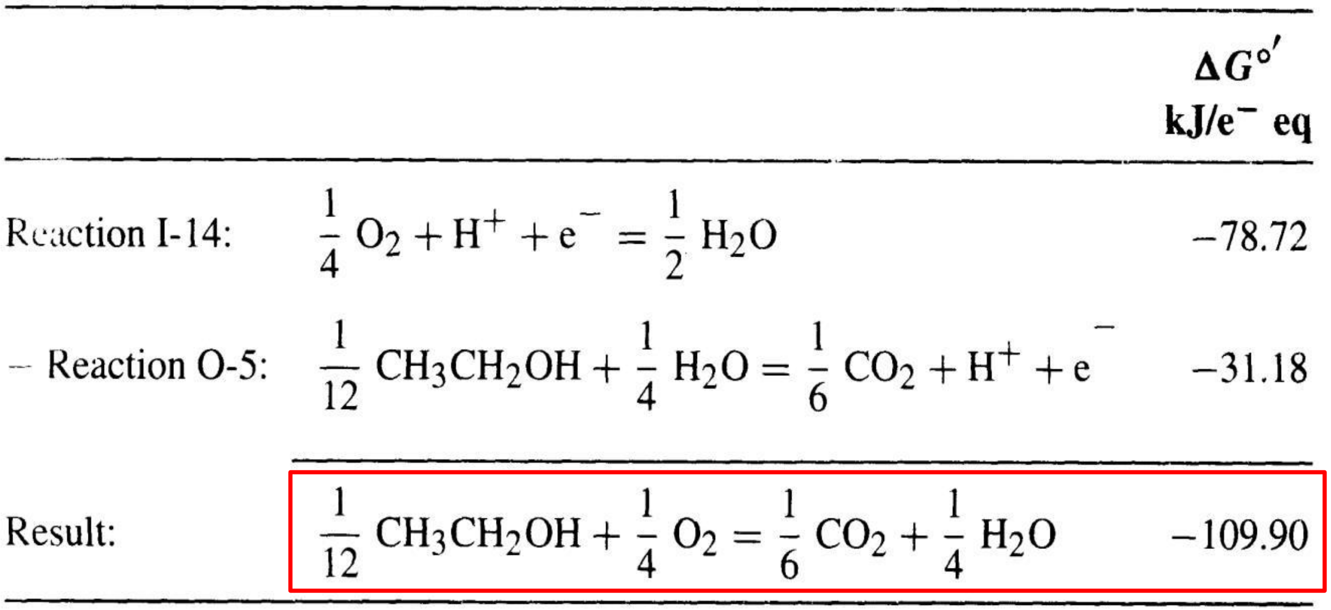
2.6.1 Free Energy of the Energy Reaction

√ Standard Gibb's free energy at neutral pH

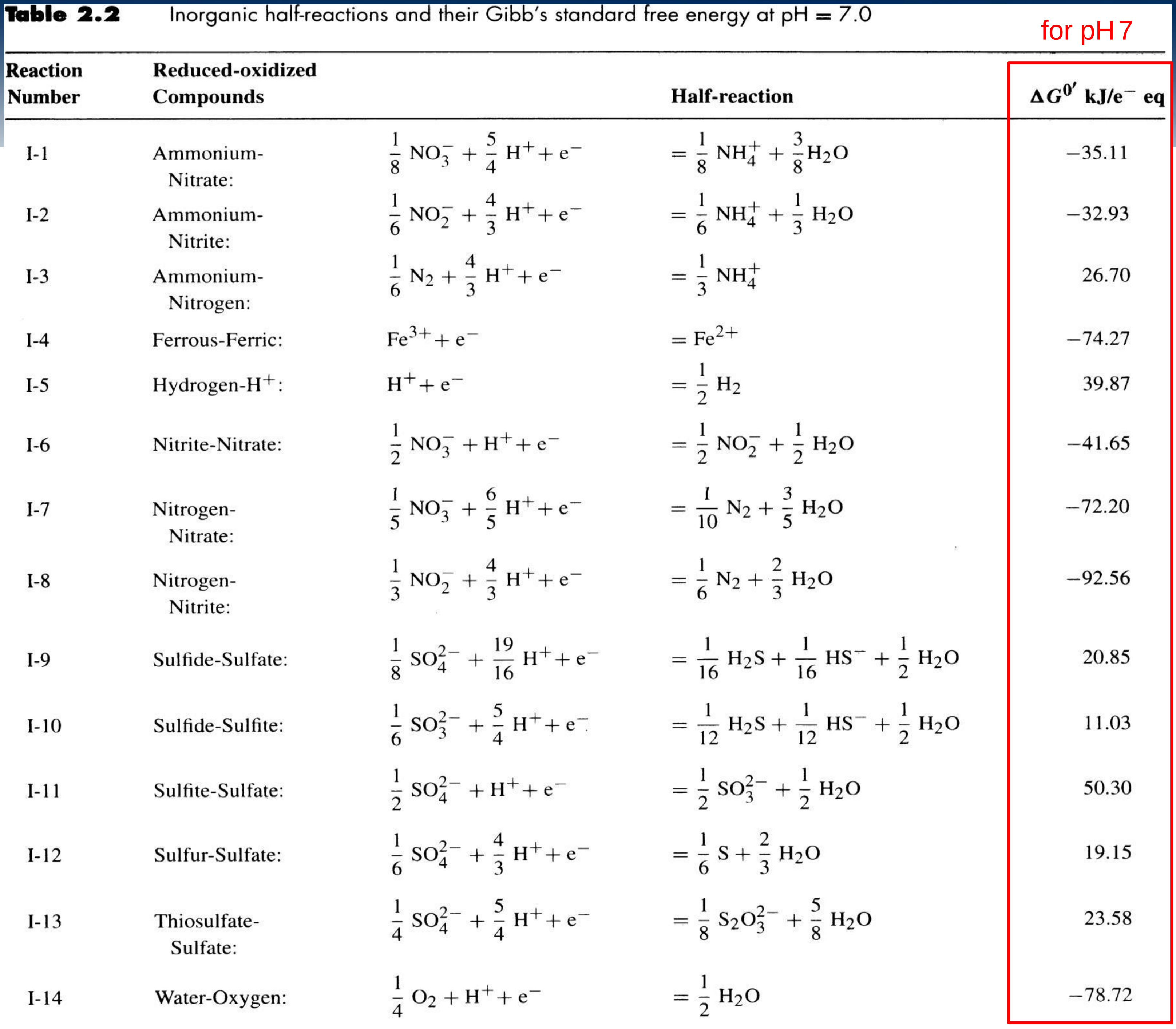
$\Delta G_r^{0\prime}$: standard free energies corrected to pH 7
: All constituents are at unit activity except [H$^+$] (pH=7.0)

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

e.g. Ethanol : $\Delta G_r^{0\prime} = \Delta G_r^{0}$, *because H$^+$ is not a component of the equation*



		$\Delta G^{\circ\prime}$ kJ/e$^-$ eq
Reaction I-14:	$\frac{1}{4}O_2 + H^+ + e^- = \frac{1}{2}H_2O$	−78.72
− Reaction O-5:	$\frac{1}{12}CH_3CH_2OH + \frac{1}{4}H_2O = \frac{1}{6}CO_2 + H^+ + e^-$	−31.18
Result:	$\frac{1}{12}CH_3CH_2OH + \frac{1}{4}O_2 = \frac{1}{6}CO_2 + \frac{1}{4}H_2O$	−109.90



for pH 7

Table 2.2 Inorganic half-reactions and their Gibb's standard free energy at pH = 7.0

