TOPIC: Enzymes & common metabolic pathways Learning Objectives

1. Calculate $\Delta G^{0'}$ and $E^{0'}$ values for coupled redox reactions and adjust $\Delta G^{0'}$ and $E^{o'}$ for "real" concentrations of substrates & products (know how to use the Nernst equation)
2. Trace the net flow of carbon & electrons in cells that are:
   a. Respiring
   b. Fermenting
3. Utilize thermodynamics/bioenergetics calculations for organisms with different physiologies to predict growth Yield. (i.e. via the "A" equation in Gossett's handout)
4. Describe the roles of ATP & NADH/NADPH/FADH as "currencies" in metabolism
5. List and briefly explain the 6 functional categories of enzymes ("OTHLyIL"). Given an enzymatic reaction, determine which class it belongs to.
6. Explain what is meant by primary, secondary, tertiary and quartenary structure of enzymes.
7. Recognize the difference between apoenzymes and holoenzymes
8. Effectively use online enzyme/pathway databases (**KEGG, Brenda, eawag Biodeg & Biocatalysis website)** to obtain information on enzymes and to incorporate kinetic parameters into models
9. Predict degradation pathways for chemicals by examining molecular structure coupled with general "rules" for biochemistry (eawag B&B Pathway Prediction)

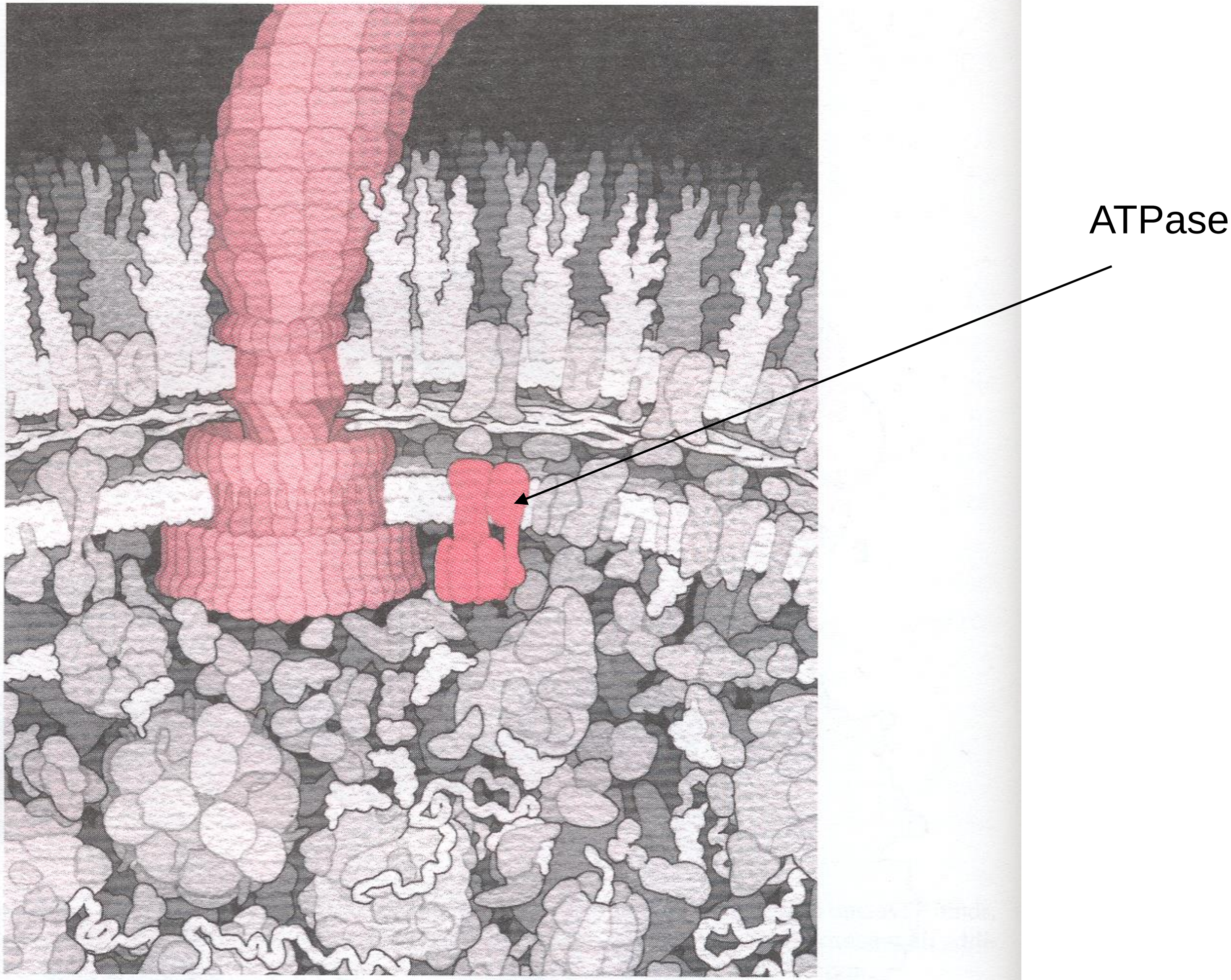
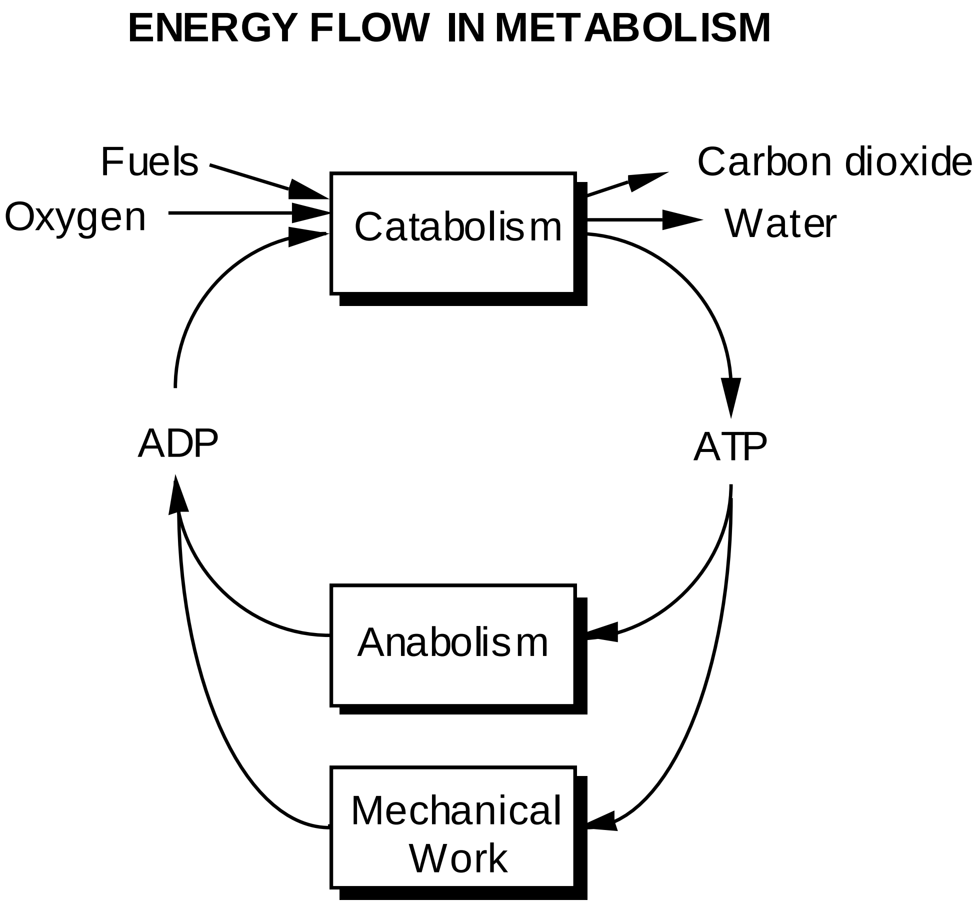
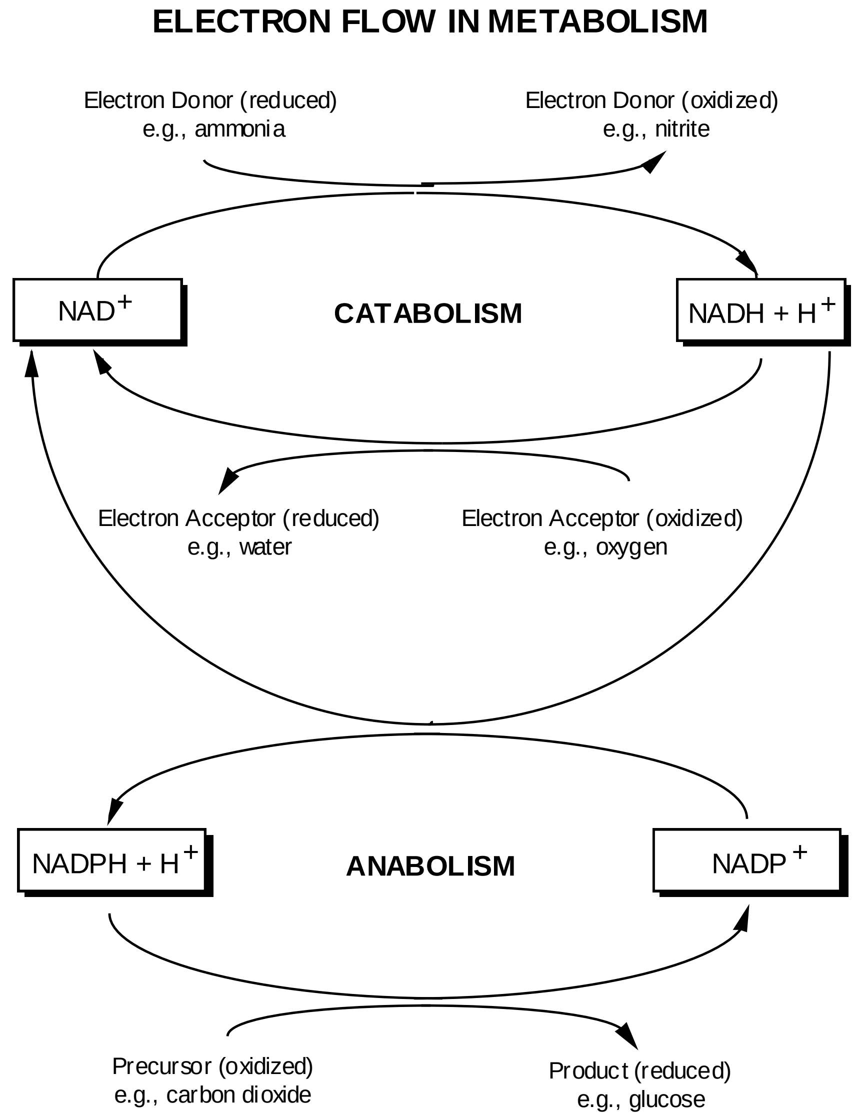


Figure 5-33 The flagellar motor of *Escherichia coli* spans the two-layered cell wall of the bacterium and turns the long corkscrew-shaped flagellum. The other rotary motor of the cell, ATP synthase, is also found spanning the cell wall, shown in darker pink in this illustration.

from: *Bionanotechnology* by David Goodsell (2004)

