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CEE 370
Environmental Engineering Principles

Lecture #13
Environmental Biology II

Metabolism

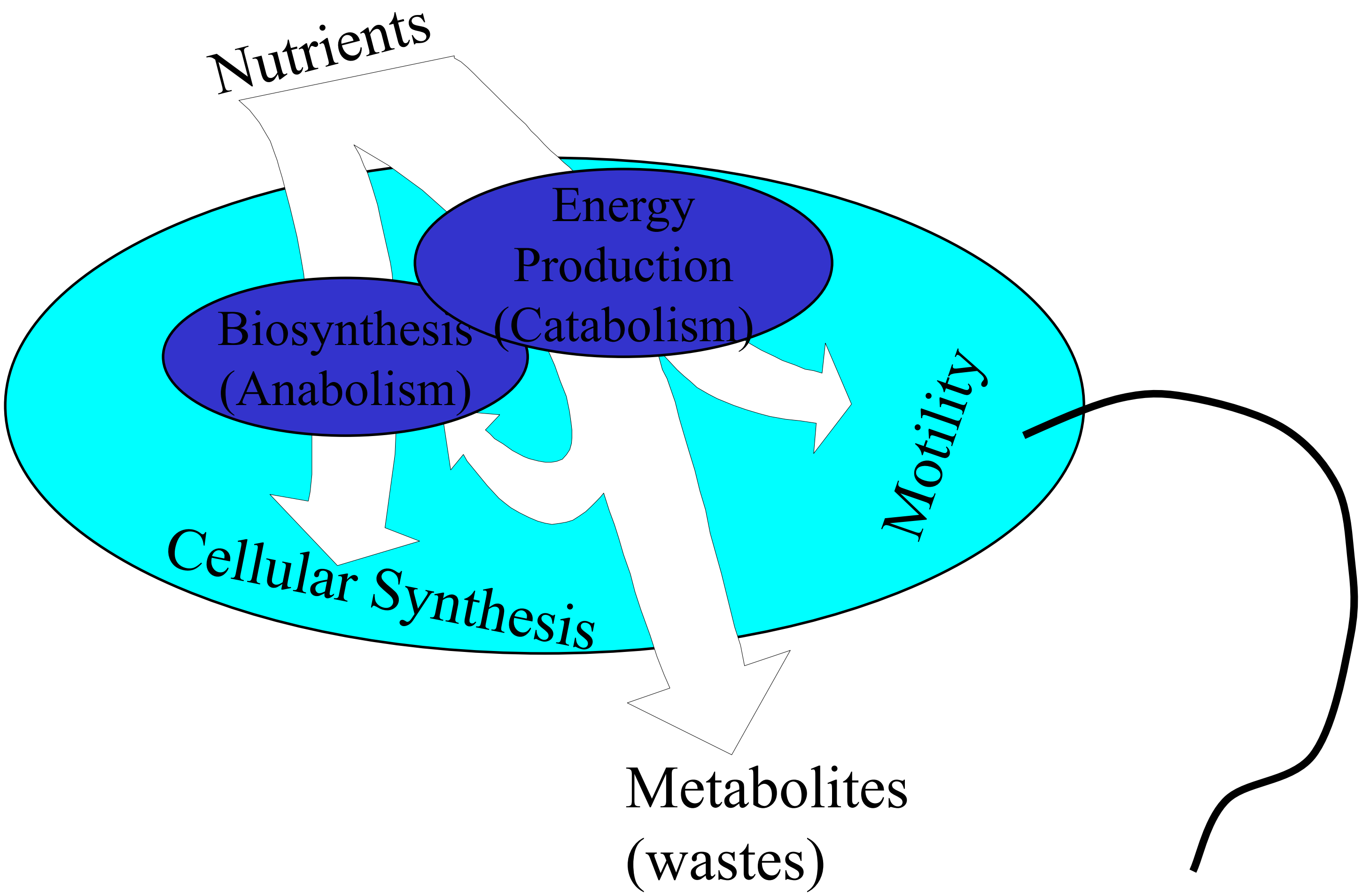
Reading: Mihelcic & Zimmerman, Chapter 5

Davis & Masten, Chapter 3

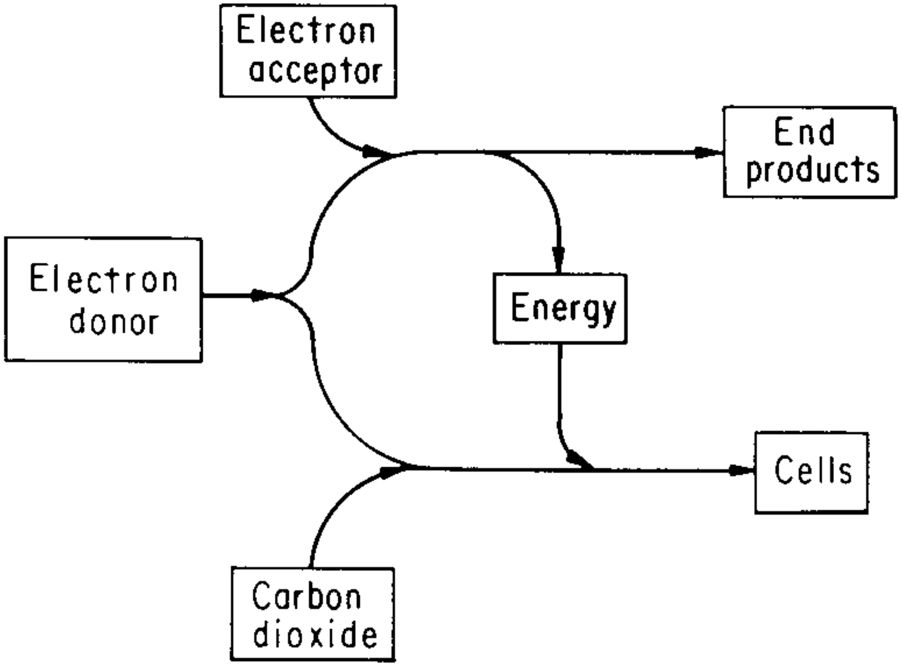
Environmental Microbiology

- Types of Microorganisms
  - Bacteria
  - Viruses
  - Protozoa
  - Rotifers
  - Fungi
- Metabolism
- Microbial Disease
- Microbial Growth

Metabolism


Nutrients
Energy Production (Catabolism)
Biosynthesis (Anabolism)
Motility
Cellular Synthesis
Metabolites (wastes)