Reaction Number	Reduced-oxidized Compounds	Half-reaction		$\Delta G^{0'}$ 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:	$\frac{1}{6}SO_4^{2-} + \frac{4}{3}H^+ + e^-$	$= \frac{1}{6}S + \frac{2}{3}H_2O$	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

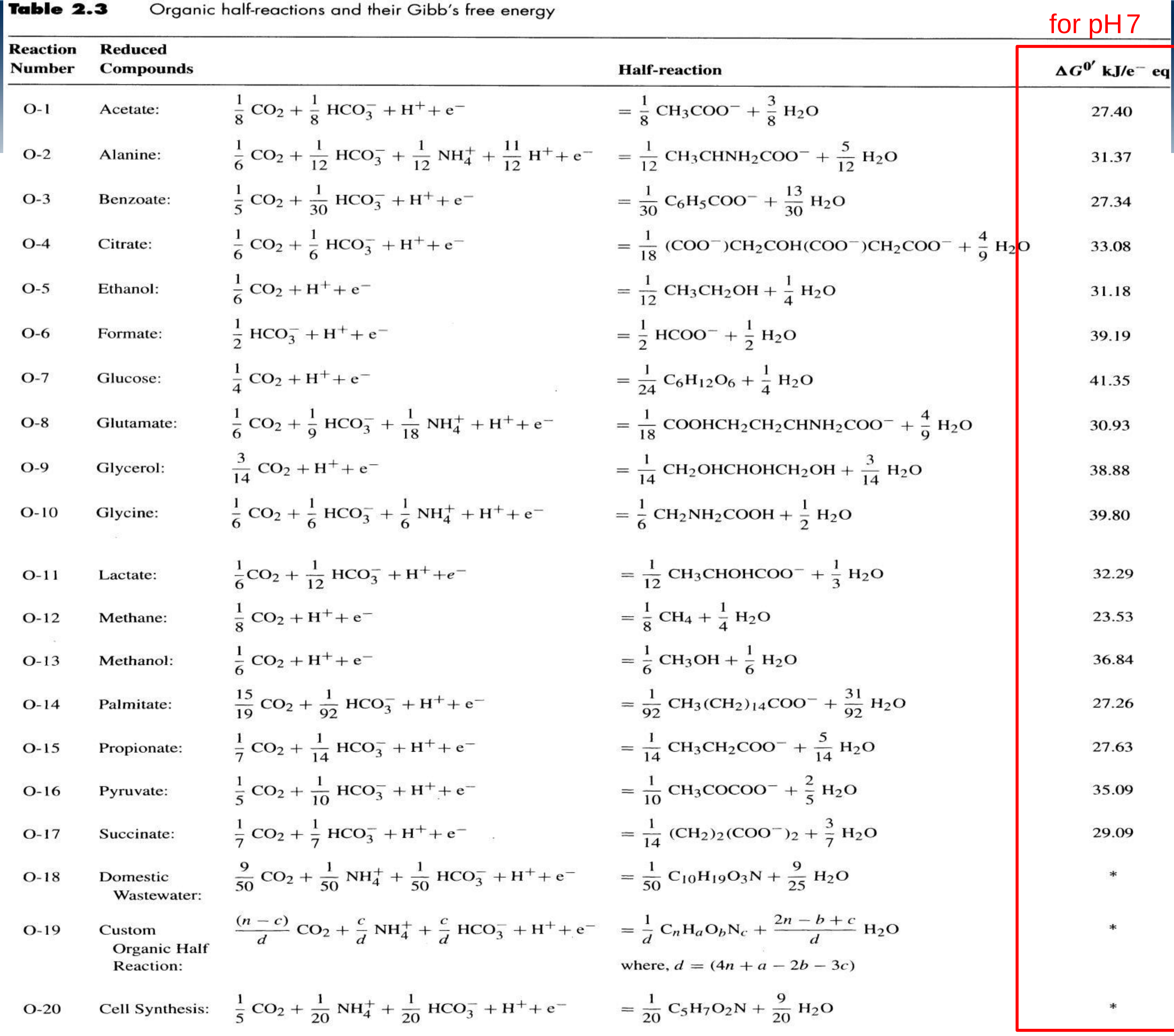


Table 2.3 Organic half-reactions and their Gibb's free energy

for pH 7

Reaction Number	Reduced Compounds	Half-reaction	$\Delta G^{0'}$ 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
O-11	Lactate:	$\frac{1}{6} CO_2 + \frac{1}{12} HCO_3^- + H^+ + e^- = \frac{1}{12} CH_3CHOHCOO^- + \frac{1}{3} H_2O$	32.29
O-12	Methane:	$\frac{1}{8} CO_2 + H^+ + e^- = \frac{1}{8} CH_4 + \frac{1}{4} H_2O$	23.53
O-13	Methanol:	$\frac{1}{6} CO_2 + H^+ + e^- = \frac{1}{6} CH_3OH + \frac{1}{6} H_2O$	36.84
O-14	Palmitate:	$\frac{15}{19} CO_2 + \frac{1}{92} HCO_3^- + H^+ + e^- = \frac{1}{92} CH_3(CH_2)_{14}COO^- + \frac{31}{92} H_2O$	27.26
O-15	Propionate:	$\frac{1}{7} CO_2 + \frac{1}{14} HCO_3^- + H^+ + e^- = \frac{1}{14} CH_3CH_2COO^- + \frac{5}{14} H_2O$	27.63
O-16	Pyruvate:	$\frac{1}{5} CO_2 + \frac{1}{10} HCO_3^- + H^+ + e^- = \frac{1}{10} CH_3COCOO^- + \frac{2}{5} H_2O$	35.09
O-17	Succinate:	$\frac{1}{7} CO_2 + \frac{1}{7} HCO_3^- + H^+ + e^- = \frac{1}{14} (CH_2)_2(COO^-)_2 + \frac{3}{7} H_2O$	29.09
O-18	Domestic Wastewater:	$\frac{9}{50} CO_2 + \frac{1}{50} NH_4^+ + \frac{1}{50} HCO_3^- + H^+ + e^- = \frac{1}{50} C_{10}H_{19}O_3N + \frac{9}{25} H_2O$	*
O-19	Custom Organic Half Reaction:	$\frac{(n-c)}{d} CO_2 + \frac{c}{d} NH_4^+ + \frac{c}{d} HCO_3^- + H^+ + e^- = \frac{1}{d} C_nH_aO_bN_c + \frac{2n-b+c}{d} H_2O$ where, $d = (4n + a - 2b - 3c)$	*
O-20	Cell Synthesis:	$\frac{1}{5} CO_2 + \frac{1}{20} NH_4^+ + \frac{1}{20} HCO_3^- + H^+ + e^- = \frac{1}{20} C_5H_7O_2N + \frac{9}{20} H_2O$	*

2.6.1 Free Energy of the Energy Reaction

√ Free Energy of ethanol oxidation

$$\frac{1}{12}CH_3CH_2OH + \frac{1}{4}O^2 = \frac{1}{6}CO_2 + \frac{1}{4}H_2O$$

Conditions :

- Ethanol : 0.002 M,
- O_2 partial pressure : 0.21 atm
- CO_2 partial pressure : 0.0003 atm
- Temp. : 20 °C
- All activity coefficients : 1.0

$$\Delta G_r = ?$$

$$\Delta G_r = \Delta G_r^0 + RT \ln \frac{[CO_2]^{1/6}[H_2O]^{1/4}}{[CH_3CH_2OH]^{1/12}[O_2]^{1/4}}$$

2.6.1 Free Energy of the Energy Reaction

$$\Delta G_r = \Delta G_r^0 + RT \ln \frac{[CO_2]^{1/6}[H_2O]^{1/4}}{[CH_3CH_2OH]^{1/12}[O_2]^{1/4}}$$

$$\Delta G_r = -109{,}900 + 8.314(273 + 20) \ln \frac{[0.0003]^{1/6}[1]^{1/4}}{[0.002]^{1/12}[0.21]^{1/4}}$$

$$= -109{,}900 + 1{,}100 (\sim 1\%)$$

$$= -111{,}000 J / e^- eq \;\; or \;\; -111 \, kJ / e^- eq$$

- **Conclusion :**

  - The reaction free energy, corrected for concentrations (1.1 kJ/e- eq) that are within a typical range for biological systems of interest, is within 1 % of the standard free energy of -109.9 kJ/e^- eq.