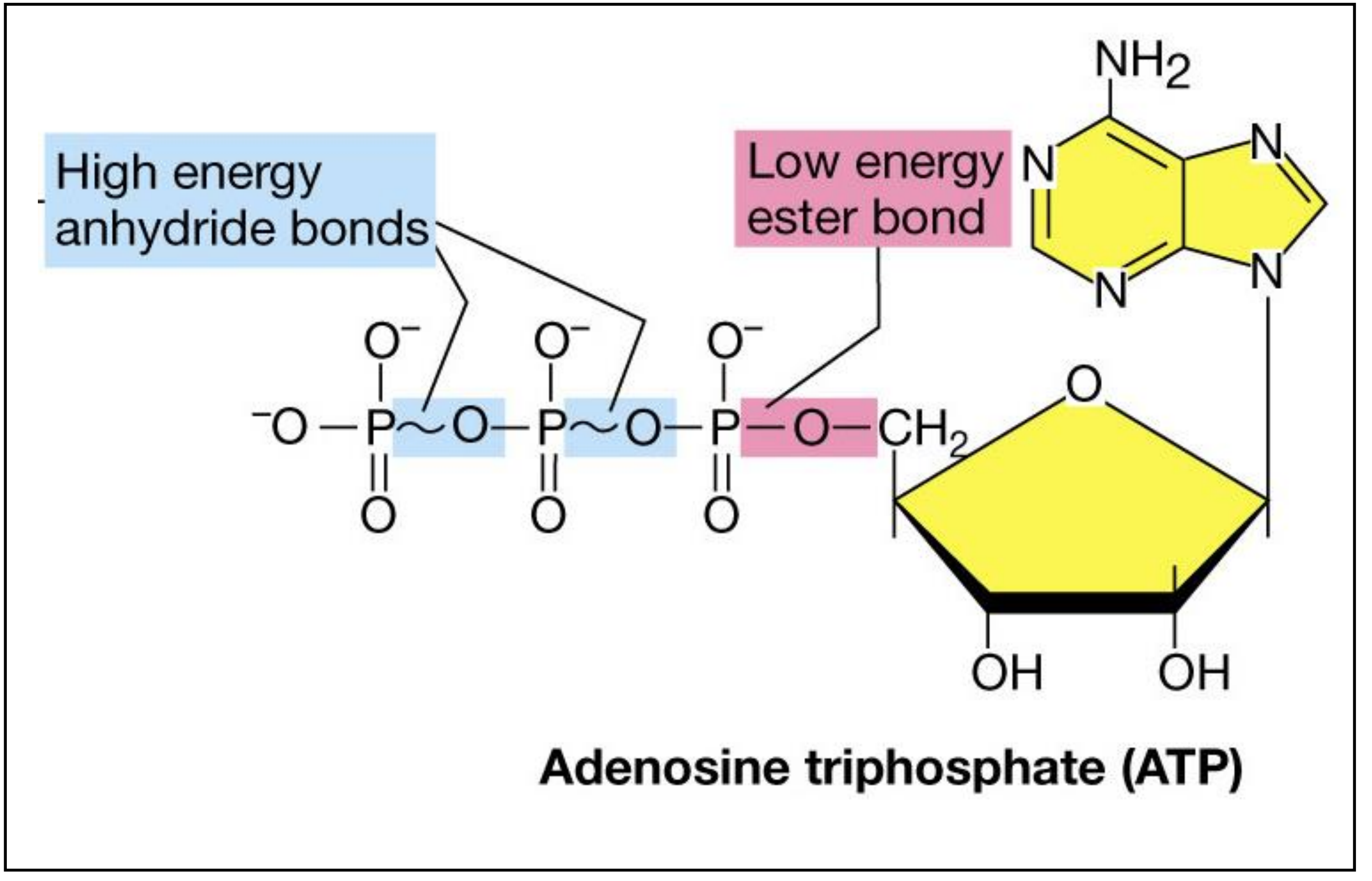
ATP & NADH as common currencies of energy and electrons, respectively





From CEE451 reader Chapter 17

High energy anhydride phosphate bonds in ATP

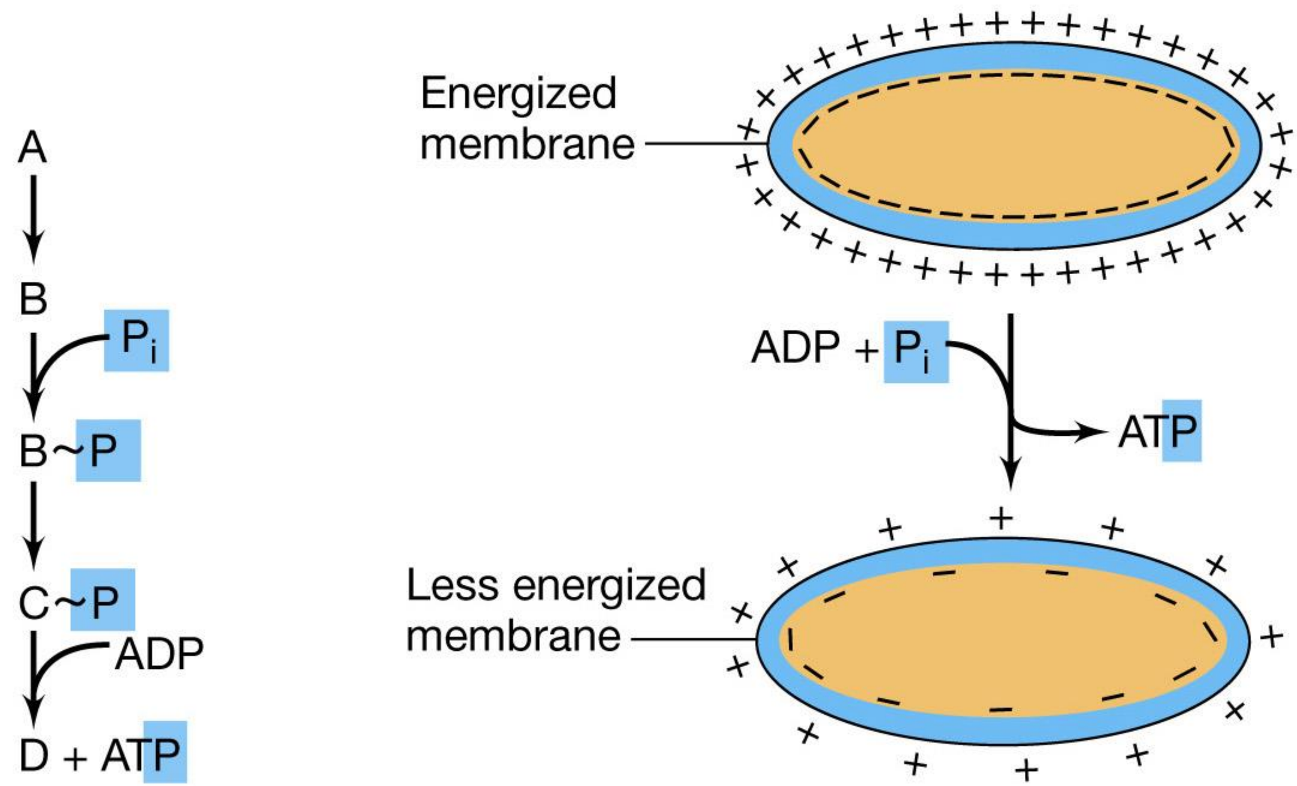


$$ATP + H2O \rightarrow ADP + Pi$$

$$\Delta G^{o'} = -31 kJ / mole \ (= -7.3 kcal / mole)$$

$$\Delta G = -52 kJ / mole (-12.5 kcal / mole) \ at \ physio\log ical \ conditions$$

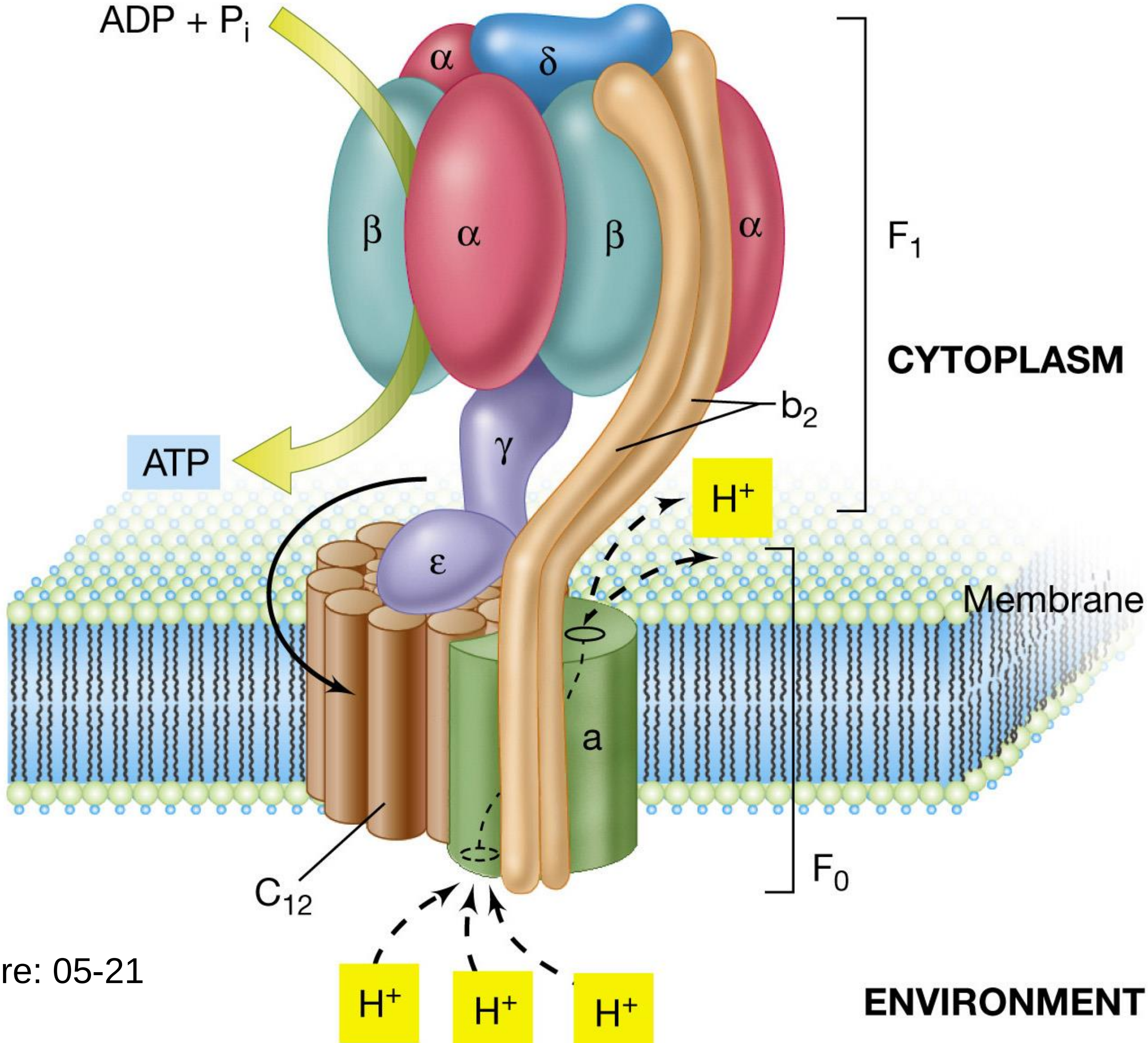
Two methods of ATP production



***(a)* Substrate-level phosphorylation**

***(b)* Oxidative phosphorylation**

Brock Figure: 05-13a-b



ADP + P_i
α
δ
β
α
β
α
F_1
CYTOPLASM
b_2
γ
ATP
H^+
ε
Membrane
a
C_{12}
F_0
H^+
H^+
H^+
ENVIRONMENT

Figure: 05-21

Cell membrane-bound Electron Transport Chain in typical aerobes:

- -shuttles e-s to O2 (forming water)
- -pumps protons (H+) out of the cell

Note: both e-s and H+ are brought by NADH + H+

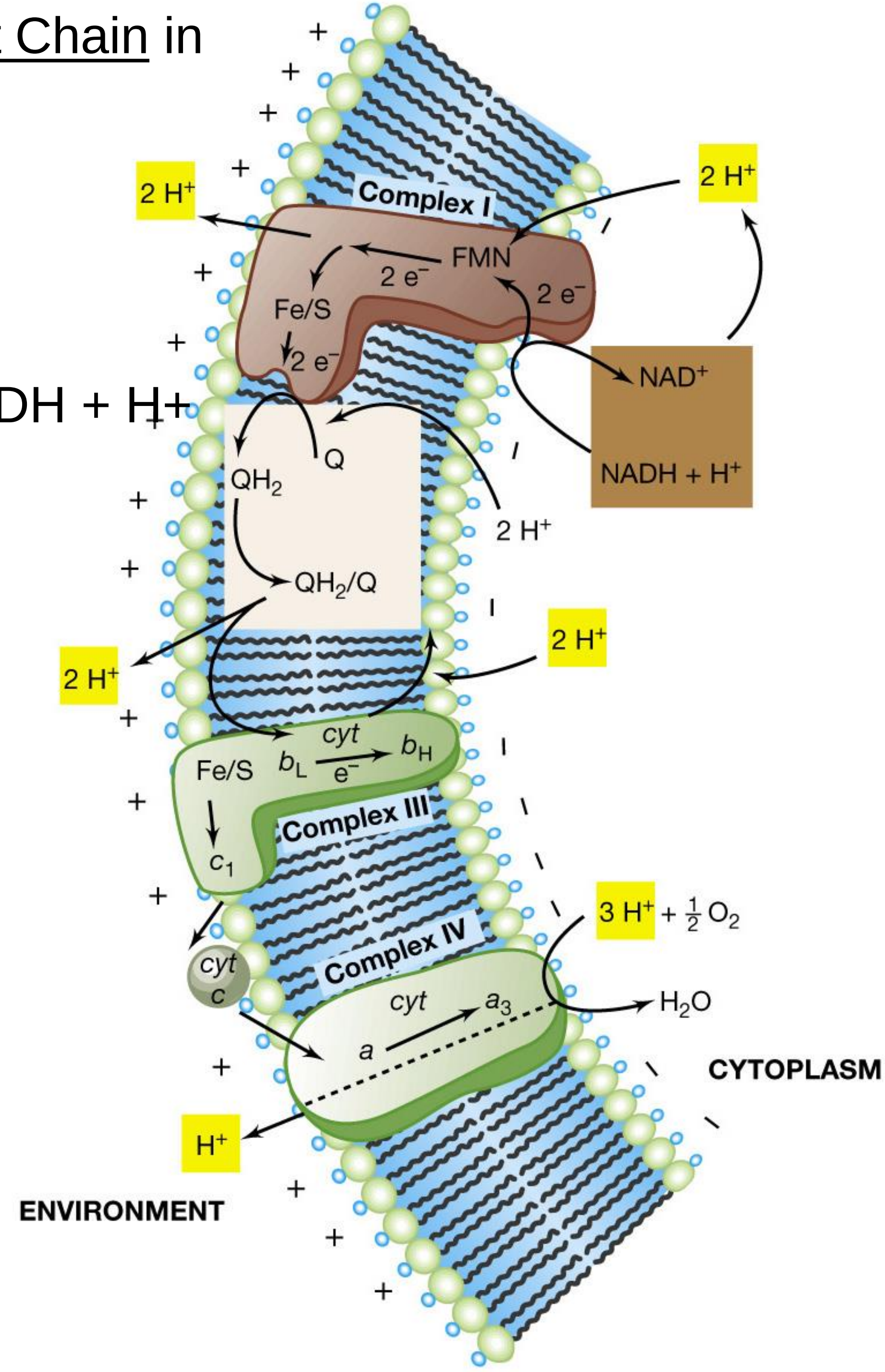


Figure: 05-20

Redox tower for common ETC components

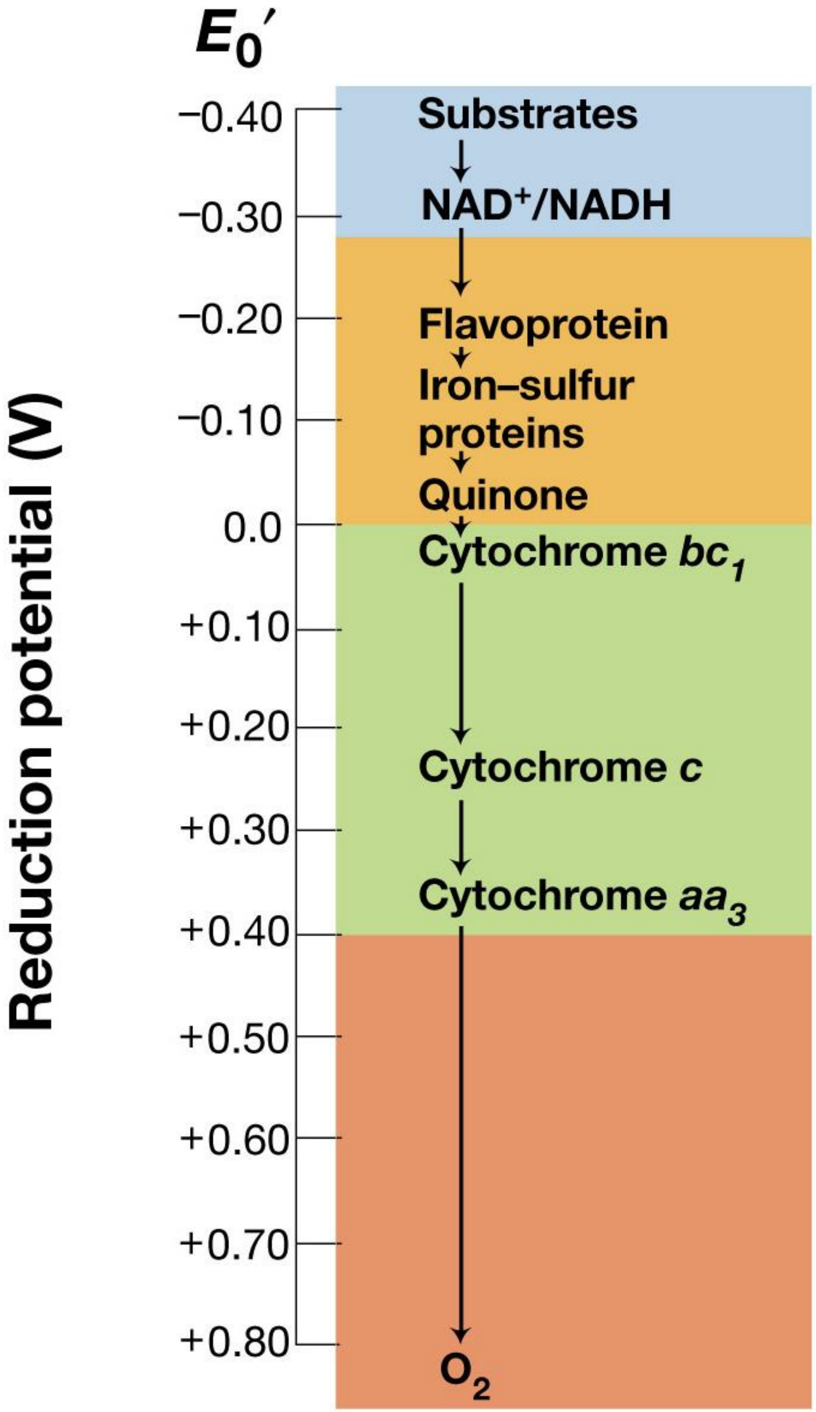


Figure: 05-19

Free energies of reactions

- The free energy associated with any catabolic reaction (any reaction, really) can be predicted
  - Using free energies of formation
  - Or (for redox rxns) coupling of standard half reactions
- For catabolic redox reactions (i.e. respirations) eeq are
  - donated by electron donors
  - accepted by electron acceptors
  - Electron balance
- Sum of half reactions involved equals the standard free energy per eeq transferred from donor to acceptor

HALF REACTIONS

kcal/eeq

Reactions for Bacterial Cell Synthesis (R_c)

Ammonia as Nitrogen Source:

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

Nitrate as Nitrogen Source:

2. $\frac{1}{28} C_5H_7O_2N + \frac{11}{28} H_2O = \frac{1}{28} NO_3^- + \frac{5}{28} CO_2 + \frac{29}{28} H^+ + e^-$

Reactions for Electron Acceptors (R_a)

Oxygen:

3. $\frac{1}{2} H_2O = \frac{1}{4} O_2 + H^+ + e^-$ 18.675

Nitrate:

4. $\frac{1}{10} N_2 + \frac{3}{5} H_2O = \frac{1}{5} NO_3^- + \frac{6}{5} H^+ + e^-$ 17.128

Sulfate:

5. $\frac{1}{16} H_2S + \frac{1}{16} HS^- + \frac{1}{2} H_2O = \frac{1}{8} SO_4^{=} + \frac{19}{16} H^+ + e^-$ –5.085

Carbon Dioxide:

6. $\frac{1}{8} CH_4 + \frac{1}{4} H_2O = \frac{1}{8} CO_2 + H^+ + e^-$ –5.763

Reactions for Electron Donors (R_d)

Organic Donors

Domestic Wastewater:

7. $\frac{1}{50} C_{10}H_{19}O_3N + \frac{9}{25} H_2O = \frac{9}{50} CO_2 + \frac{1}{50} NH_4^+ + \frac{1}{50} HCO_3^- + H^+ + e^-$ –7.6

Protein:

8. $\frac{1}{66} C_{16}H_{24}O_5N_4 + \frac{27}{66} H_2O = \frac{8}{33} CO_2 + \frac{2}{33} NH_4^+ + \frac{31}{33} H^+ + e^-$ –7.7

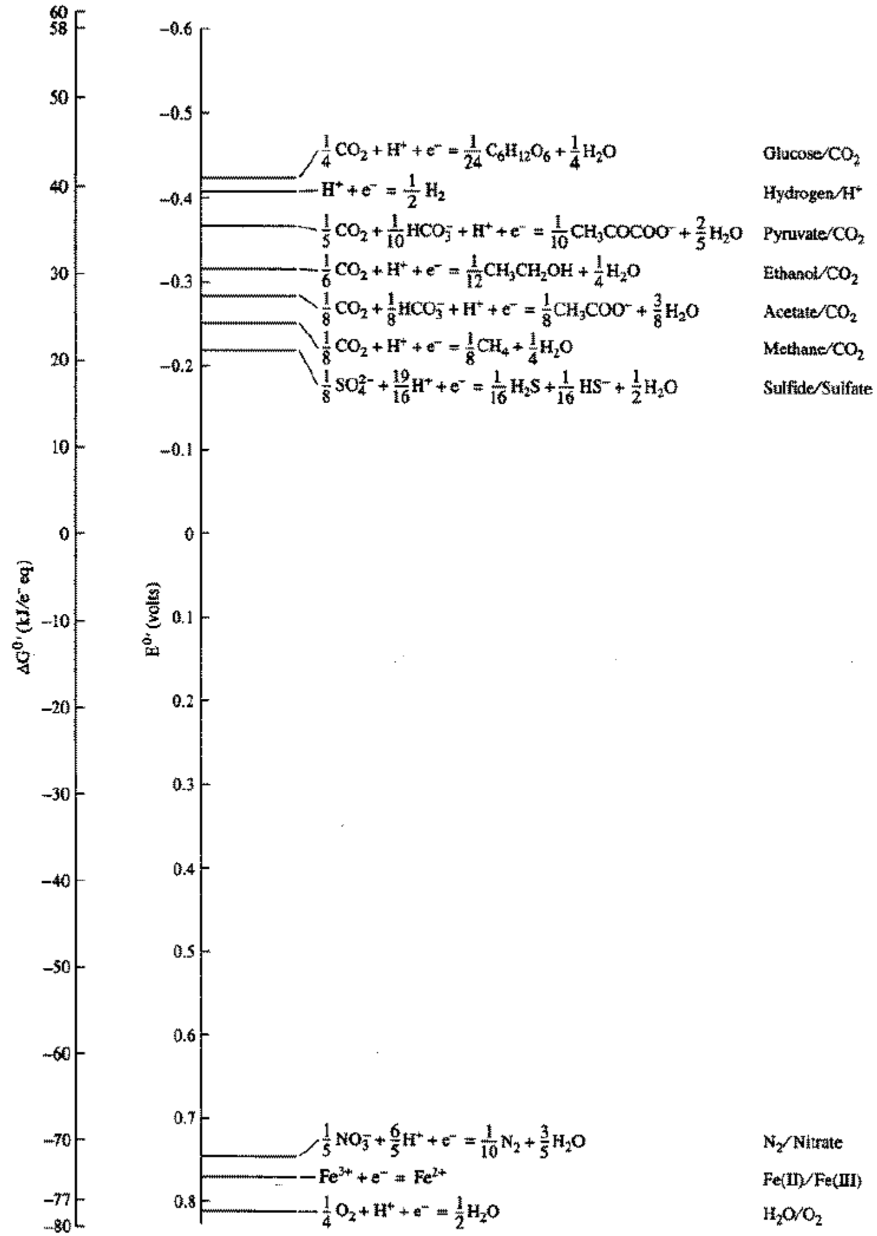
Carbohydrate (Cellulose, Starch, Sugars):