An overview of metabolism

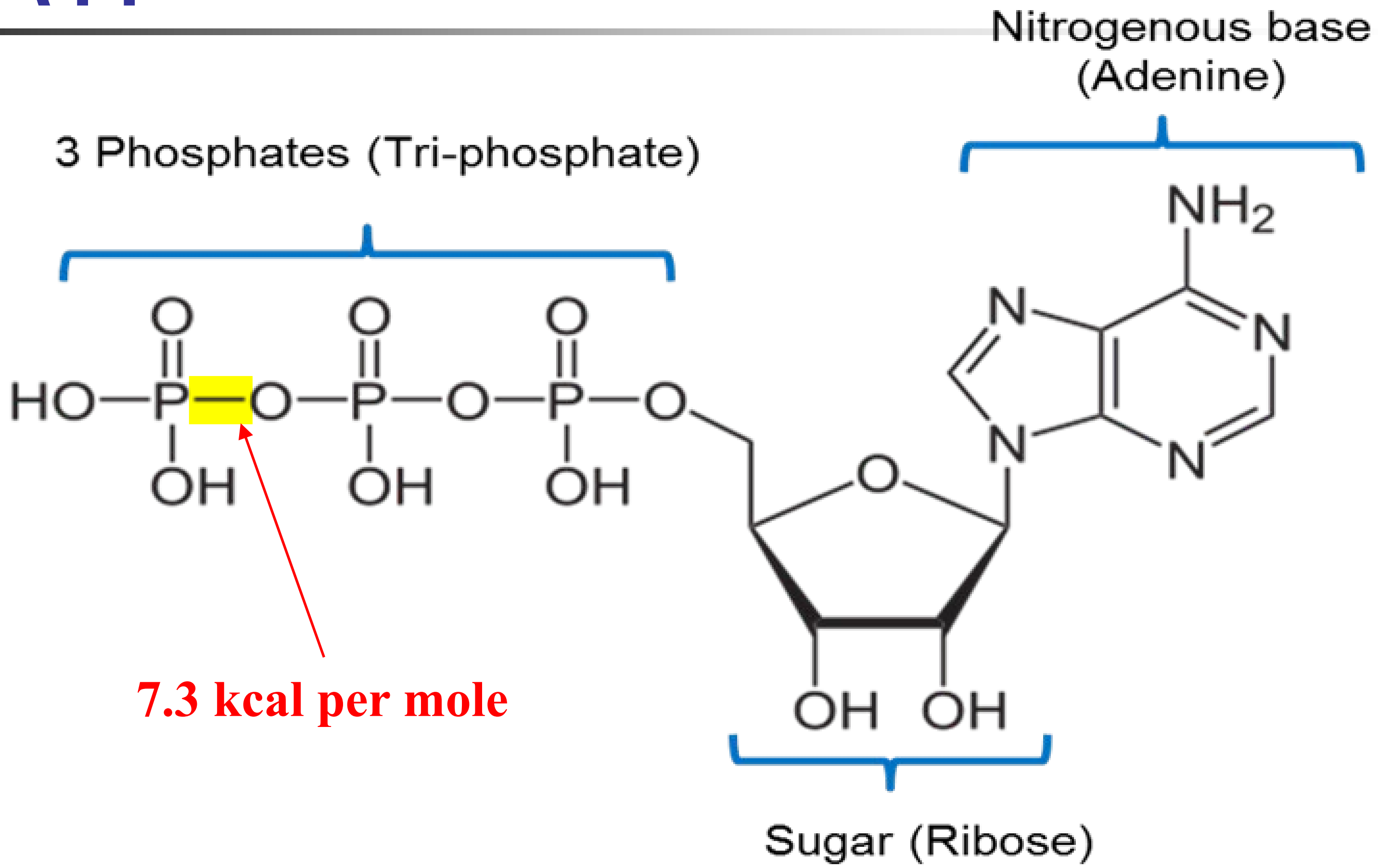


From: Sawyer, McCarty & Parkin, 1994;
also: Sawyer & McCarty, 1978

Energy

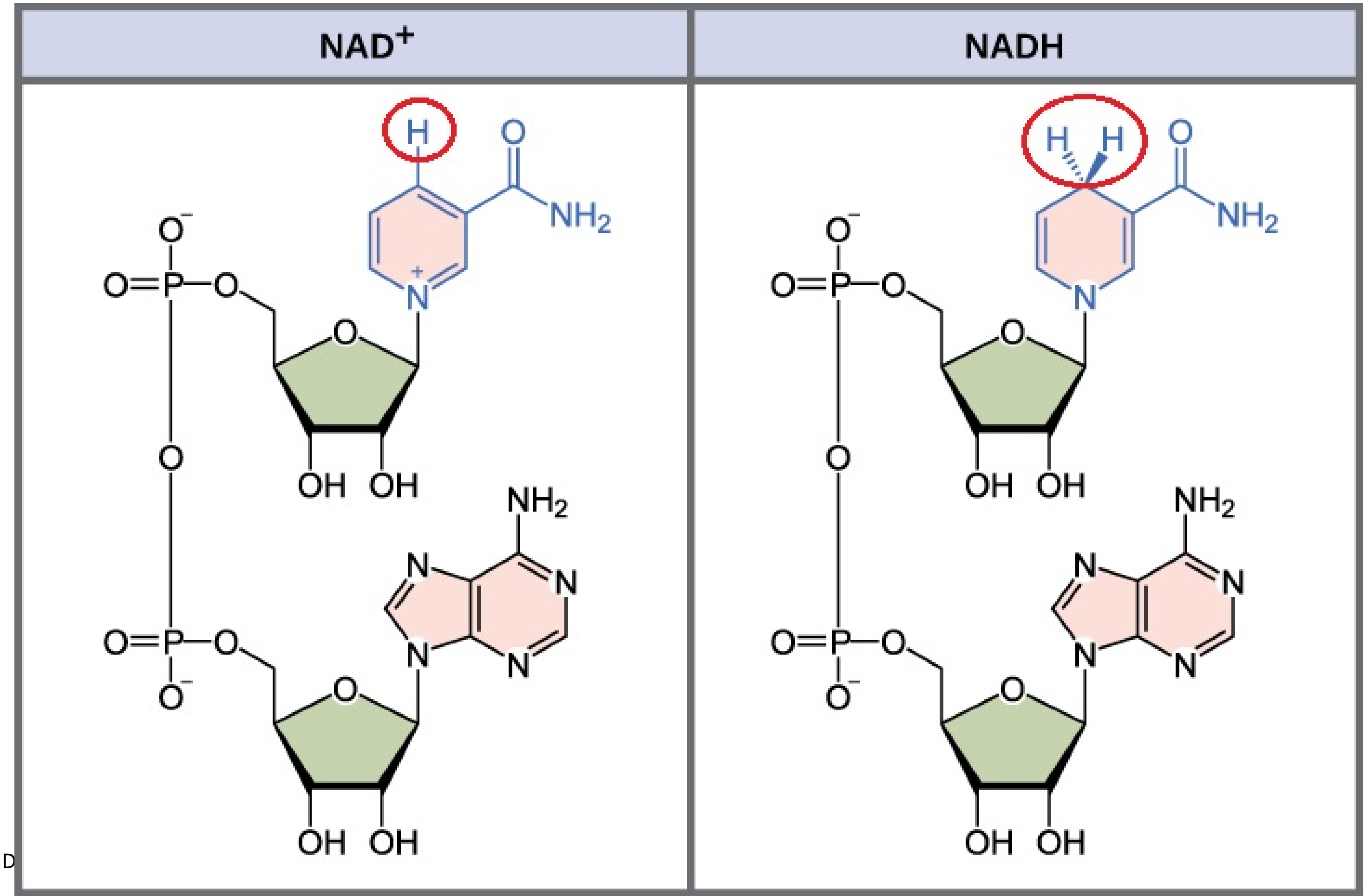
- Source
  - Light
  - Chemicals (e.g., glucose)
- Storage
  - ATP
  - NAD+
- Advantages of oxygen as a terminal electron acceptor
  - aerobic
  - anaerobic
  - facultative