  - Concentration corrections become important when the concentration of e- donor or acceptor is very low or ΔG_r^0 *is < -10 kJ/e- eq*

2.6.1 Free Energy of the Energy Reaction

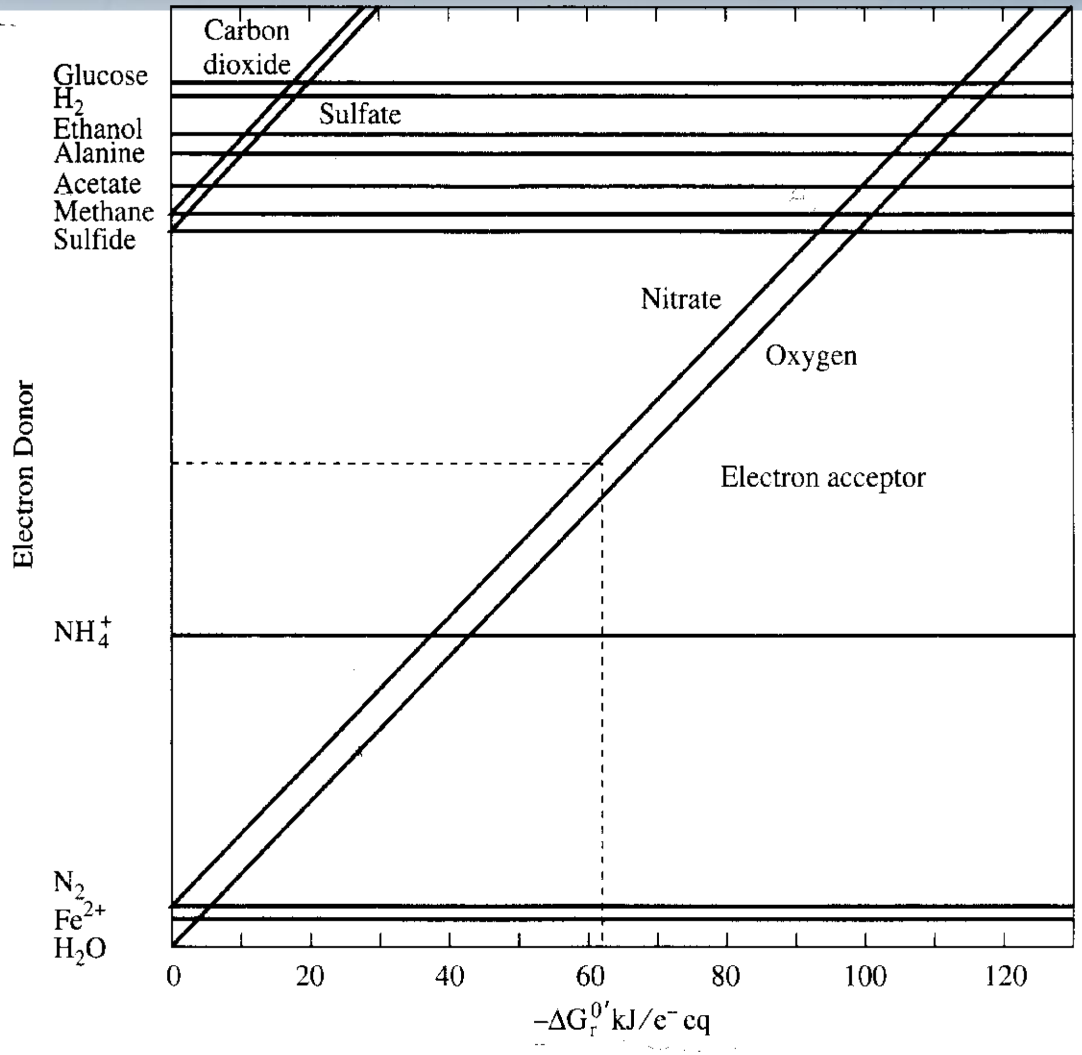


Figure 2.2 Relationship between various electron donors and acceptors and resulting reaction free energy.

√ Aerobic oxidation
(Oxygen as electron acceptor)

Ex 1.1 The range of organic materials from Methane to glucose - from -96 to -120 kJ/e⁻ eq (∆ 24)

Ex 1.2 The range of inorganic materials from iron oxidation to hydrogen oxidation (fuel cell)

- from -5 to -119 kJ/e⁻ eq (∆ 114)

√ Anaerobic oxidation
(Carbon dioxide as electron acceptor)

Ex 2.1 The range of organic materials from acetate oxidation to glucose oxidation

- from -3.87 to -18.82 kJ/e⁻ eq (∆14.95)

The great differences in reaction free energies for aerobic verse anaerobic and organic verse inorganic reaction **have great effects on resulting bacterial yields**

2.7 Yield Coefficient and Reaction Energetics

√ The microbial yield from substrate utilization

- **The energy reaction creates high-energy carriers, such as ATP**
- **The energy carriers are "spent" to drive cell synthesis or cell maintenance**
- **A certain amount of thermodynamic free energy is lost with each transfer**

- **How to compute the energy costs of cell synthesis and how to account for the energy lost in transfers**

(= How to estimate f_s^0 and the true yield (Y) based on thermodynamic principles)

- **For determining true yield (Y), the energy for maintenance is set to zero, so that all energy is used for cell synthesis.**

2.7 Yield Coefficient and Reaction Energetics

√ How to estimate f_s^0 and Y based on thermodynamic principle

How much energy is needed to synthesize an equivalent of cells from carbon source?

ΔG_s : the energy required to synthesize one equivalent of cells from a given carbon source (when NH_4^+ is N-source)

ΔG_p : the energy required to convert the carbon source to pyruvate

ΔG_{pc}: the energy required to convert the pyruvate carbon to cellular source

$\Delta G_c^{0\prime}$: free energy of half reaction for the given e- donnor (= carbon source)

- First, we must determine the energy change resulting from the conversion of the carbon source to the common organic intermediates that the cell uses for synthesizing macromolecules (use pyruvate as a representative intermediate)

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

- Free energy of half reaction for pyruvate synthesis (O-16, in Table 2.3) is 35.09 kJ /e^- eq.