9. $\frac{1}{24} C_6H_{12}O_6 + \frac{1}{4} H_2O = \frac{1}{4} CO_2 + H^+ + e^-$ –10.0

Grease, Fat, and Oil:

10. $\frac{1}{46} C_8H_{16}O + \frac{15}{46} H_2O = \frac{4}{23} CO_2 + H^+ + e^-$ –6.6

- From Rittman & McCarty Env'l Biotech 2001



Balanced catabolic summary reaction for aerobic glucose respiration

		$\Delta G^o(w)^*$ (kcal/eeq)
Rxn (9)	$^1/_{24}\,C_6H_{12}O_6 + {^1/_4}\,H_2O = {^1/_4}\,CO_2 + H^+ + e^-$	–10.0
–Rxn (3)	$^1/_4\,O_2 + H^+ + e^- = {^1/_2}\,H_2O$	–18.675
	$^1/_{24}\,C_6H_{12}O_6 + {^1/_4}\,O_2 = {^1/_4}\,CO_2 + {^1/_4}\,H_2O$	–28.675

Note: 1 eeq reduces ¼ mole O2 to water (1/4 mole O2 = 32/4 = 8 g O2)

Therefore 1 eeq of an electron donor imposes 8 g of Oxygen Demand (OD)

Other examples of chemoorganotrophy

- Fermentation of sugar to lactate

Lactate:

15. $\frac{1}{12} CH_3CHOHCOO^- + \frac{1}{3} H_2O = \frac{1}{6} CO_2 + \frac{1}{12} HCO_3^- + H^+ + e^-$ −7.873 x (-1)

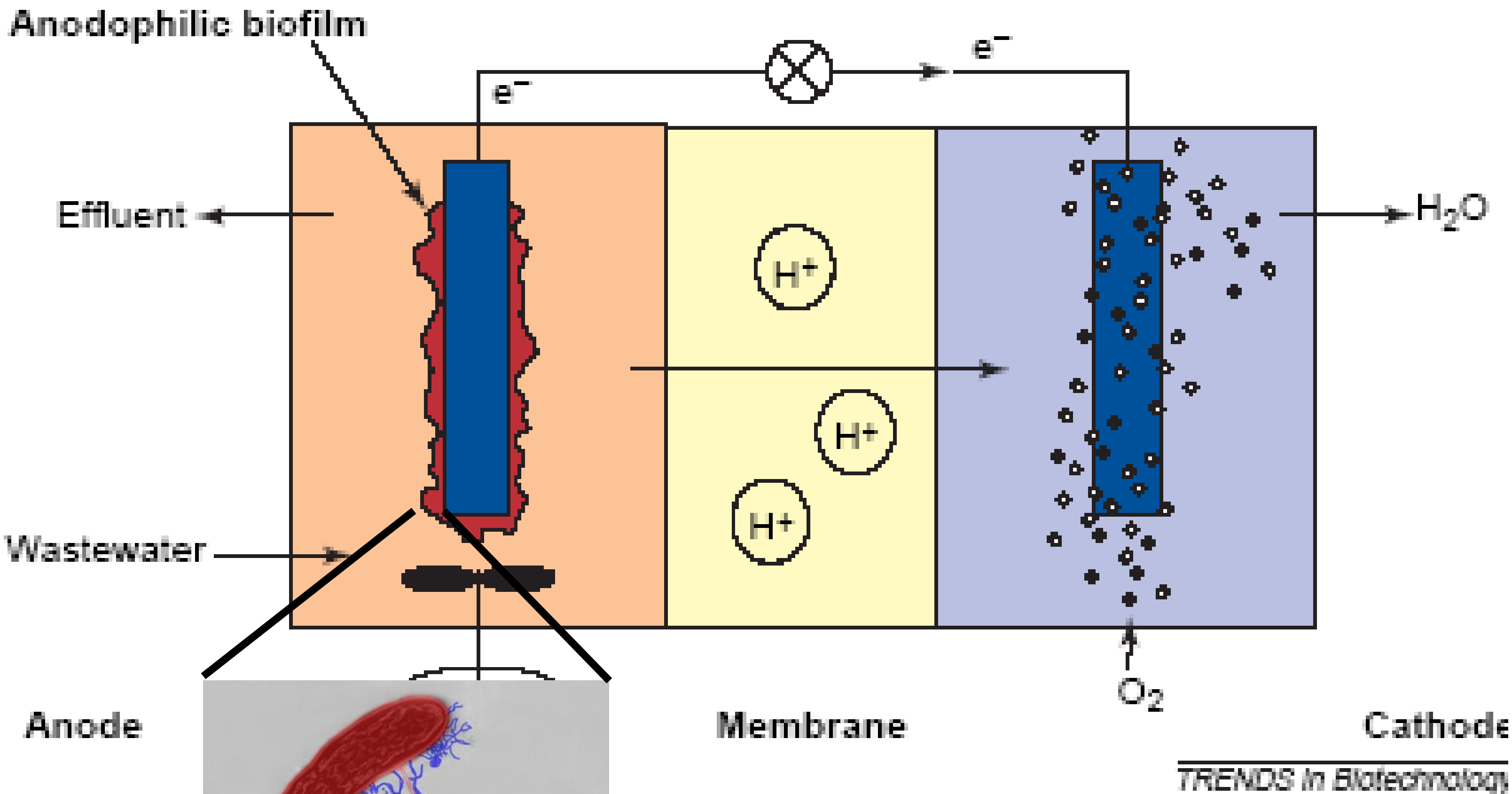
Carbohydrate (Cellulose, Starch, Sugars):

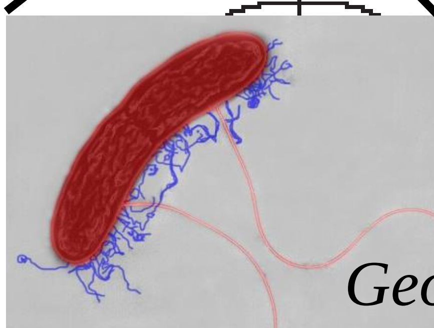
9. $\frac{1}{24} C_6H_{12}O_6 + \frac{1}{4} H_2O = \frac{1}{4} CO_2 + H^+ + e^-$ −10.0

Sum: $$\frac{1}{24} C_6H_{12}O_6 + \frac{1}{12} HCO_3^- = \frac{1}{12} H_2O + \frac{1}{12} CH_3CHOHCOO^- + \frac{1}{12} CO_2$$

$\Delta G^{o(w)}$= -2.127 kcal/ee

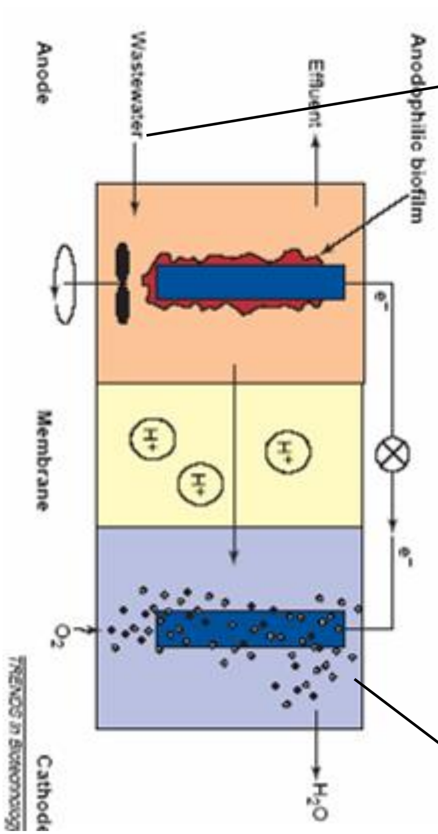
Microbial Fuel Cells tap into electron flow directly





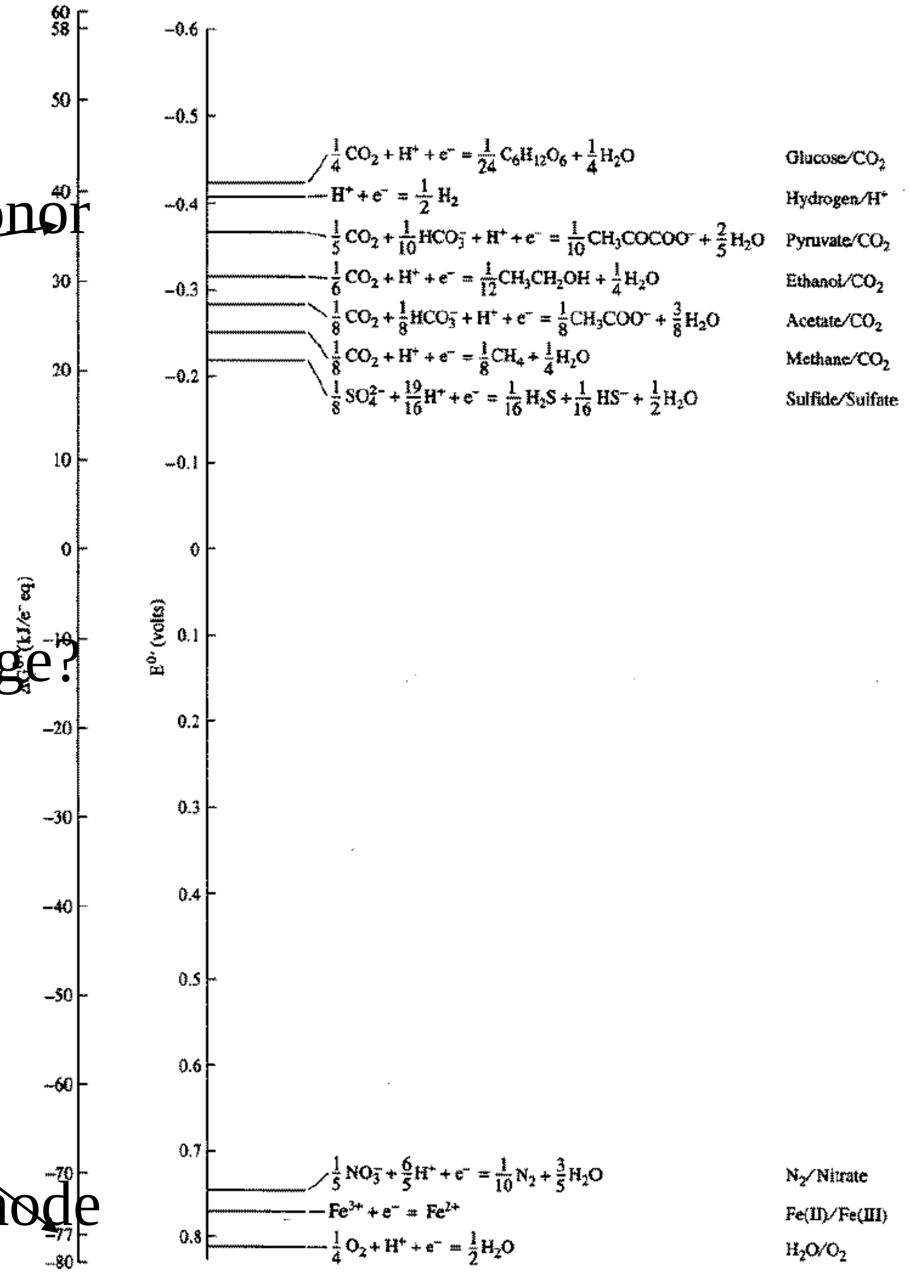
Geobacter with naonowires (blue) and flagella

E donor



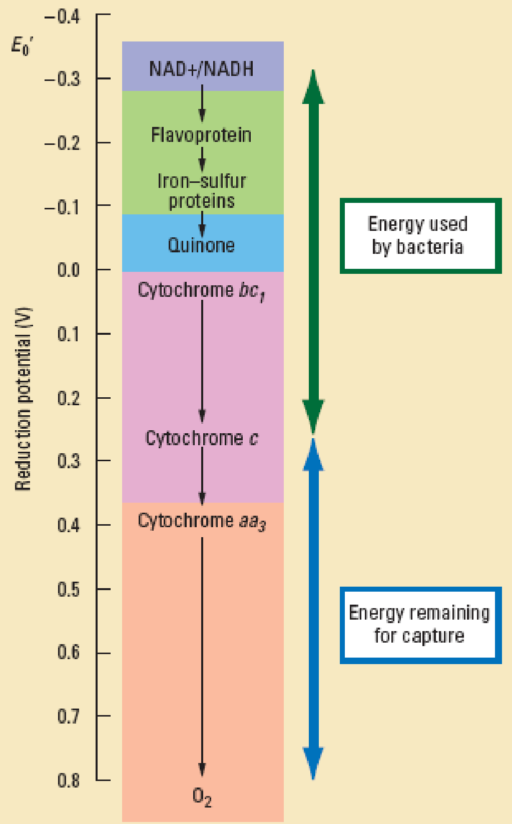
Anode voltage?