ATP

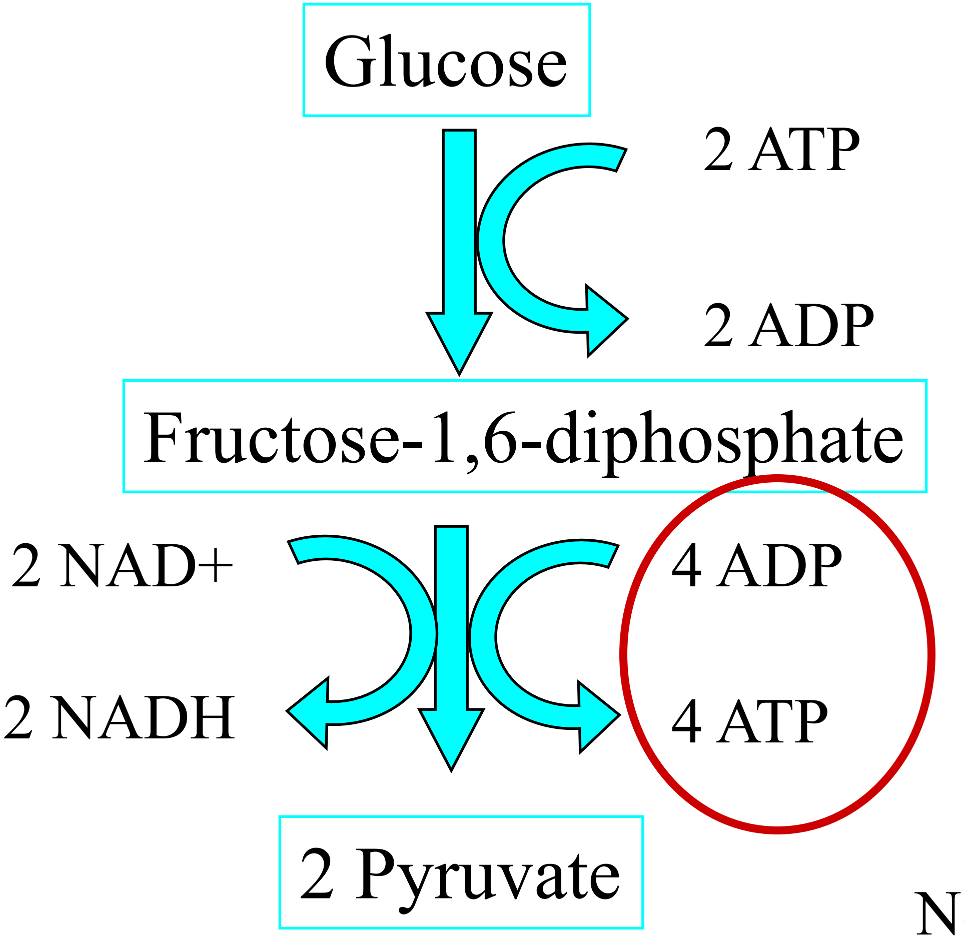

Nitrogenous base
(Adenine)
3 Phosphates (Tri-phosphate)
7.3 kcal per mole
Sugar (Ribose)

Nicotinamide Adenine Dinucleotide

$$NAD^+ + H^+ + 2e^- \rightleftharpoons NADH$$



Embden-Meyerhof Pathway



Glucose

2 ATP

2 ADP

Fructose-1,6-diphosphate

2 NAD+

2 NADH

4 ADP

4 ATP

2 Pyruvate

Investment

Pay-back

Net result: 2 ATPs
or 14.6 kcal/mole

Advantages of Aerobic Systems

If we have aerobic metabolism, rather than fermentation, energy from NADH may be harvested.

$$NADH + H^{+} + 3\,PO_4^{3-} + 3ADP + \tfrac{1}{2}O_2 \rightarrow NAD+ + 3ATP + H_2O$$