2.7 Yield Coefficient and Reaction Energetics

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

- For heterotrophic bacteria, the carbon source almost always is the e- donor.

e.g., Heterotrophic bacterial growth using organics

- Acetate (carbon & e- source)

$\Delta G_c^{0\prime} = 27.40$ (Table 2.3), $\Delta G_p = 35.09 - 27.40 = 7.69$ kJ/e- eq

- Glucose (carbon & e- source)

$\Delta G_c^{0\prime} = 41.35$ (Table 2.3), $\Delta G_p = 35.09 - 41.35 = -6.26$ kJ/e- eq

- In autotrophic reactions, inorganic carbon is used as the carbon source.
- In photosynthesis, the H^+ and e^- for reducing CO_2 to form cellular organic matter comes from water.

e.g., Photosynthesis

Carbon source (CO_2), e- source (H_2O)

We take $\frac{1}{4} O_2 + H^+ + e^- = \frac{1}{2} H_2O$ -78.72 kJ/e- eq (Table2.2)

$\Delta G_c^{0\prime} = -78.72$ (Table 2.2), $\Delta G_p = 35.09 - (-78.72) = 113.8$ kJ/e- eq

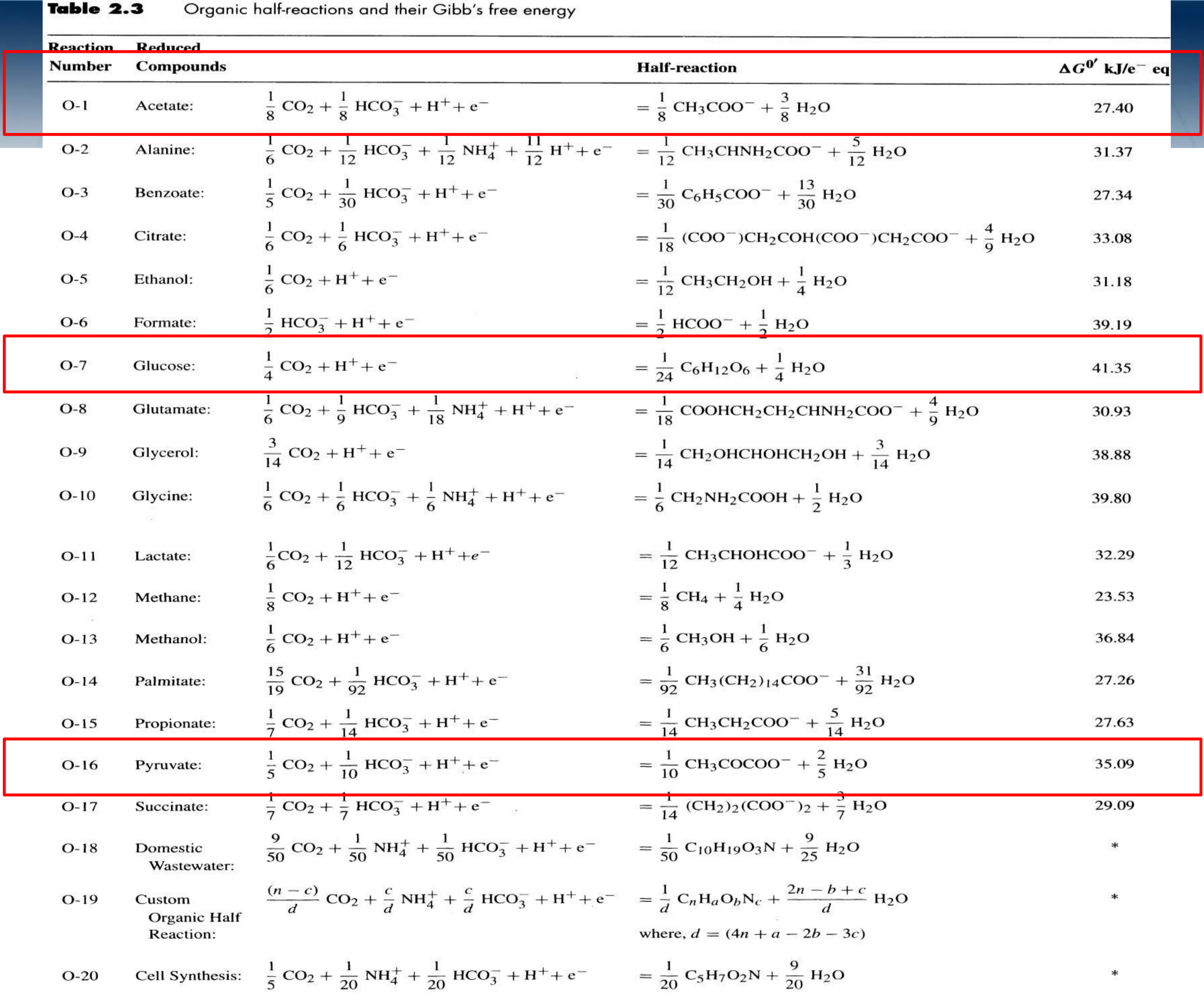


Table 2.3 Organic half-reactions and their Gibb's free energy

Reaction Number	Reduced Compounds	Half-reaction		$\Delta G^{0'}$ 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
O-11	Lactate:	$\frac{1}{6}CO_2 + \frac{1}{12}HCO_3^- + H^+ + e^-$	$= \frac{1}{12}CH_3CHOHCOO^- + \frac{1}{3}H_2O$	32.29
O-12	Methane:	$\frac{1}{8}CO_2 + H^+ + e^-$	$= \frac{1}{8}CH_4 + \frac{1}{4}H_2O$	23.53
O-13	Methanol:	$\frac{1}{6}CO_2 + H^+ + e^-$	$= \frac{1}{6}CH_3OH + \frac{1}{6}H_2O$	36.84
O-14	Palmitate:	$\frac{15}{19}CO_2 + \frac{1}{92}HCO_3^- + H^+ + e^-$	$= \frac{1}{92}CH_3(CH_2)_{14}COO^- + \frac{31}{92}H_2O$	27.26
O-15	Propionate:	$\frac{1}{7}CO_2 + \frac{1}{14}HCO_3^- + H^+ + e^-$	$= \frac{1}{14}CH_3CH_2COO^- + \frac{5}{14}H_2O$	27.63
O-16	Pyruvate:	$\frac{1}{5}CO_2 + \frac{1}{10}HCO_3^- + H^+ + e^-$	$= \frac{1}{10}CH_3COCOO^- + \frac{2}{5}H_2O$	35.09
O-17	Succinate:	$\frac{1}{7}CO_2 + \frac{1}{7}HCO_3^- + H^+ + e^-$	$= \frac{1}{14}(CH_2)_2(COO^-)_2 + \frac{3}{7}H_2O$	29.09
O-18	Domestic Wastewater:	$\frac{9}{50}CO_2 + \frac{1}{50}NH_4^+ + \frac{1}{50}HCO_3^- + H^+ + e^-$	$= \frac{1}{50}C_{10}H_{19}O_3N + \frac{9}{25}H_2O$	*
O-19	Custom Organic Half Reaction:	$\frac{(n-c)}{d}CO_2 + \frac{c}{d}NH_4^+ + \frac{c}{d}HCO_3^- + H^+ + e^-$	$= \frac{1}{d}C_nH_aO_bN_c + \frac{2n-b+c}{d}H_2O$ where, $d = (4n + a - 2b - 3c)$	*
O-20	Cell Synthesis:	$\frac{1}{5}CO_2 + \frac{1}{20}NH_4^+ + \frac{1}{20}HCO_3^- + H^+ + e^-$	$= \frac{1}{20}C_5H_7O_2N + \frac{9}{20}H_2O$	*

Table 2.2 Inorganic half-reactions and their Gibb's standard free energy at pH = 7.0

Reaction Number	Reduced-oxidized Compounds	Half-reaction	$\Delta G^{0'}$ 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:	$\frac{1}{6} SO_4^{2-} + \frac{4}{3} H^+ + e^- = \frac{1}{6} S + \frac{2}{3} H_2O$	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

2.7 Yield Coefficient and Reaction Energetics

- Second, Pyruvate carbon is converted to cellular carbon.