E acceptor in cathode



Respiratory chain shows how the voltage that could be recovered in a microbial fuel cell (MFC) is dependent on where electrons exit the chain of respiratory enzymes

In the case shown here, bacteria could derive energy from the potential between NADH (the reduced form of nicotinamide adenine dinucleotide) and cytochrome *c* (green arrow), whereas the MFC could be used to recover energy from the potential between cytochrome *c* and oxygen (blue arrow). Actual potentials depend on concentrations and potentials of specific enzymes and electron acceptors. (Respiratory-chain and standard potentials shown here are adapted with permission from a figure in Ref. 51 for *Paracoccus denitrificans*.)



From logan et al 2006, ES&T

eeq conversions

- 1 eeq (1 mole e's)= 8 g OD (oxygen demand) = 96,400 Coulombs

- In electrical engineering:
  - 1 Coulomb/s = 1 Ampere (a unit of current w symbol I)

- Power in an electrical system = Volts*Current = Watts

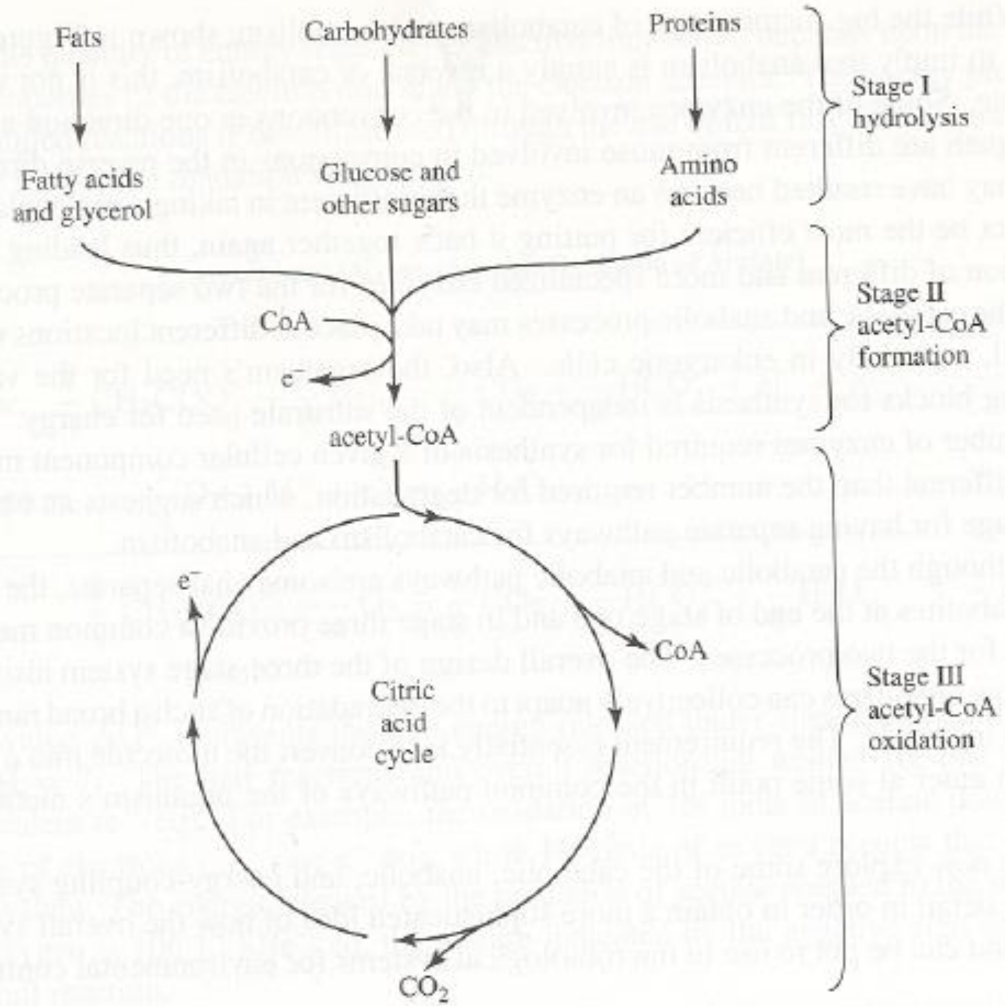


Figure 1.14 The three general stages of catabolism of fats, carbohydrates, and proteins under aerobic conditions. Reversing the processes gives anabolism.

From Rittmann & McCarty *Environmental Biotechnology*

Metabolic Maps: representing enzymatic pathways

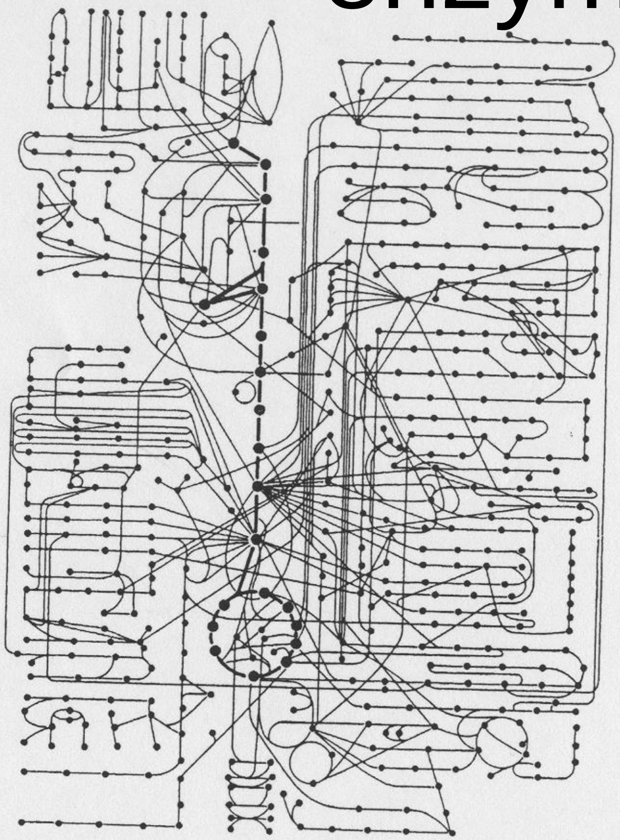


Figure 8.1 Network of intermediary metabolism showing compounds as nodes ar interconnecting reactions as lines. Highlighted in boldface is the central linear pat way flowing into the tricarboxylic acid cycle (circle). (From *Proteins, Energy and Meta olism* [Rawn, © 1989], by permission of Prentice-Hall, Inc., Upper Saddle River, N.

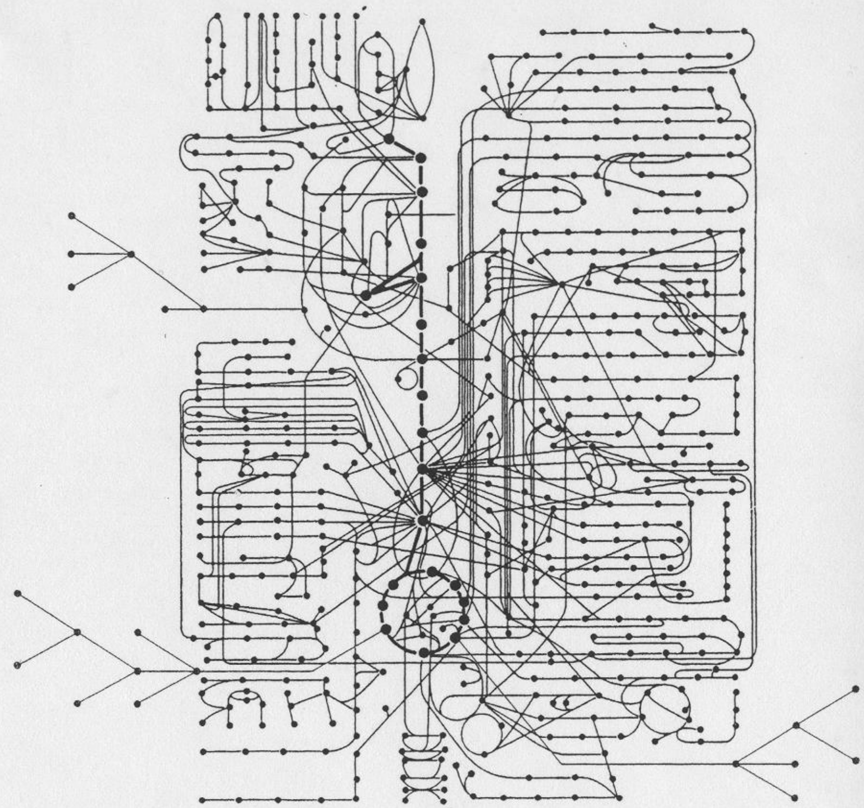
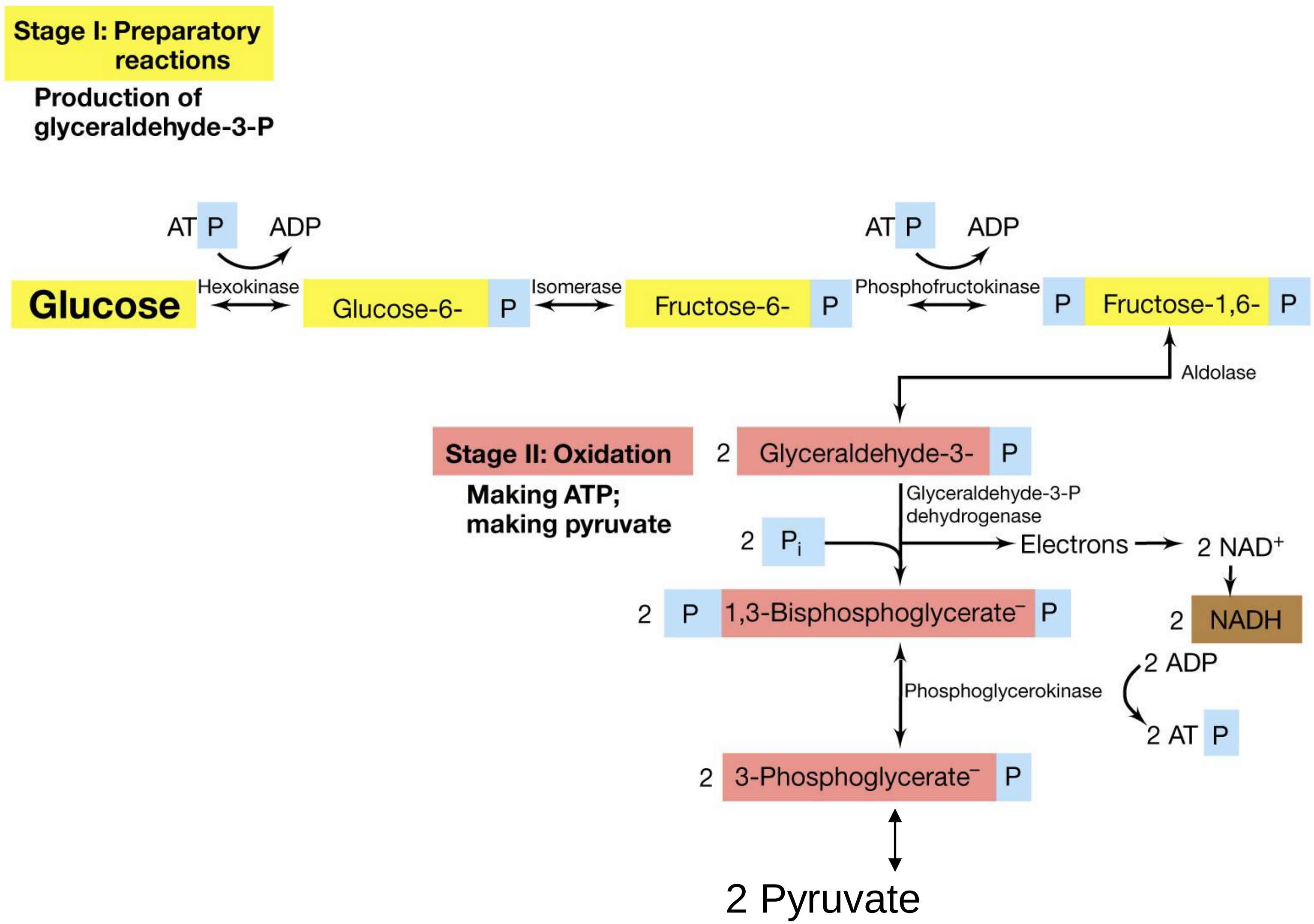


Figure 8.2 Network of intermediary metabolism with novel catabolic reactions (shown in green) which funnel into intermediary metabolites.