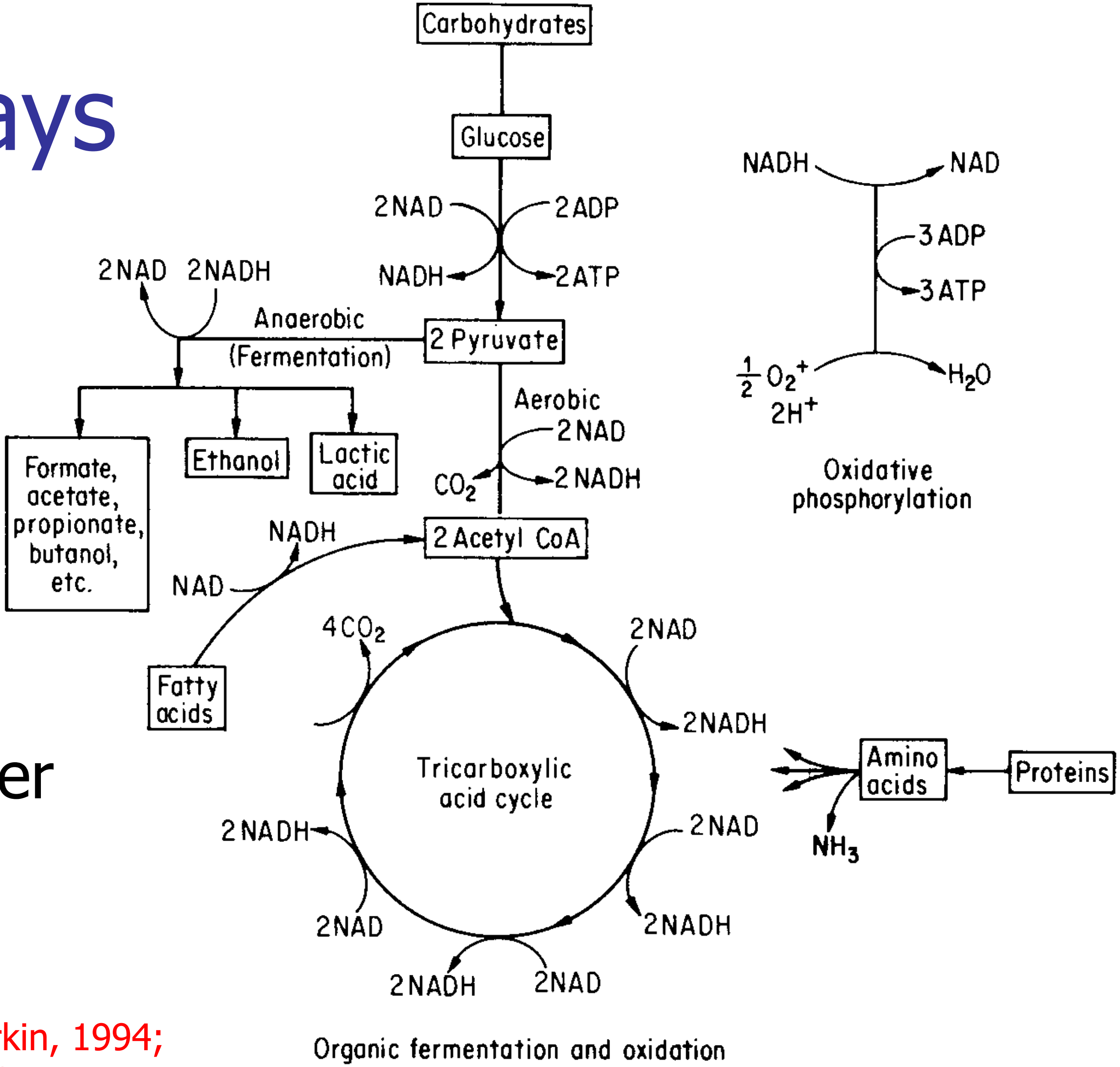
This gives us 6 more ATPs. Then the pyruvate may be further oxidized to carbon dioxide and water, producing 30 more ATPs. The final tally is 38 ATPs or <u>277 kcal/mole</u> of glucose.

Don't need to "know" this

Pathways

- Generalized view of both aerobic and fermentative pathways
- Also showing energy transfer
    - ATP
    - NAD

From: Sawyer, McCarty & Parkin, 1994; also: Sawyer & McCarty, 1978



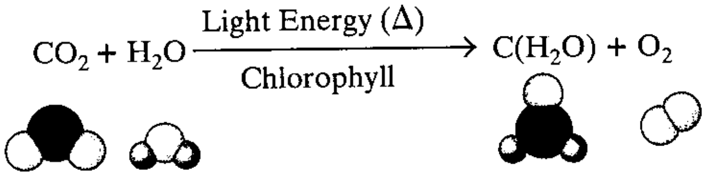
Organic fermentation and oxidation

Metabolic Classification

- Carbon Source
  - Heterotrophic: other organic matter
  - Autotrophic: inorganic carbon (CO_2)
- Energy Source (electron donor)
  - Chemosynthetic: chemical oxidation
  - Photosynthetic: light energy
- Terminal Electron Acceptor
  - Aerobic: oxygen
  - Anaerobic: nitrate, sulfate
  - Fermentative: organic compounds

Energy Flow

- Storage of Energy
  - Photosynthesis
- Release of Energy
  - Respiration
- Energy transfers by organisms are inherently inefficient
  - 5-50% capture