  The energy required here is

ΔG_{pc} = [an estimated value of 3.33 kJ/g cell (McCarty, 1971)] x [5.65 g/e$^-$ eq]

**from Table 2.4, ammonium as Nitrogen source, 113.8/20 = 5.65*

= 18.8 kJ/e$^-$ eq

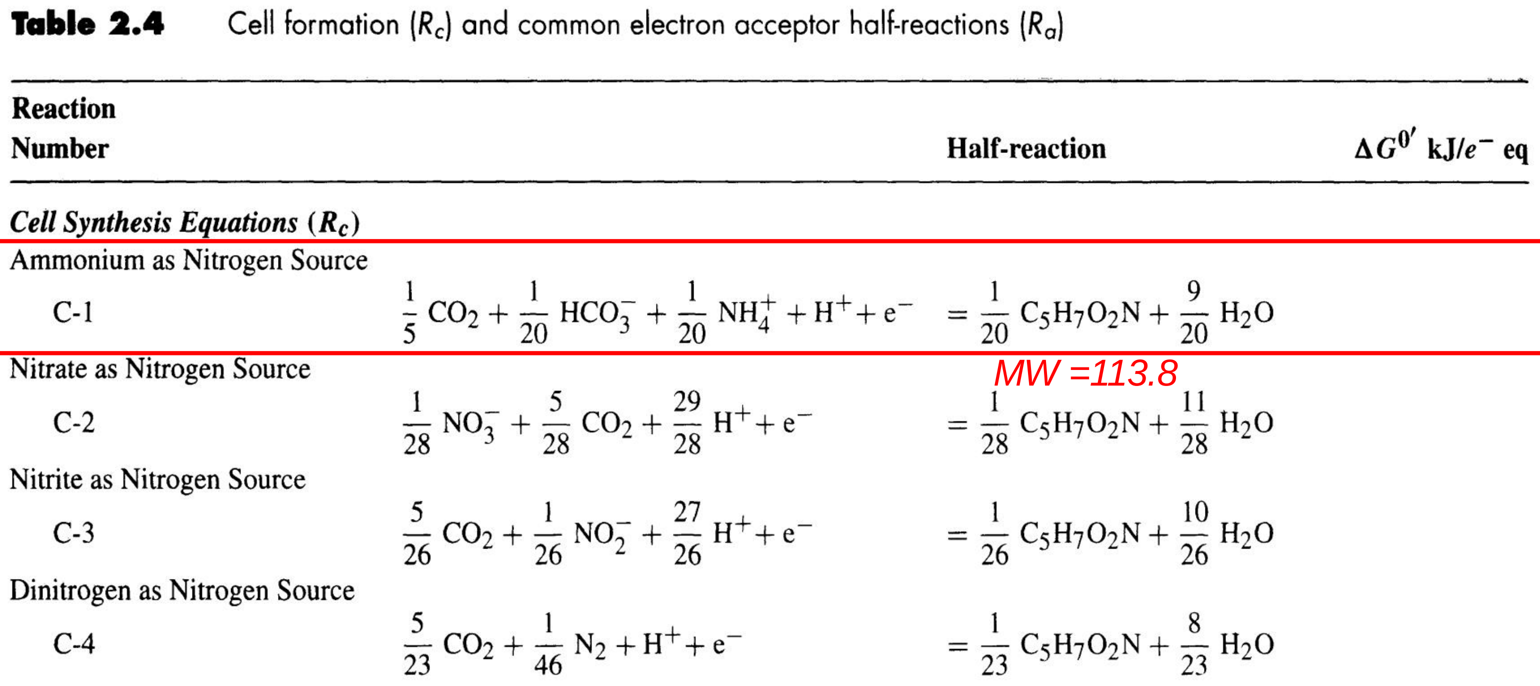


Table 2.4 Cell formation (R_c) and common electron acceptor half-reactions (R_a)

Reaction Number		Half-reaction	$\Delta G^{0'}$ kJ/e^- eq
Cell Synthesis Equations **(R_c)**			
Ammonium as Nitrogen Source			
C-1	$\frac{1}{5}CO_2 + \frac{1}{20}HCO_3^- + \frac{1}{20}NH_4^+ + H^+ + e^-$	$= \frac{1}{20}C_5H_7O_2N + \frac{9}{20}H_2O$	
Nitrate as Nitrogen Source			
C-2	$\frac{1}{28}NO_3^- + \frac{5}{28}CO_2 + \frac{29}{28}H^+ + e^-$	$= \frac{1}{28}C_5H_7O_2N + \frac{11}{28}H_2O$	
Nitrite as Nitrogen Source			
C-3	$\frac{5}{26}CO_2 + \frac{1}{26}NO_2^- + \frac{27}{26}H^+ + e^-$	$= \frac{1}{26}C_5H_7O_2N + \frac{10}{26}H_2O$	
Dinitrogen as Nitrogen Source			
C-4	$\frac{5}{23}CO_2 + \frac{1}{46}N_2 + H^+ + e^-$	$= \frac{1}{23}C_5H_7O_2N + \frac{8}{23}H_2O$	

MW =113.8

2.7 Yield Coefficient and Reaction Energetics

- Finally, the energy that is lost in the electron transfers is considered by including a term for energy-transfer efficiency (**ϵ**)

$$\Delta G_s = \frac{\Delta G_p}{\varepsilon^n} + \frac{\Delta G_{pc}}{\varepsilon}$$

= energy required
for cell synthesis
from carbon source

n = +1, when ΔG_p is positive n(energy is required in its conversion to pyruvate)

e.g., **Conversion of <u>acetate</u> to pyruvate**

More energy is required than the thermodynamic amount

n = -1, when ΔG_p is negative (energy is released by its conversion to pyruvate)

e.g., Conversion of <u>glucose</u> to pyruvate >

Some of the thermodynamic energy is lost.

2.7 Yield Coefficient and Reaction Energetics

√ *Energy balance for energy generation and consumption (for cell synthesis)*

ΔG_r : free energy released per equivalent of donor oxidized for energy generation

A : equivalents of electron donor
(required to supply energy to synthesize an equivalent of cells)

$\mathrm{A}\Delta G_r$: the energy released by donor oxidized for energy generation

$A\varepsilon\,\Delta G_r$: the energy transferred to the carrier;

when this energy is transferred to the energy carrier, a portion is lost through transfer inefficiencies. If transfer efficiency here is the same as that for transferring energy (ε)

- An energy balance must be maintained around the energy carrier at steady state:

$$A\varepsilon\Delta G_r + \Delta G_s = 0 \quad \Longrightarrow \quad A = \frac{\dfrac{\Delta G_p}{\varepsilon^n} + \dfrac{\Delta G_{pc}}{\varepsilon}}{-\varepsilon\Delta G_r}$$

$$A = f_e^0 / f_s^0$$

2.7 Yield Coefficient and Reaction Energetics

√ *Now, we can estimate f_s^0 and Y using A*

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

- The above equation does not include energy of maintenance, the resulting value of A is for the situation of the true yield or Y

$$f_s^0 = \frac{1}{1+A} \quad and \quad f_e^0 = 1 - f_s^0 = \frac{A}{1+A}$$

$$Y = \frac{f_s^0 (M_c)}{(n_e)(8)}$$

(refer to Slide 11)

Example 2.10

EFFECTS OF ε ON HETEROTROPHIC YIELD Compare estimates for f_s^0 and Y for aerobic oxidation of acetate, assuming $\varepsilon = 0.4$, 0.6, and 0.7, that pH = 7, and that all other reactants and products are at unit activity. Ammonium is available for synthesis.