From Wackett

Glycolysis (Embden-Meyerhoff pathway)



Krebs cycle or TCA cycle or citric acid cycle (CAC).

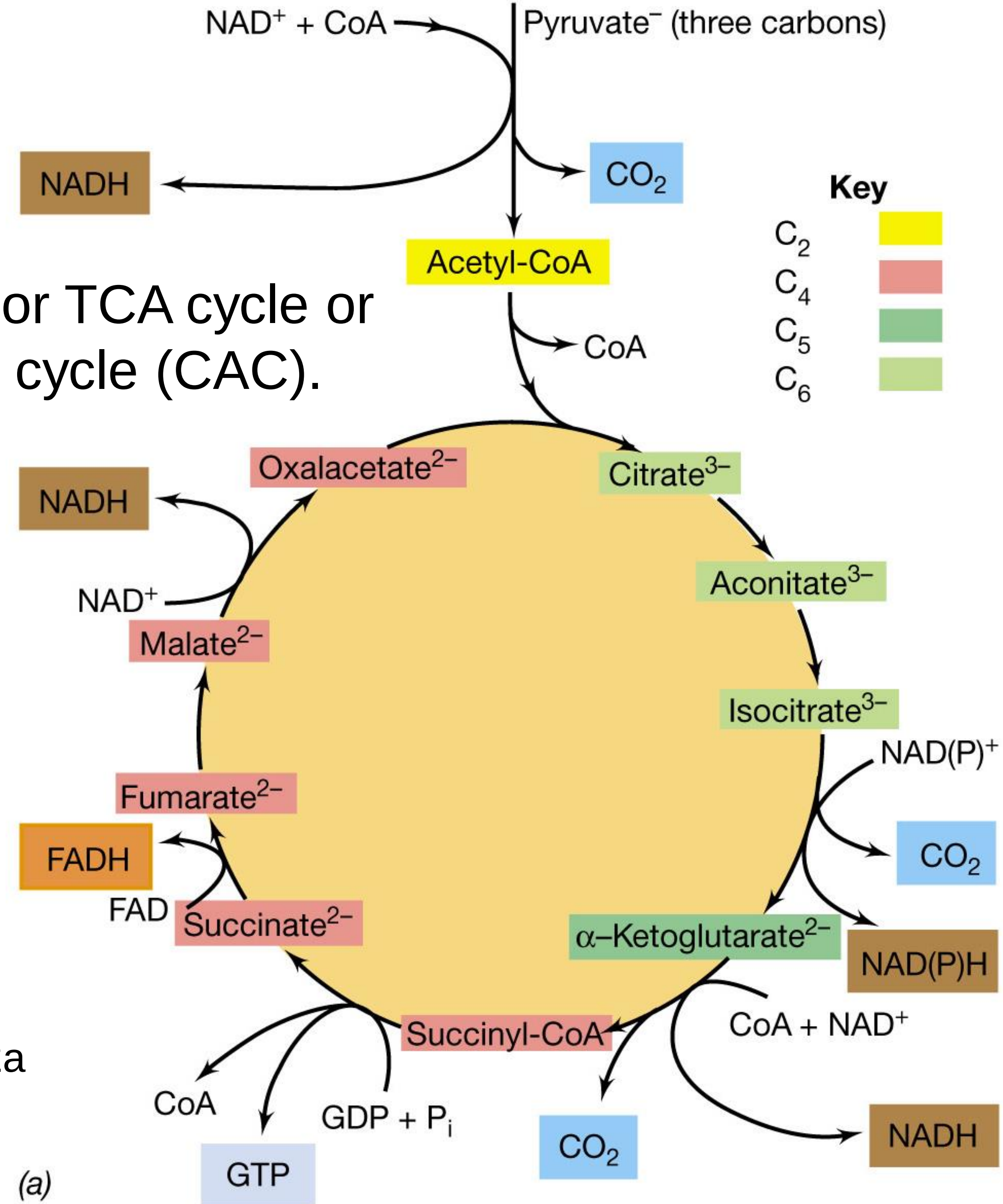


Figure: 05-22a

Beta-oxidation of organic acids

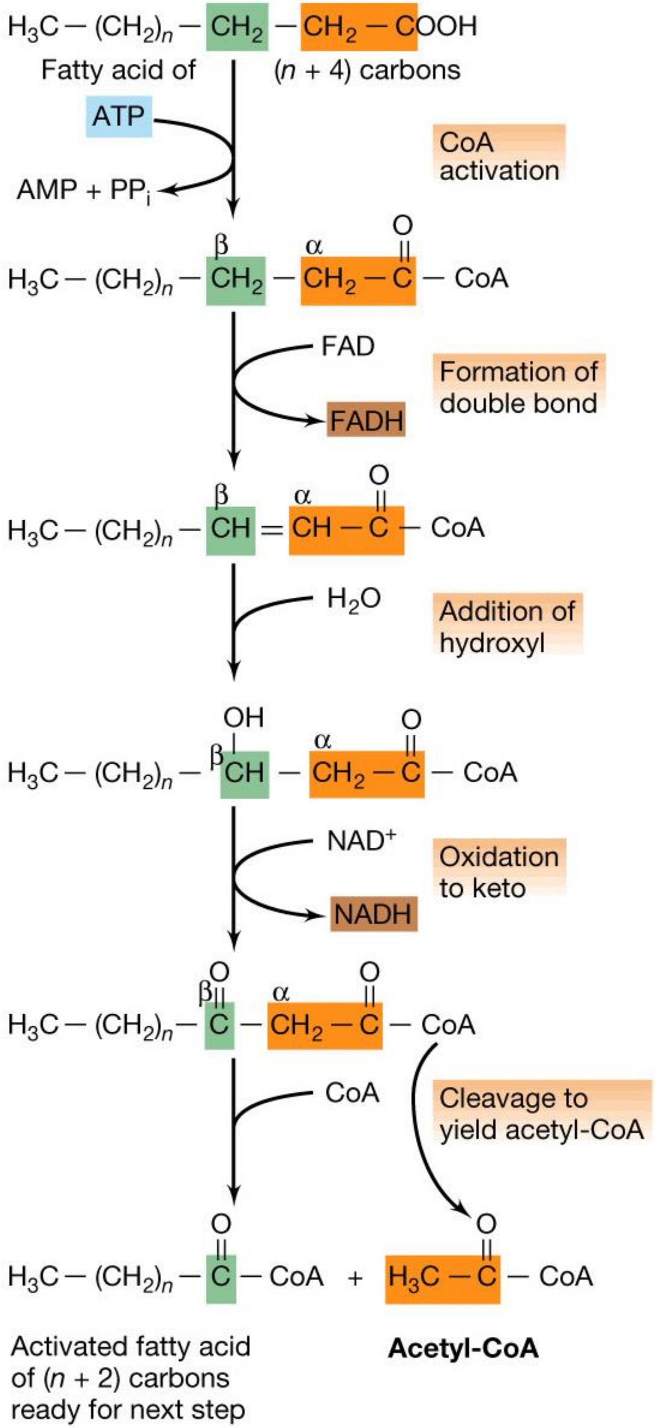


Figure: 17-68

Examples of fermentation reactions from pyruvate

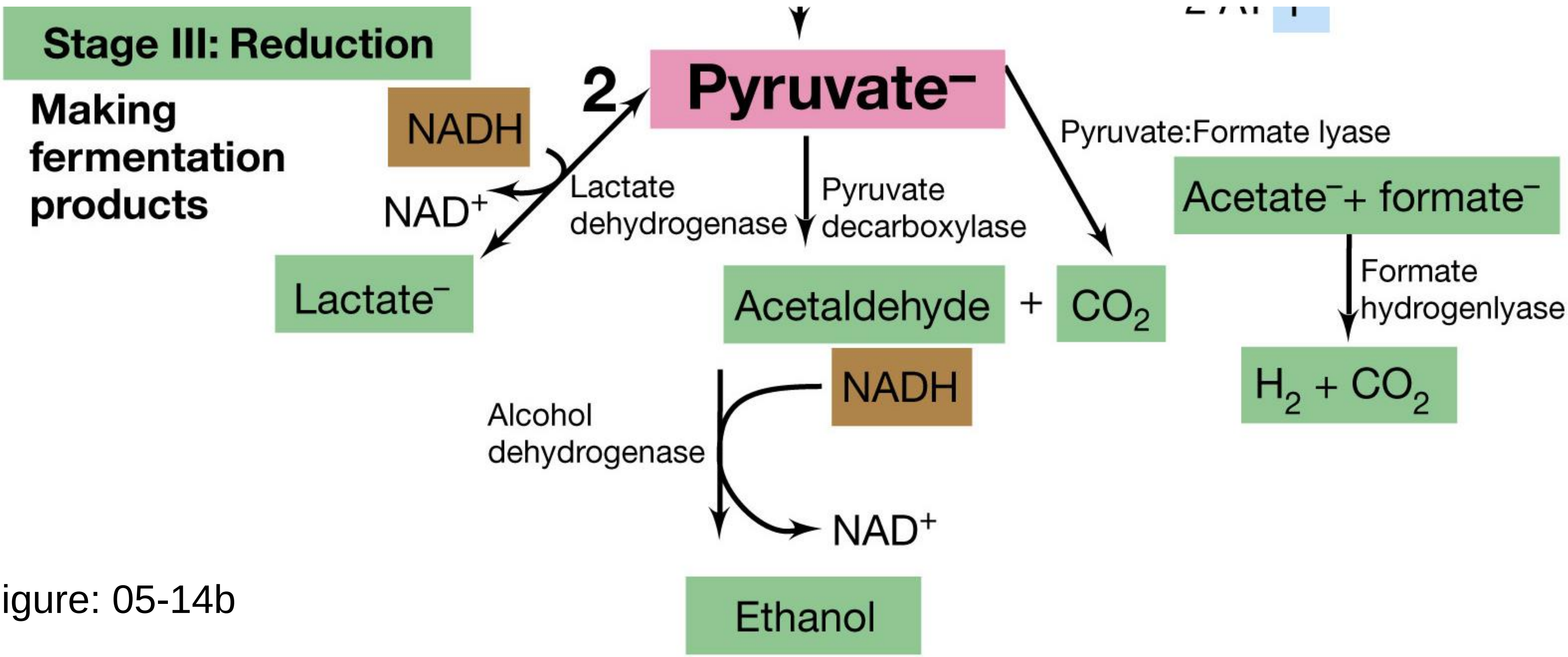
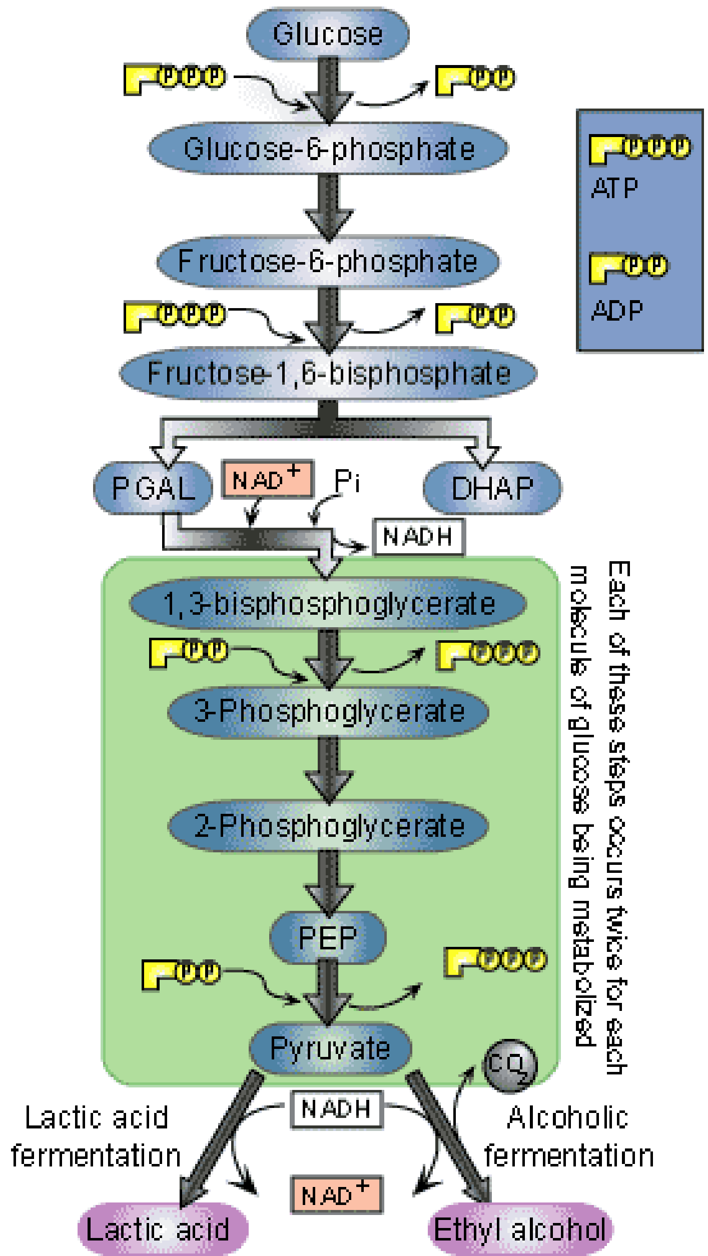