(*a*) Photosynthesis

(*b*) Respiration

Aerobic Respiration

(*b*) Respiration

- A Redox reaction
  - Oxidation of Carbon

$$C(H_2O) + H_2O \rightarrow CO_2 + 4H^+ + 4e^-$$

  - Reduction of oxygen or some other terminal electron acceptor

$$O_2 + 4H^+ + 4e^- \rightarrow 2H_2O$$

Other TEA: Anaerobic Respiration

- Nitrate

$$C(H_2O)+NO_3^- \rightarrow N_2+CO_2+HCO_3^-+H_2O$$

- Manganese

$$C(H_2O)+Mn^{+4} \rightarrow Mn^{+2}+CO_2+H_2O$$

- Iron

$$C(H_2O)+Fe^{+3} \rightarrow Fe^{+2}+CO_2+H_2O$$

- Sulfate

$$C(H_2O)+SO_4^{-2} \rightarrow H_2S+CO_2+H_2O$$

- Fermentation

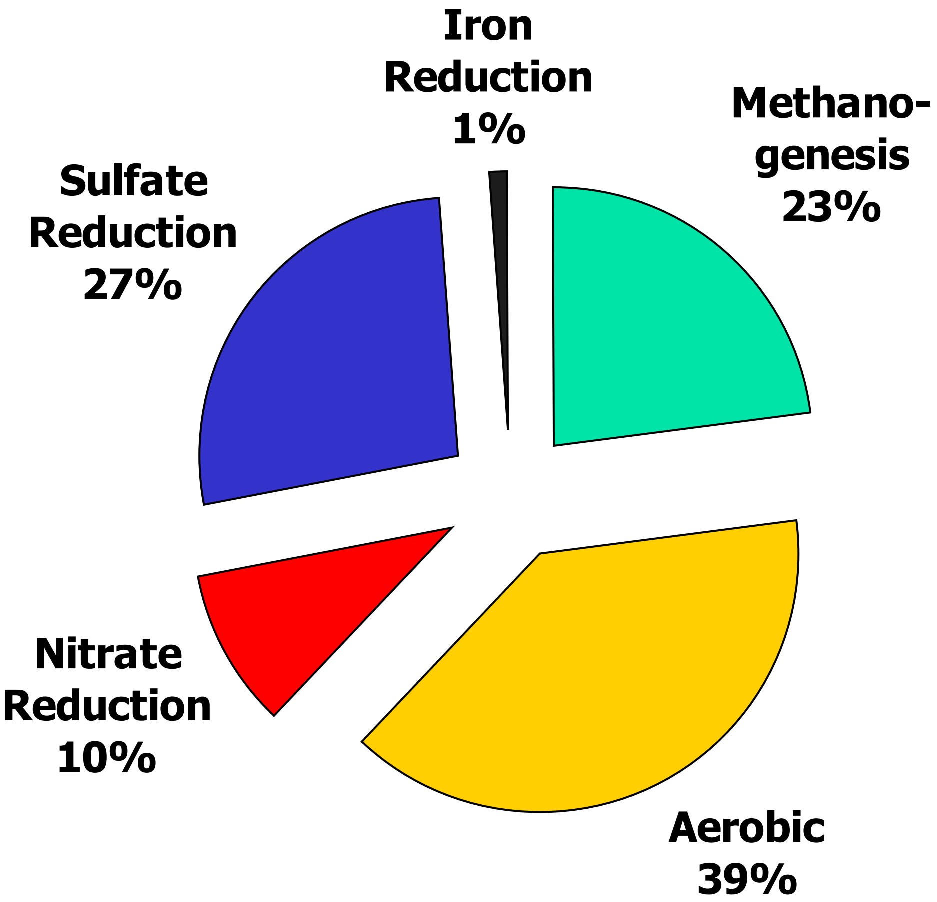
$$C(H_2O) \rightarrow CH_4+CO_2$$

methanogenesis

Ecological
Redox
Sequence

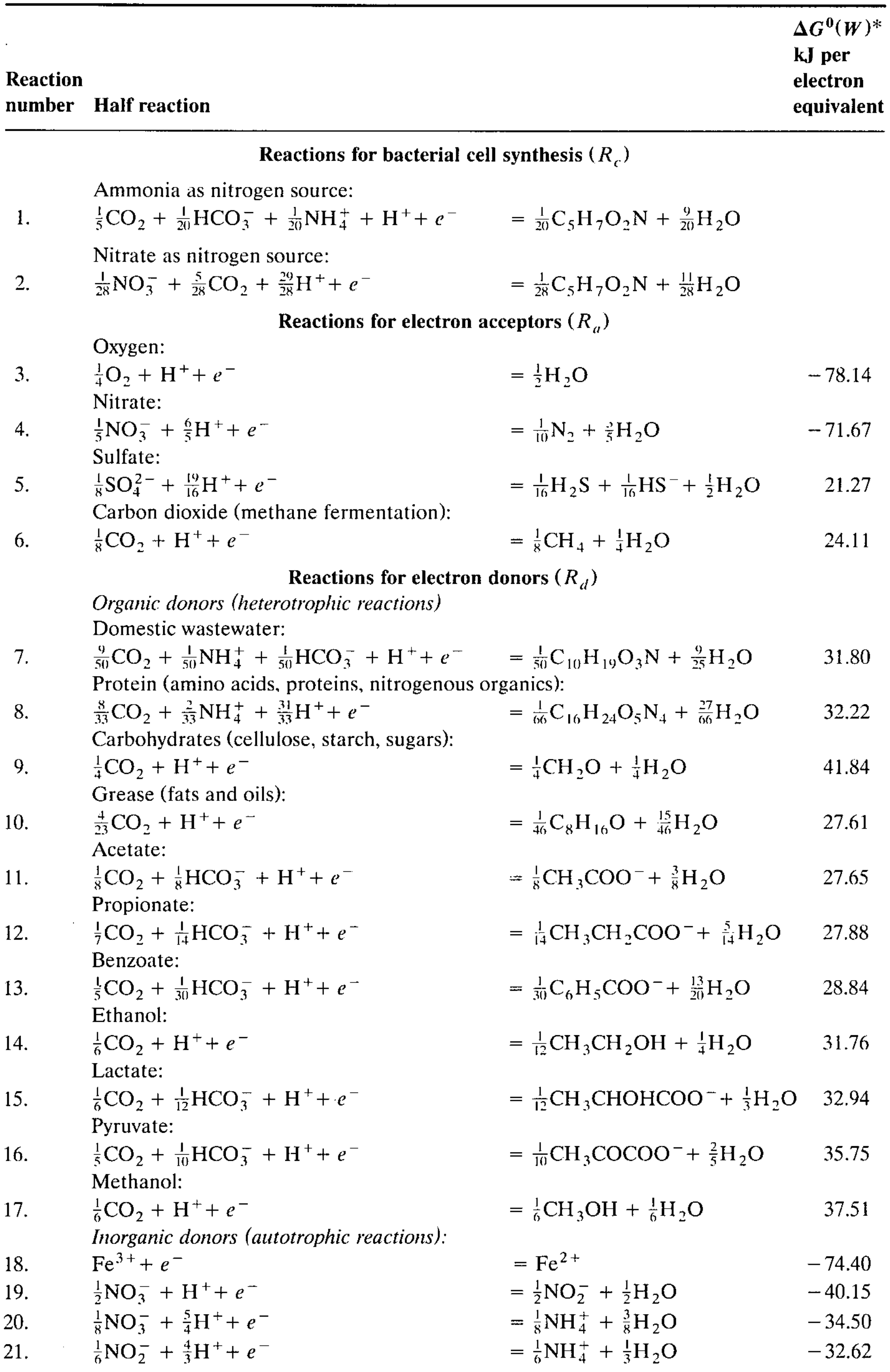
Terminal Electron Acceptors

- Contribution to the oxidation of organic matter
  - Bottom waters of Onondaga Lake, NY
  - (Effler, 1997)



Energetics

- Principles of Gibbs Free Energy and Energy Balance can be applied to microbial growth