Since the reaction is heterotrophic, Equation 2.45 applies. Using reaction O-1 from Table 2.3, $\Delta G_d^{0\prime} = 27.40$ kJ/e⁻ eq. Therefore,

$$\Delta G_p = 35.09 - 27.40 = 7.69 \text{ kJ/e}^- \text{ eq}$$

Since this is an aerobic reaction, $\Delta G_a^{0\prime} = -78.72$ kJ/e⁻ eq. Thus,

$$\Delta G_r = \Delta G_a^{0\prime} - \Delta G_d^{0\prime} = -78.72 - 27.40 = -106.12 \text{ kJ/e}^- \text{ eq}$$

Since ΔG_p is positive, $n = +1$. Also, since ammonium is available for cell synthesis, ΔG_{pc} equals 18.8 kJ/e^- eq. Hence,

$$A = -\frac{\dfrac{7.69}{\varepsilon^{+1}} + \dfrac{18.8}{\varepsilon}}{-106.12\varepsilon}$$

Letting $\varepsilon = 0.4$, 0.6, and 0.7, $A = 1.56$, 0.69, and 0.51, respectively. Using Equation 2.49, values for f_s^0 that result are:

ε	f_s^0	Y g cells/mol donor	Y g cells/g donor	Y g cells/g COD′
0.4	0.39	18	0.30	0.28
0.6	0.59	27	0.45	0.42
0.7	0.66	30	0.51	0.47

In order to determine bacterial yield, it is well to write a balanced stoichiometric equation. This will be done for illustration only for the case where $\varepsilon = 0.6$, for which $f_s^0 = 0.59$ and $f_e^0 = 1 - 0.59 = 0.41$:

$$-\text{Reaction O-1:}\quad 0.125\ CH_3COO^- + 0.375\ H_2O \rightarrow 0.125\ CO_2 + 0.125\ HCO_3^- + H^+ + e^-$$

$$0.41(\text{Reaction I-14}):\quad 0.1025\ O_2 + 0.41\ H^+ + 0.41\ e^- \rightarrow 0.205\ H_2O$$

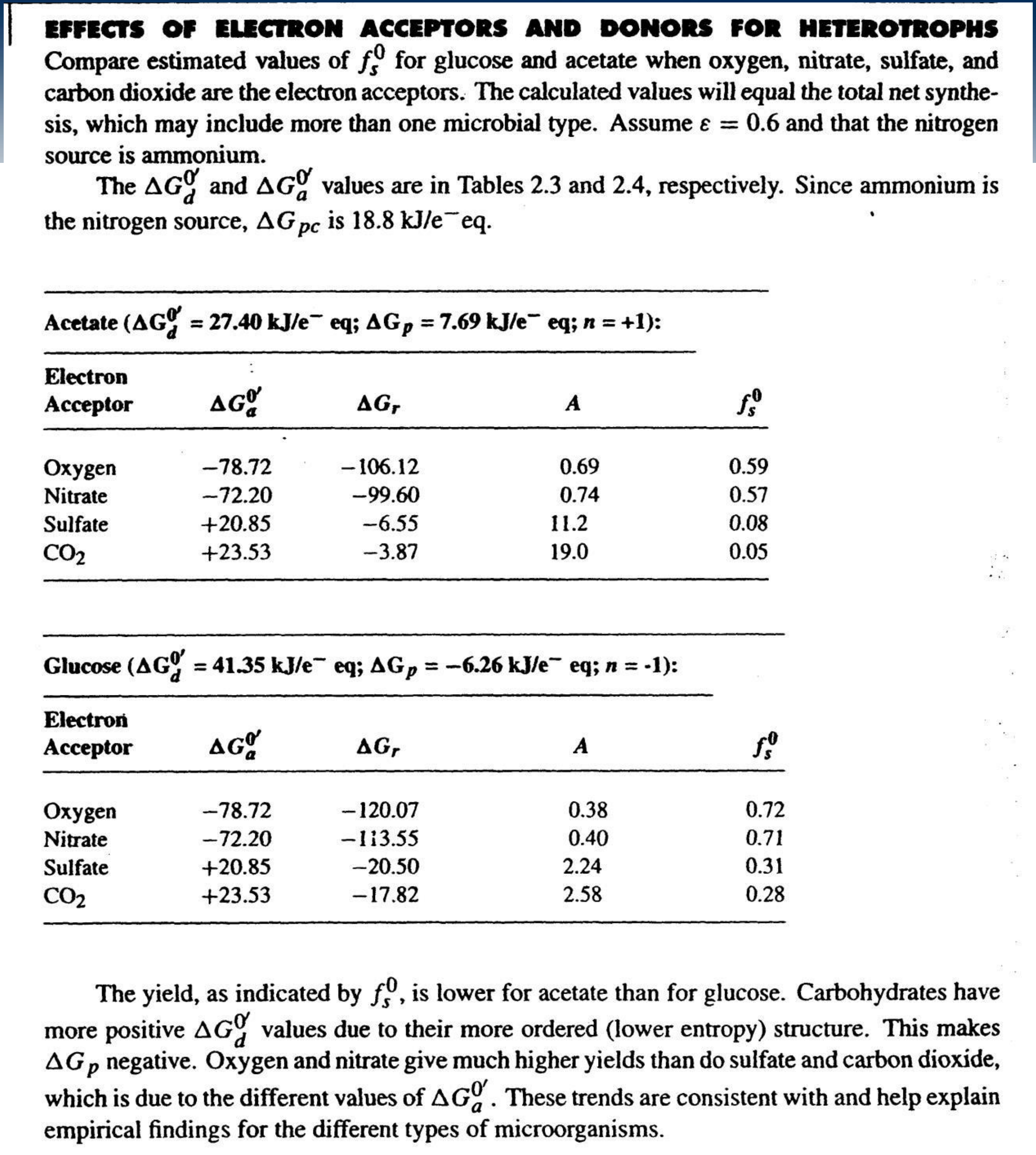
$$0.59(\text{Reaction C-1}):\quad 0.118\ CO_2 + 0.0295\ HCO_3^- + 0.0295\ NH_4^+ + 0.59\ H^+ + 0.59\ e^- \rightarrow 0.0295\ C_5H_7O_2N + 0.2655\ H_2O$$

$$\text{Overall:}\quad 0.125\ CH_3COO^- + 0.1025\ O_2 + 0.0295\ NH_4^+ \rightarrow 0.0295\ C_5H_7O_2N + 0.007\ CO_2 + 0.0955\ HCO_3^- + 0.0955\ H_2O$$

From this balanced reaction:

$$Y = 0.0295(113)/0.125 = 27 \text{ g cells/mol acetate}$$
$$= 0.0295(113)/0.125(59) = 0.45 \text{ g cells/g acetate}$$
$$= 0.0295(113)/8 = 0.42 \text{ g cells/g COD}'$$

Example 2.11



EFFECTS OF ELECTRON ACCEPTORS AND DONORS FOR HETEROTROPHS

Compare estimated values of f_s^0 for glucose and acetate when oxygen, nitrate, sulfate, and carbon dioxide are the electron acceptors. The calculated values will equal the total net synthesis, which may include more than one microbial type. Assume $\varepsilon = 0.6$ and that the nitrogen source is ammonium.