Figure: 05-14b

Note how pyruvate is the central "hub" of glycolysis, all fermentation products are made from pyruvate, and just a few common examples are given.



Glucose
Glucose-6-phosphate
Fructose-6-phosphate
Fructose-1,6-bisphosphate
PGAL
NAD+
Pi
DHAP
NADH
1,3-bisphosphoglycerate
3-Phosphoglycerate
2-Phosphoglycerate
PEP
Pyruvate
CO2
NADH
Lactic acid fermentation
Alcoholic fermentation
NAD+
Lactic acid
Ethyl alcohol
ATP
ADP
Each of these steps occurs twice for each molecule of glucose being metabolized

Hydrocarbon oxidation (leads to carboxylic acid which undergoes Beta-oxidation)

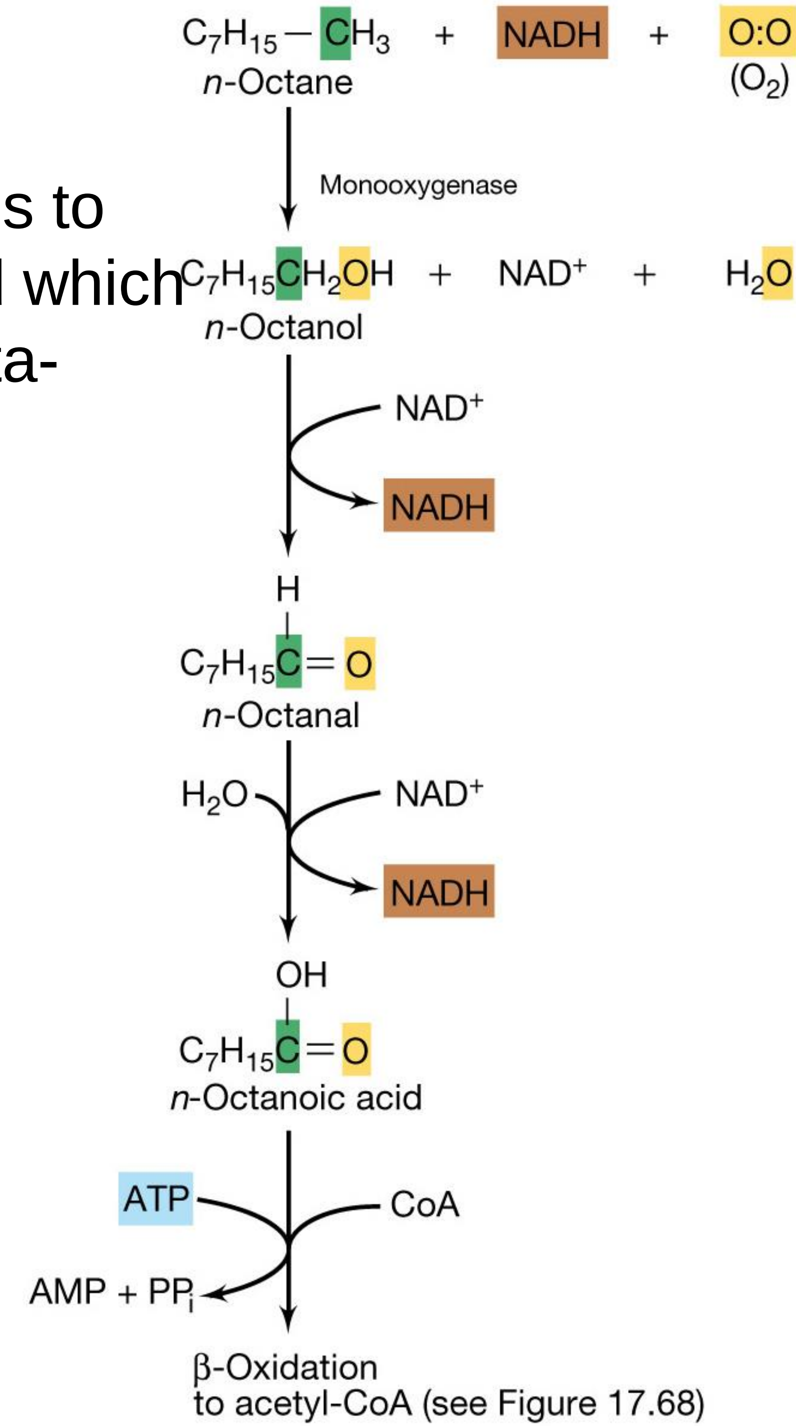
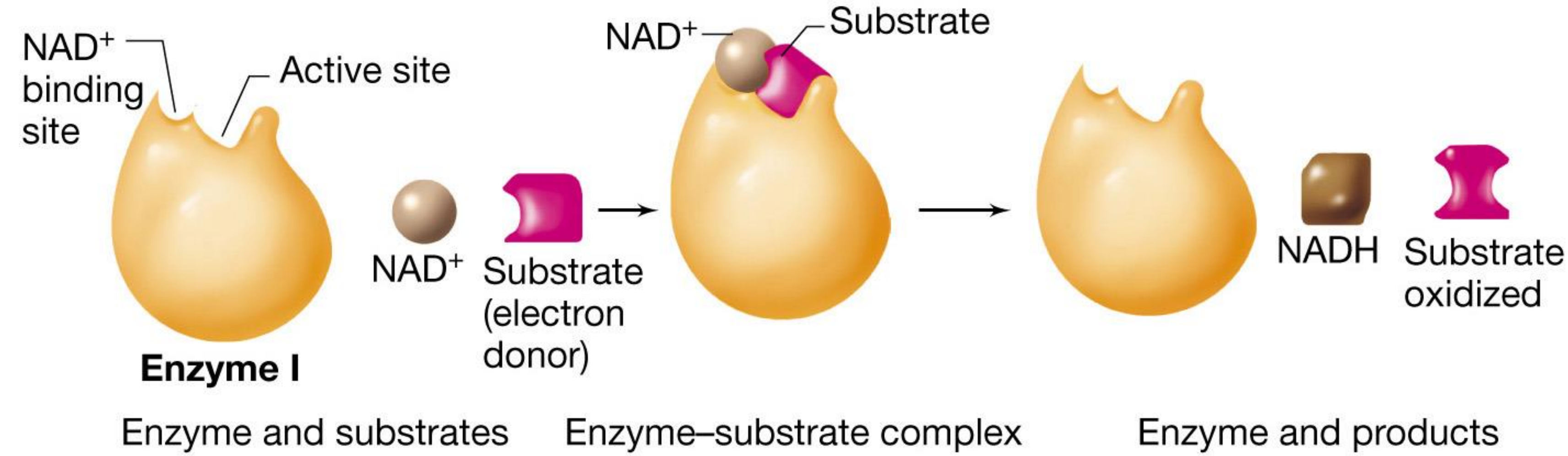
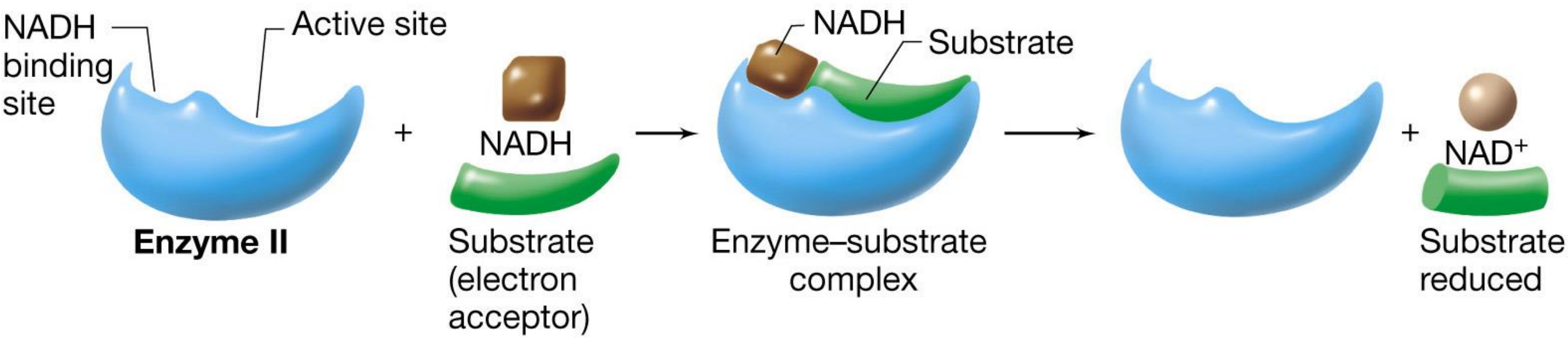


Figure: 17-55

NADH is a coenzyme!!