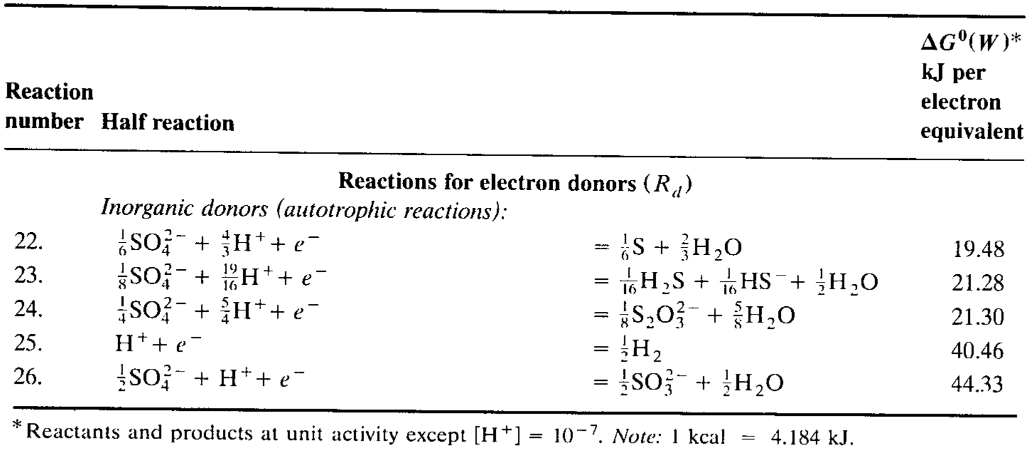


Reaction number	Half reaction		$\Delta G^0(W)$* kJ per electron equivalent
	Reactions for bacterial cell synthesis (R_c)		
	Ammonia as nitrogen source:		
1.	$\frac{1}{5}CO_2 + \frac{1}{20}HCO_3^- + \frac{1}{20}NH_4^+ + H^+ + e^-$	$= \frac{1}{20}C_5H_7O_2N + \frac{9}{20}H_2O$	
	Nitrate as nitrogen source:		
2.	$\frac{1}{28}NO_3^- + \frac{5}{28}CO_2 + \frac{29}{28}H^+ + e^-$	$= \frac{1}{28}C_5H_7O_2N + \frac{11}{28}H_2O$	
	Reactions for electron acceptors (R_a)		
	Oxygen:		
3.	$\frac{1}{4}O_2 + H^+ + e^-$	$= \frac{1}{2}H_2O$	−78.14
	Nitrate:		
4.	$\frac{1}{5}NO_3^- + \frac{6}{5}H^+ + e^-$	$= \frac{1}{10}N_2 + \frac{3}{5}H_2O$	−71.67
	Sulfate:		
5.	$\frac{1}{8}SO_4^{2-} + \frac{19}{16}H^+ + e^-$	$= \frac{1}{16}H_2S + \frac{1}{16}HS^- + \frac{1}{2}H_2O$	21.27
	Carbon dioxide (methane fermentation):		
6.	$\frac{1}{8}CO_2 + H^+ + e^-$	$= \frac{1}{8}CH_4 + \frac{1}{4}H_2O$	24.11
	Reactions for electron donors (R_d)		
	Organic donors (heterotrophic reactions)		
	Domestic wastewater:		
7.	$\frac{9}{50}CO_2 + \frac{1}{50}NH_4^+ + \frac{1}{50}HCO_3^- + H^+ + e^-$	$= \frac{1}{50}C_{10}H_{19}O_3N + \frac{9}{25}H_2O$	31.80
	Protein (amino acids, proteins, nitrogenous organics):		
8.	$\frac{8}{33}CO_2 + \frac{2}{33}NH_4^+ + \frac{31}{33}H^+ + e^-$	$= \frac{1}{66}C_{16}H_{24}O_5N_4 + \frac{27}{66}H_2O$	32.22
	Carbohydrates (cellulose, starch, sugars):		
9.	$\frac{1}{4}CO_2 + H^+ + e^-$	$= \frac{1}{4}CH_2O + \frac{1}{4}H_2O$	41.84
	Grease (fats and oils):		
10.	$\frac{4}{23}CO_2 + H^+ + e^-$	$= \frac{1}{46}C_8H_{16}O + \frac{15}{46}H_2O$	27.61
	Acetate:		
11.	$\frac{1}{8}CO_2 + \frac{1}{8}HCO_3^- + H^+ + e^-$	$= \frac{1}{8}CH_3COO^- + \frac{3}{8}H_2O$	27.65
	Propionate:		
12.	$\frac{1}{7}CO_2 + \frac{1}{14}HCO_3^- + H^+ + e^-$	$= \frac{1}{14}CH_3CH_2COO^- + \frac{5}{14}H_2O$	27.88
	Benzoate:		
13.	$\frac{1}{5}CO_2 + \frac{1}{30}HCO_3^- + H^+ + e^-$	$= \frac{1}{30}C_6H_5COO^- + \frac{13}{20}H_2O$	28.84
	Ethanol:		
14.	$\frac{1}{6}CO_2 + H^+ + e^-$	$= \frac{1}{12}CH_3CH_2OH + \frac{1}{4}H_2O$	31.76
	Lactate:		
15.	$\frac{1}{6}CO_2 + \frac{1}{12}HCO_3^- + H^+ + e^-$	$= \frac{1}{12}CH_3CHOHCOO^- + \frac{1}{3}H_2O$	32.94
	Pyruvate:		
16.	$\frac{1}{5}CO_2 + \frac{1}{10}HCO_3^- + H^+ + e^-$	$= \frac{1}{10}CH_3COCOO^- + \frac{2}{5}H_2O$	35.75
	Methanol:		
17.	$\frac{1}{6}CO_2 + H^+ + e^-$	$= \frac{1}{6}CH_3OH + \frac{1}{6}H_2O$	37.51
	Inorganic donors (autotrophic reactions):		
18.	$Fe^{3+} + e^-$	$= Fe^{2+}$	−74.40
19.	$\frac{1}{2}NO_3^- + H^+ + e^-$	$= \frac{1}{2}NO_2^- + \frac{1}{2}H_2O$	−40.15
20.	$\frac{1}{8}NO_3^- + \frac{5}{4}H^+ + e^-$	$= \frac{1}{8}NH_4^+ + \frac{3}{8}H_2O$	−34.50
21.	$\frac{1}{6}NO_2^- + \frac{4}{3}H^+ + e^-$	$= \frac{1}{6}NH_4^+ + \frac{1}{3}H_2O$	−32.62

From: Sawyer, McCarty & Parkin, 1994;
also: Sawyer & McCarty, 1978

Energetics Cont.

- Energy Balance
  - Cell synthesis (R_c)
  - Energy (R_a)
  - Electron acceptor (R_d)



Reaction number	Half reaction		$\Delta G^0(W)$* kJ per electron equivalent
	Reactions for electron donors (R_d)		
	Inorganic donors (autotrophic reactions):		
22.	$\frac{1}{6}SO_4^{2-} + \frac{4}{3}H^+ + e^-$	$= \frac{1}{6}S + \frac{2}{3}H_2O$	19.48
23.	$\frac{1}{8}SO_4^{2-} + \frac{19}{16}H^+ + e^-$	$= \frac{1}{16}H_2S + \frac{1}{16}HS^- + \frac{1}{2}H_2O$	21.28
24.	$\frac{1}{4}SO_4^{2-} + \frac{5}{4}H^+ + e^-$	$= \frac{1}{8}S_2O_3^{2-} + \frac{5}{8}H_2O$	21.30
25.	$H^+ + e^-$	$= \frac{1}{2}H_2$	40.46
26.	$\frac{1}{2}SO_4^{2-} + H^+ + e^-$	$= \frac{1}{2}SO_3^{2-} + \frac{1}{2}H_2O$	44.33

*Reactants and products at unit activity except $[H^+] = 10^{-7}$. *Note:* 1 kcal = 4.184 kJ.