The $\Delta G_d^{0'}$ and $\Delta G_a^{0'}$ values are in Tables 2.3 and 2.4, respectively. Since ammonium is the nitrogen source, ΔG_{pc} is 18.8 kJ/e$^-$eq.

Acetate ($\Delta G_d^{0'} = 27.40$ kJ/e$^-$ eq; $\Delta G_p = 7.69$ kJ/e$^-$ eq; $n = +1$):

Electron Acceptor	$\Delta G_a^{0'}$	ΔG_r	A	f_s^0
Oxygen	−78.72	−106.12	0.69	0.59
Nitrate	−72.20	−99.60	0.74	0.57
Sulfate	+20.85	−6.55	11.2	0.08
CO_2	+23.53	−3.87	19.0	0.05

Glucose ($\Delta G_d^{0'} = 41.35$ kJ/e$^-$ eq; $\Delta G_p = -6.26$ kJ/e$^-$ eq; $n = -1$):

Electron Acceptor	$\Delta G_a^{0'}$	ΔG_r	A	f_s^0
Oxygen	−78.72	−120.07	0.38	0.72
Nitrate	−72.20	−113.55	0.40	0.71
Sulfate	+20.85	−20.50	2.24	0.31
CO_2	+23.53	−17.82	2.58	0.28

The yield, as indicated by f_s^0, is lower for acetate than for glucose. Carbohydrates have more positive $\Delta G_d^{0'}$ values due to their more ordered (lower entropy) structure. This makes ΔG_p negative. Oxygen and nitrate give much higher yields than do sulfate and carbon dioxide, which is due to the different values of $\Delta G_a^{0'}$. These trends are consistent with and help explain empirical findings for the different types of microorganisms.

2.8 Oxidized Nitrogen Sources

- Microorganisms prefer to use ammonium nitrogen as an inorganic nitrogen source for cell synthesis because it already is in the (-Ⅲ) oxidation state ***(next slide)***

- But, when ammonium is not available for synthesis, many prokaryotic cells can use oxidized forms of nitrogen as alternatives (N_2, NO_2^-, NO_3^-)

$$\Delta G_s = \frac{\Delta G_p}{\varepsilon^n} + \frac{\Delta G_{pc}}{\varepsilon}$$

- The ΔG_{pc} part of ΔG_s depends on the nitrogen source, since a mole of cells ($C_5H_7O_2N$) contains different of electron equivalents

ΔG_{pc} for **NO_3^-** = [an estimated value of 3.33kJ/g cell] X [4.06 g/e- eq (=113.8/28)]

= 13.5 kJ/e^- eq

ΔG_{pc} = 18.8 (NH_4^+), 13.5 (NO_3^-), 14.5 (NO_2^-) , 16.4(N_2) kJ/e- eq

2.8 Oxidized Nitrogen Sources

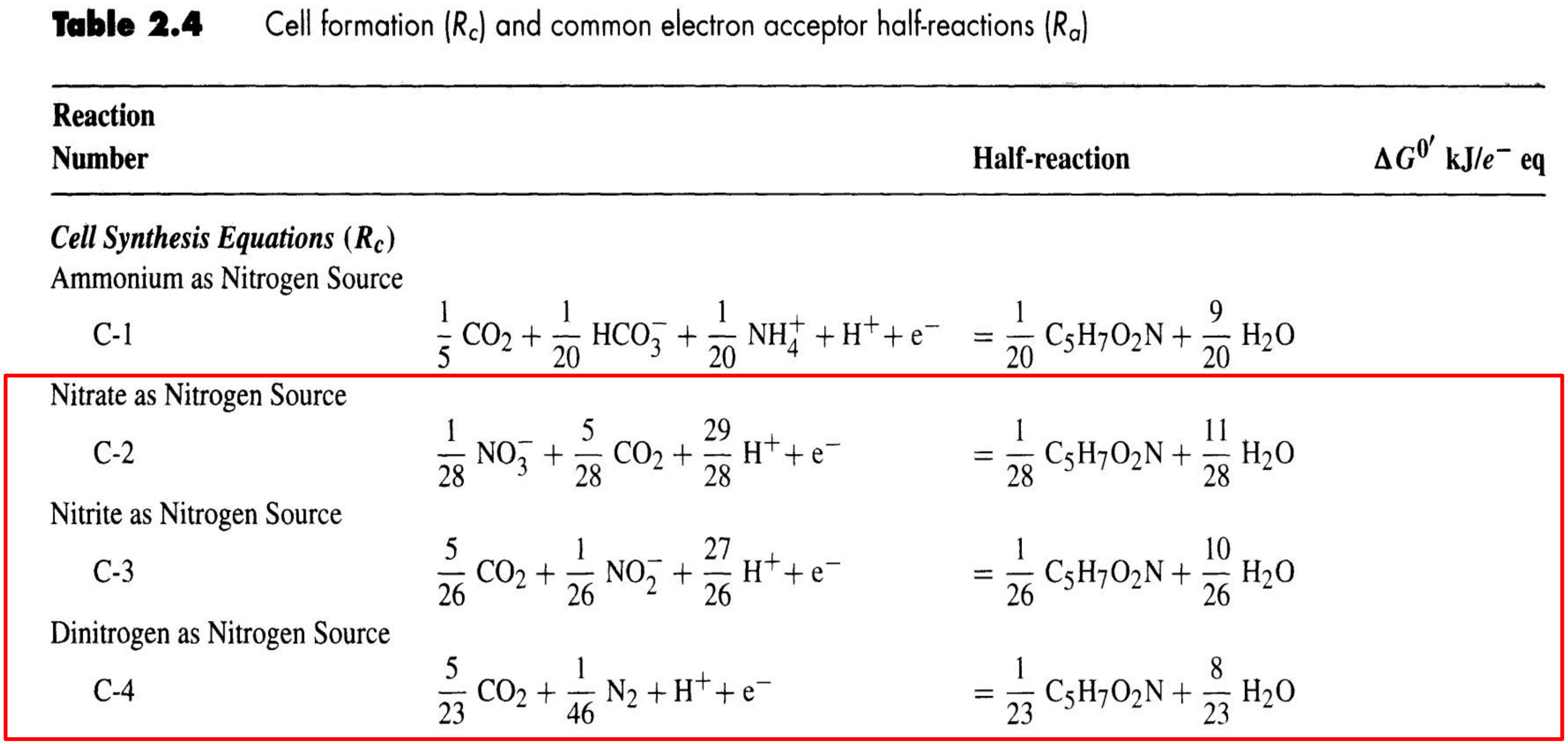
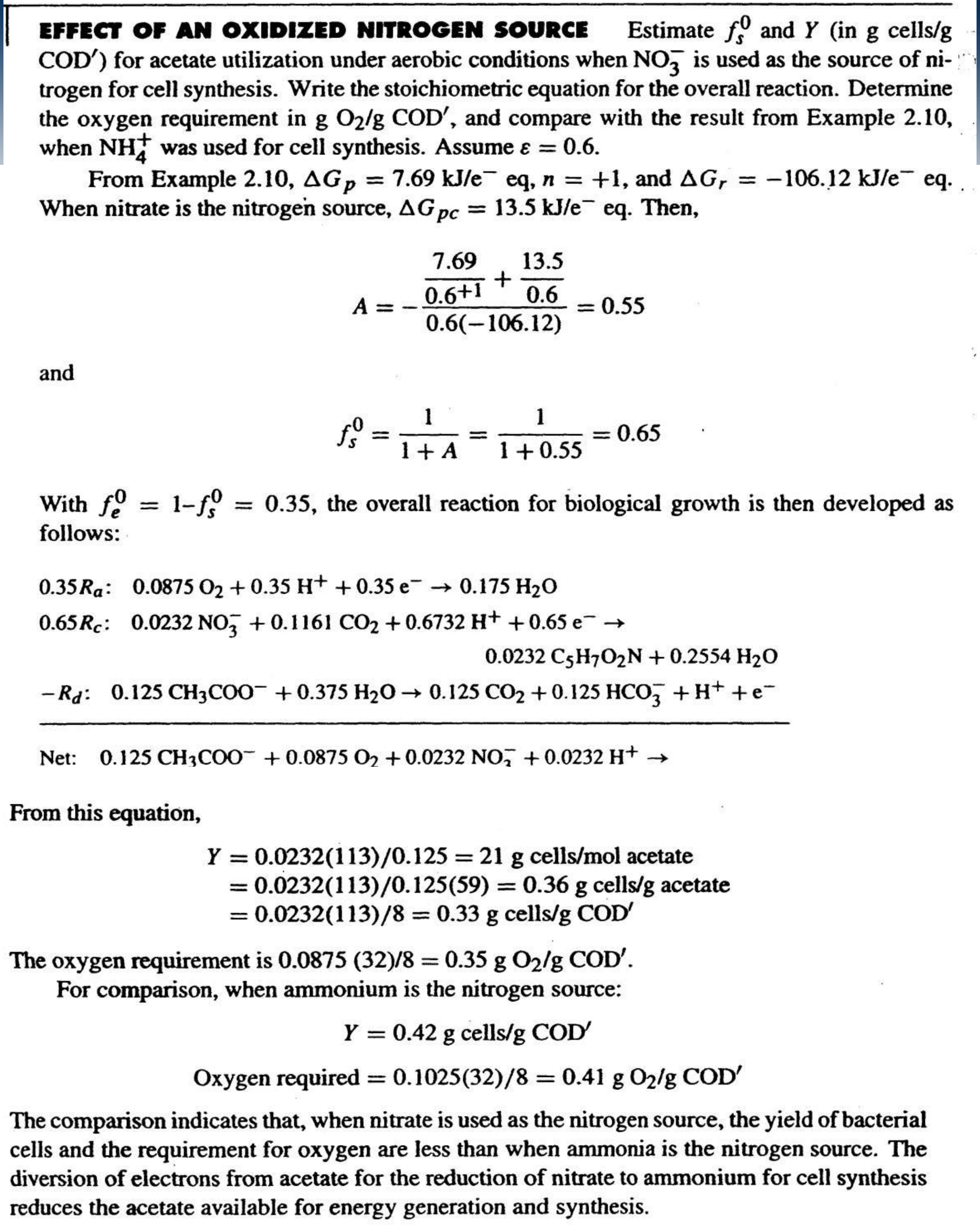