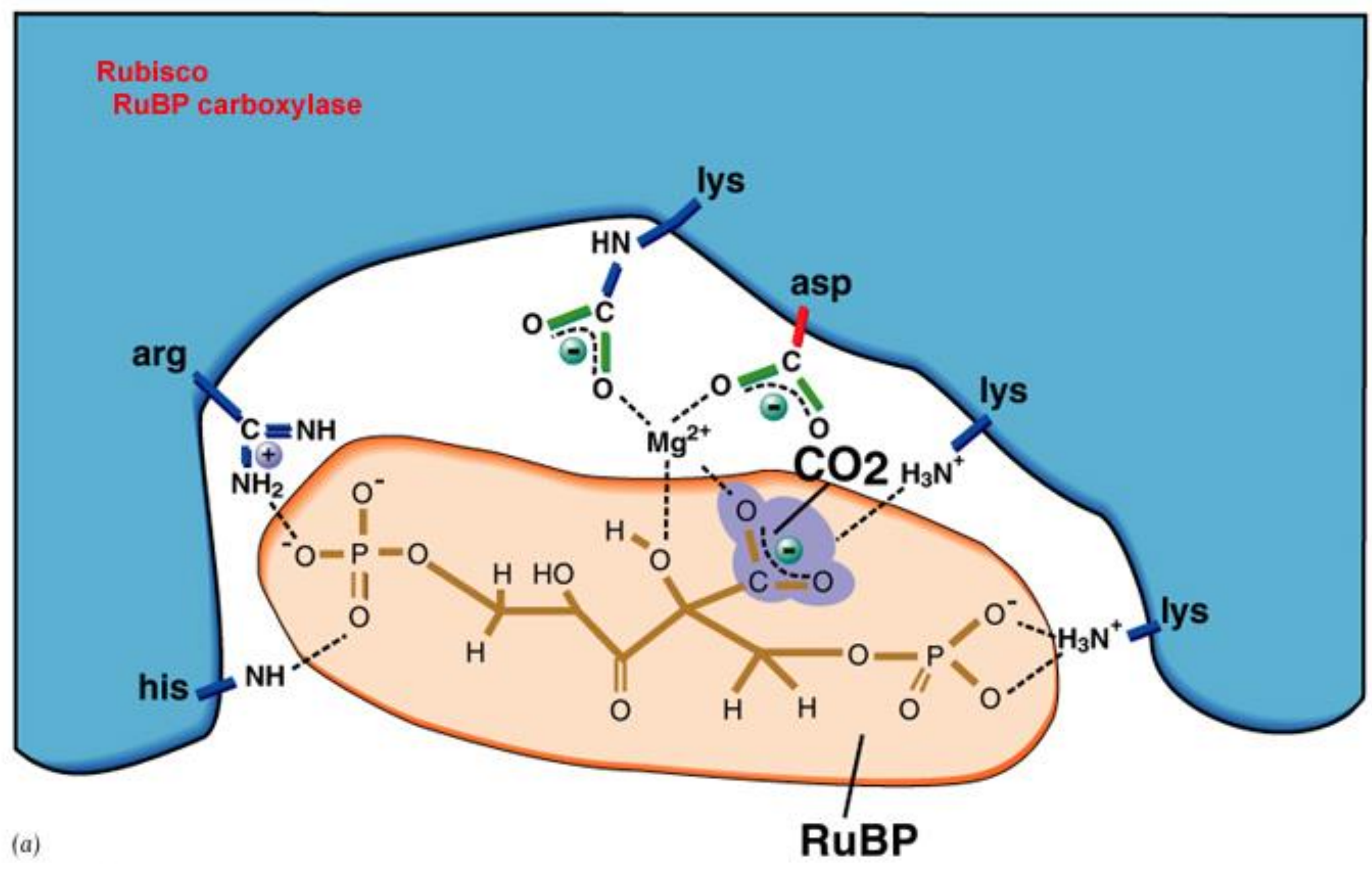
Reaction 1. Enzyme I reacts with substrate (electron donor) and oxidized form of coenzyme, NAD^+.



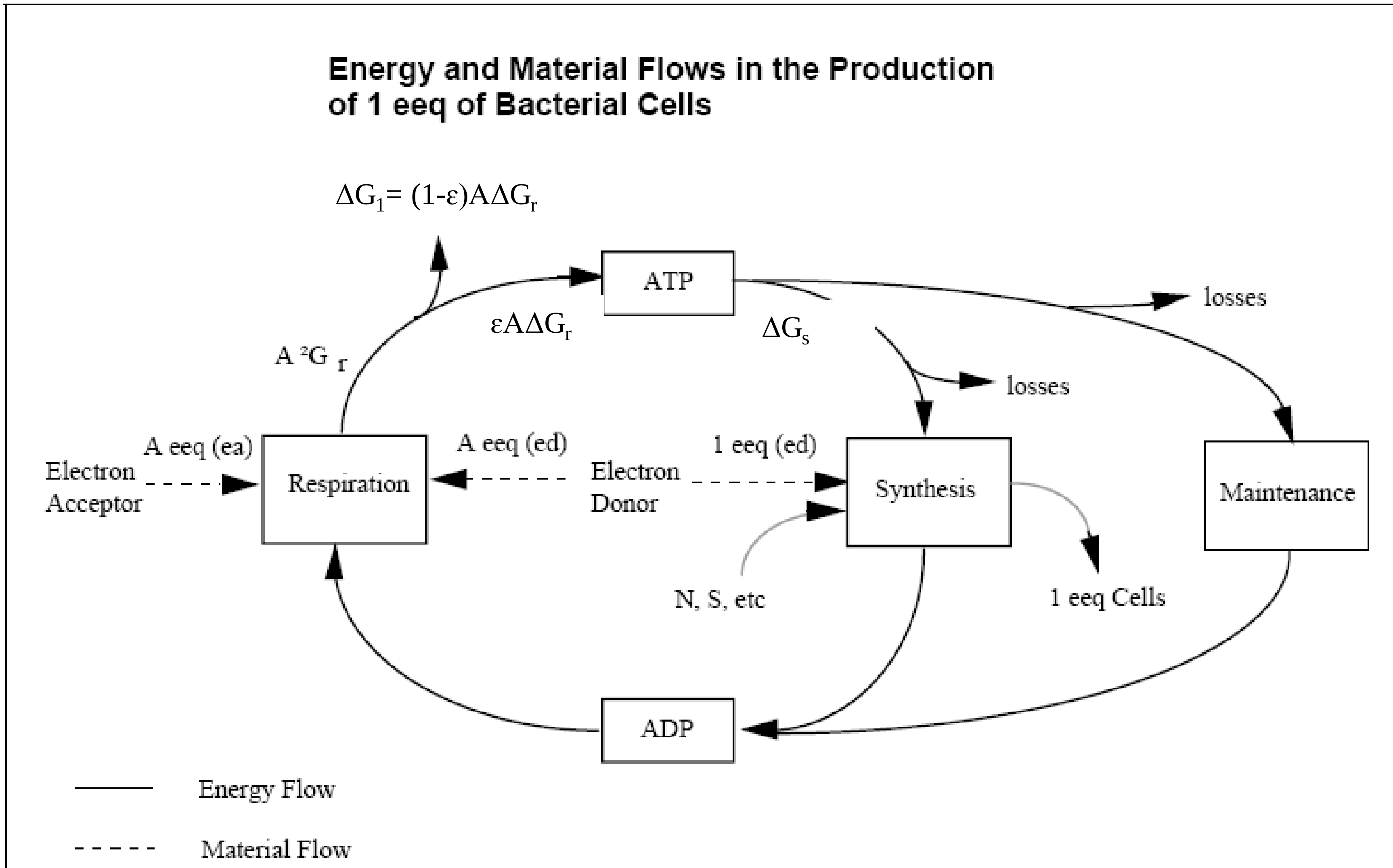
Reaction 2. Enzyme II reacts with substrate (electron acceptor) and reduced form of coenzyme, NADH.

Figure: 05-11

Enzyme active sites have 3D nature – amino acid R-groups dictate Enzyme-substrate interactions



Bioenergetics packet (from Jim Gossett). Cells metabolism is balanced energetically & materially: ***example of chemoorganoheterotroph***



Balancing flow of eeqs to energy and to synthesis

$$\frac{1}{24}C_6H_{12}O_6 + \frac{1}{4}O_2 \xrightarrow[\text{aerobic heterotrophs}]{\Delta Gr \text{ per eeq ED/EA coupled}} \frac{1}{4}CO_2 + \frac{1}{4}H_2O$$

ΔGp per eeq ED

$$1/10\ CH_3COCOO^- \longrightarrow \frac{1}{20}C_5H_7O_2N\ (1\,eeq\ biomass)$$

pyruvate

ΔGc (cells from pyruvate)
= + 7.5 kcal/eeq

A equations (with ammonia as N source)

Heterotrophs

$$A = \frac{\dfrac{-\Delta G_p}{\varepsilon^m} - \Delta G_c}{\varepsilon\ \Delta G_r}$$

Autotrophs

$$A = \frac{-59.7}{0.6\ \Delta G_r}$$

$m = +1\ \ (\Delta G_p > 0)$

$m = -1\ (\Delta G_p < 0)$

$\varepsilon = 0.6$ and $\Delta Gc = 7.5$ kcal are usually assumed

From A values to yields

$$a_e \equiv \frac{\text{eeq cells formed}}{\text{eeq e.d. used}} = \frac{1}{1 + A}$$

$$Y \equiv \frac{\text{g } X_a \text{ formed}}{\text{g OD used}} = \frac{5.65\ a_e}{8} = \frac{5.65}{8\ (1 + A)}$$

Of course, ΔG values vary from standard values if…

- … you are far from "standard" conditions of pH7, 1 M all aqueous solutes and 1 atm of all gaseous compounds, 298K
- Correction term can be key:

$\Delta G = \Delta G^{o'} + RT \ln Q$, where Q=reaction quotient of products & reactants

$$\Delta G = \Delta G^{o} + RT \ln \left[\frac{\prod_j \{P_j\}^{p_j}}{\prod_i \{R_i\}^{r_i}} \right]$$

TABLE 1. Fermentation Reactions for Hydrogen Donors Examined during This Study (*25*)[a]

	$\Delta G_{35°C}$ (kJ/mol)
Fermentation to Acetate and H_2	
butyrate$^-$ + $2H_2O \rightarrow$ 2acetate$^-$ + H^+ + $2H_2$	123.16
ethanol + $H_2O \rightarrow$ acetate$^-$ + H^+ + $2H_2$	84.85
lactate$^-$ + $2H_2O \rightarrow$ acetate$^-$ + HCO_3^- + H^+ + $2H_2$	71.01
propionate$^-$ + $3H_2O \rightarrow$ acetate$^-$ + HCO_3^- + H^+ + $3H_2$	166.9
Fermentation to Propionate and Acetate	
ethanol + $^2/_3HCO_3^- \rightarrow$ $^2/_3$propionate$^-$ + $^1/_3$acetate$^-$ + $^1/_3H^+$ + H_2O	−26.41
lactate$^-$ $\rightarrow$ $^1/_3$acetate$^-$ + $^2/_3$propionate$^-$ + $^1/_3HCO_3^-$ + $^1/_3H^+$	−40.26

[a] All species as aqueous.

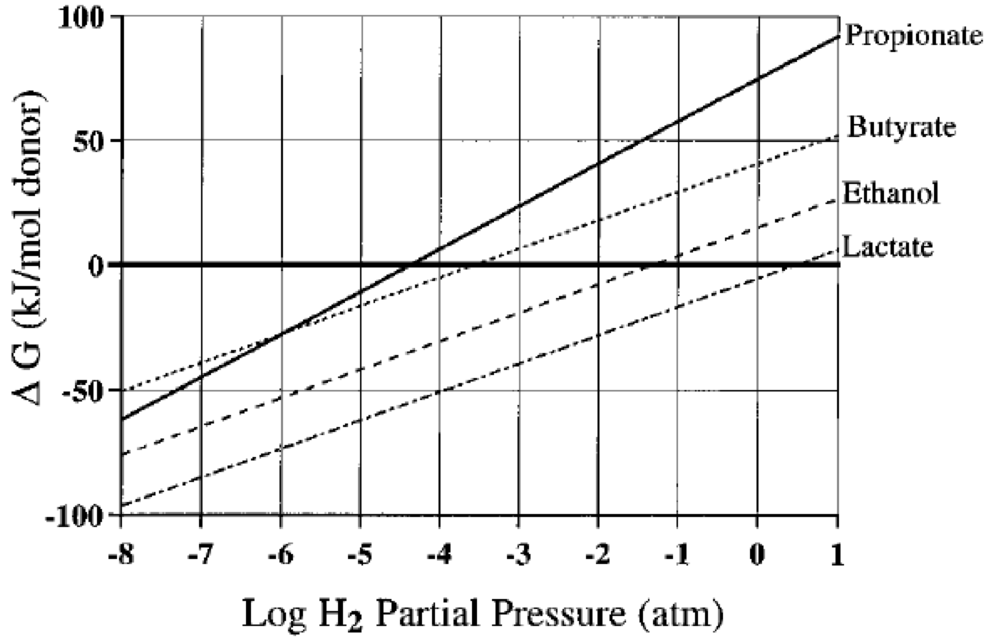


FIGURE 1. Effect of H_2 partial pressure on the free energy available from the fermentation of propionate, butyrate, ethanol, and lactate. Calculations were based on standard free energies and reactions in Thauer et al. (*22*) with temperature = 25 °C; pH = 7; HCO_3^- = 70 mM; propionate, butyrate, ethanol, and lactate concentrations = 0.5 mM; and acetate concentration = 5 mM.

Fennell et al., ES&T, 1997