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

From: Sawyer, McCarty & Parkin, 1994;
also: Sawyer & McCarty, 1978

f-values and Yield

- Portions of electron donor used for:
  - Synthesis (f_s)
  - Energy (f_e)
- Values are for rapidly growing cells

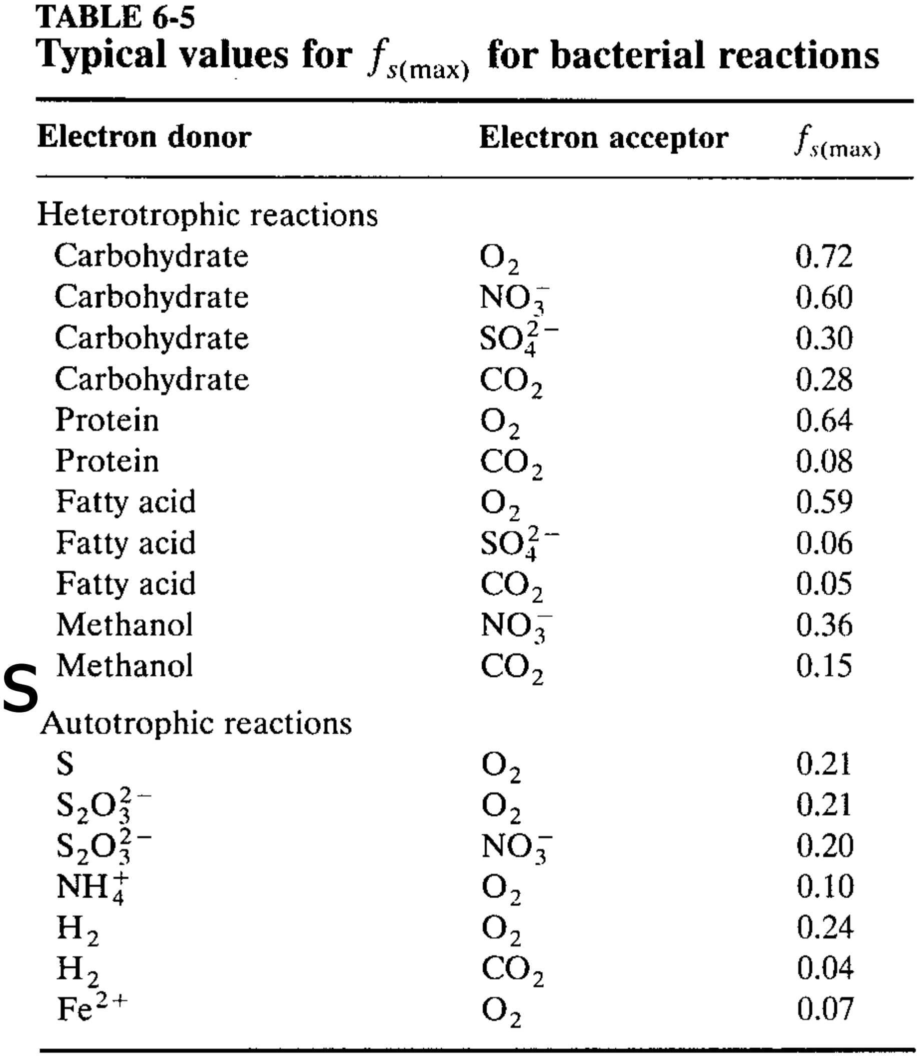


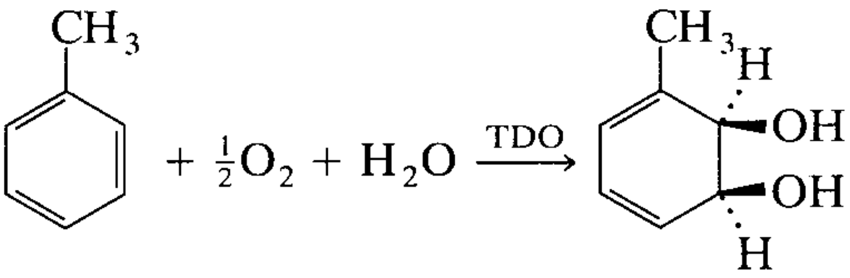
TABLE 6-5
Typical values for $f_{s(\max)}$ for bacterial reactions

Electron donor	Electron acceptor	$f_{s(\max)}$
Heterotrophic reactions		
Carbohydrate	O_2	0.72
Carbohydrate	NO_3^-	0.60
Carbohydrate	SO_4^{2-}	0.30
Carbohydrate	CO_2	0.28
Protein	O_2	0.64
Protein	CO_2	0.08
Fatty acid	O_2	0.59
Fatty acid	SO_4^{2-}	0.06
Fatty acid	CO_2	0.05
Methanol	NO_3^-	0.36
Methanol	CO_2	0.15
Autotrophic reactions		
S	O_2	0.21
$S_2O_3^{2-}$	O_2	0.21
$S_2O_3^{2-}$	NO_3^-	0.20
NH_4^+	O_2	0.10
H_2	O_2	0.24
H_2	CO_2	0.04
Fe^{2+}	O_2	0.07

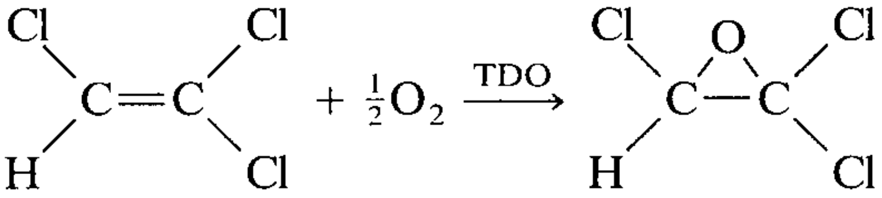
From: Sawyer, McCarty & Parkin, 1994;
also: Sawyer & McCarty, 1978

Novel Biotransformations

- Oxidation
  - Toluene dioxygenase (TDO)