Table 2.4 Cell formation (R_c) and common electron acceptor half-reactions (R_a)

Reaction Number	Half-reaction	$\Delta G^{0'}$ kJ/e^- eq
Cell Synthesis Equations (R_c)		
Ammonium as Nitrogen Source		
C-1	$\frac{1}{5}CO_2 + \frac{1}{20}HCO_3^- + \frac{1}{20}NH_4^+ + H^+ + e^- = \frac{1}{20}C_5H_7O_2N + \frac{9}{20}H_2O$	
Nitrate as Nitrogen Source		
C-2	$\frac{1}{28}NO_3^- + \frac{5}{28}CO_2 + \frac{29}{28}H^+ + e^- = \frac{1}{28}C_5H_7O_2N + \frac{11}{28}H_2O$	
Nitrite as Nitrogen Source		
C-3	$\frac{5}{26}CO_2 + \frac{1}{26}NO_2^- + \frac{27}{26}H^+ + e^- = \frac{1}{26}C_5H_7O_2N + \frac{10}{26}H_2O$	
Dinitrogen as Nitrogen Source		
C-4	$\frac{5}{23}CO_2 + \frac{1}{46}N_2 + H^+ + e^- = \frac{1}{23}C_5H_7O_2N + \frac{8}{23}H_2O$	

MW =113.8

Example 2.13



EFFECT OF AN OXIDIZED NITROGEN SOURCE Estimate f_s^0 and Y (in g cells/g COD′) for acetate utilization under aerobic conditions when NO_3^- is used as the source of nitrogen for cell synthesis. Write the stoichiometric equation for the overall reaction. Determine the oxygen requirement in g O_2/g COD′, and compare with the result from Example 2.10, when NH_4^+ was used for cell synthesis. Assume $\varepsilon = 0.6$.

From Example 2.10, $\Delta G_p = 7.69$ kJ/e⁻ eq, $n = +1$, and $\Delta G_r = -106.12$ kJ/e⁻ eq. When nitrate is the nitrogen source, $\Delta G_{pc} = 13.5$ kJ/e⁻ eq. Then,

$$A = -\frac{\frac{7.69}{0.6^{+1}} + \frac{13.5}{0.6}}{0.6(-106.12)} = 0.55$$

and

$$f_s^0 = \frac{1}{1+A} = \frac{1}{1+0.55} = 0.65$$

With $f_e^0 = 1 - f_s^0 = 0.35$, the overall reaction for biological growth is then developed as follows:

$$0.35R_a\text{:}\quad 0.0875\ O_2 + 0.35\ H^+ + 0.35\ e^- \rightarrow 0.175\ H_2O$$

$$0.65R_c\text{:}\quad 0.0232\ NO_3^- + 0.1161\ CO_2 + 0.6732\ H^+ + 0.65\ e^- \rightarrow 0.0232\ C_5H_7O_2N + 0.2554\ H_2O$$

$$-R_d\text{:}\quad 0.125\ CH_3COO^- + 0.375\ H_2O \rightarrow 0.125\ CO_2 + 0.125\ HCO_3^- + H^+ + e^-$$

$$\text{Net:}\quad 0.125\ CH_3COO^- + 0.0875\ O_2 + 0.0232\ NO_3^- + 0.0232\ H^+ \rightarrow$$

From this equation,

$$\begin{aligned} Y &= 0.0232(113)/0.125 = 21 \text{ g cells/mol acetate} \\ &= 0.0232(113)/0.125(59) = 0.36 \text{ g cells/g acetate} \\ &= 0.0232(113)/8 = 0.33 \text{ g cells/g COD}' \end{aligned}$$

The oxygen requirement is 0.0875 (32)/8 = 0.35 g O_2/g COD′.

For comparison, when ammonium is the nitrogen source:

$$Y = 0.42 \text{ g cells/g COD}'$$

$$\text{Oxygen required} = 0.1025(32)/8 = 0.41 \text{ g } O_2\text{/g COD}'$$

The comparison indicates that, when nitrate is used as the nitrogen source, the yield of bacterial cells and the requirement for oxygen are less than when ammonia is the nitrogen source. The diversion of electrons from acetate for the reduction of nitrate to ammonium for cell synthesis reduces the acetate available for energy generation and synthesis.