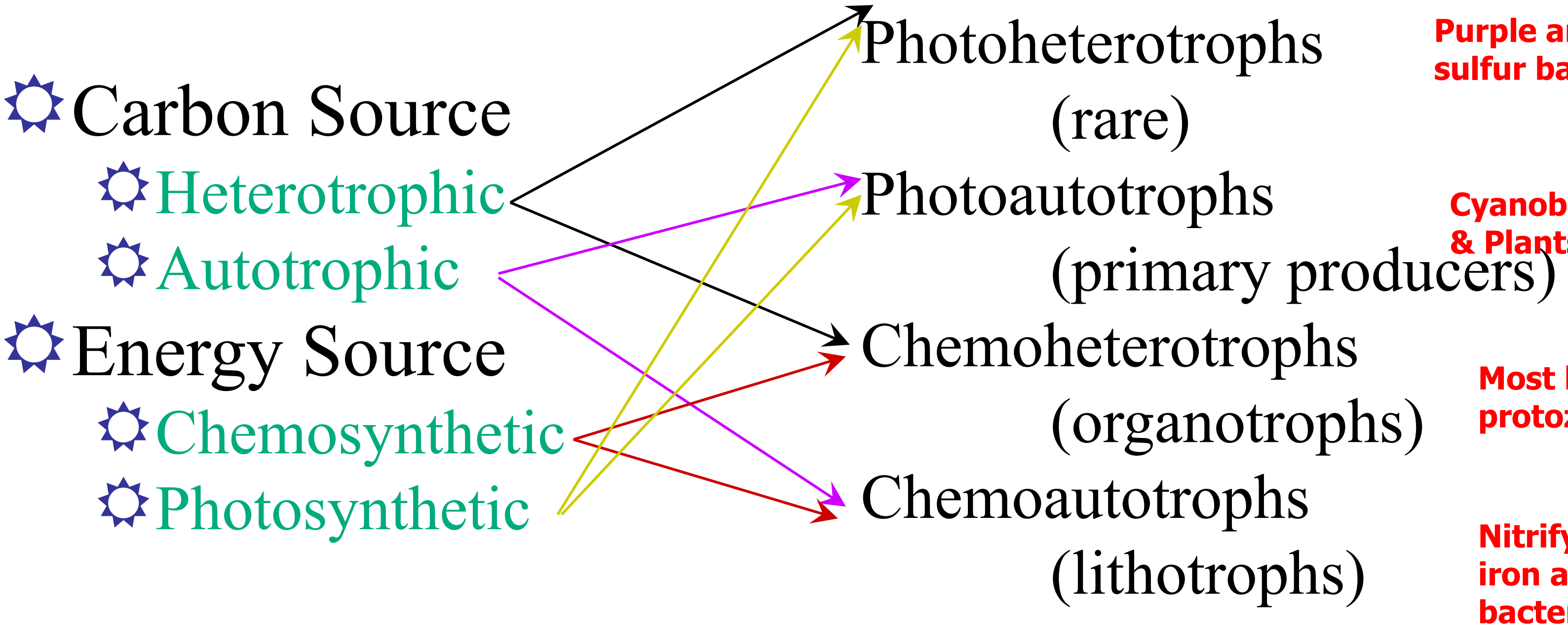


This enzyme will also oxidize TCE to its epoxide:



From: Sawyer, McCarty & Parkin, 1994;
also: Sawyer & McCarty, 1978

Overall Types



- Carbon Source
  - Heterotrophic
  - Autotrophic
- Energy Source
  - Chemosynthetic
  - Photosynthetic

Photoheterotrophs (rare)

Purple and green non-sulfur bacteria

Photoautotrophs (primary producers)

Cyanobacteria, algae & Plants

Chemoheterotrophs (organotrophs)

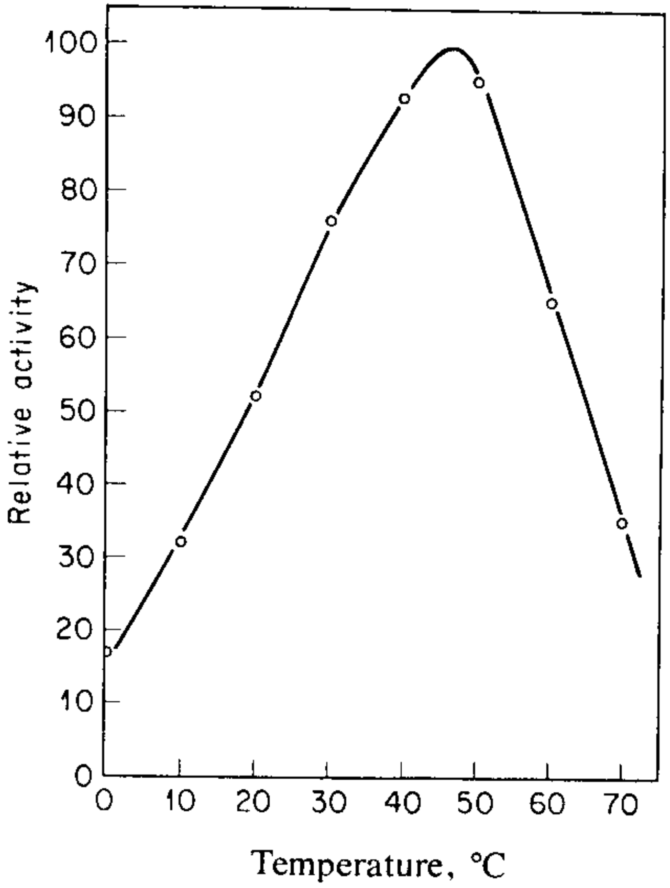
Most bacteria, fungi, protozoa & animals

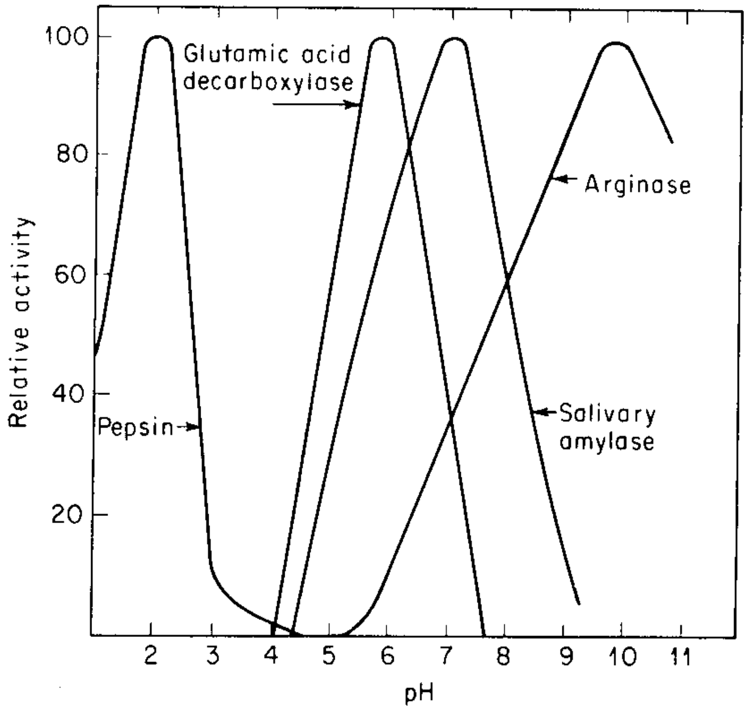
Chemoautotrophs (lithotrophs)

Nitrifying, hydrogen , iron and sulfur bacteria

Enzyme Chemistry

- Highly dependent on:
  - Temperature
  - pH

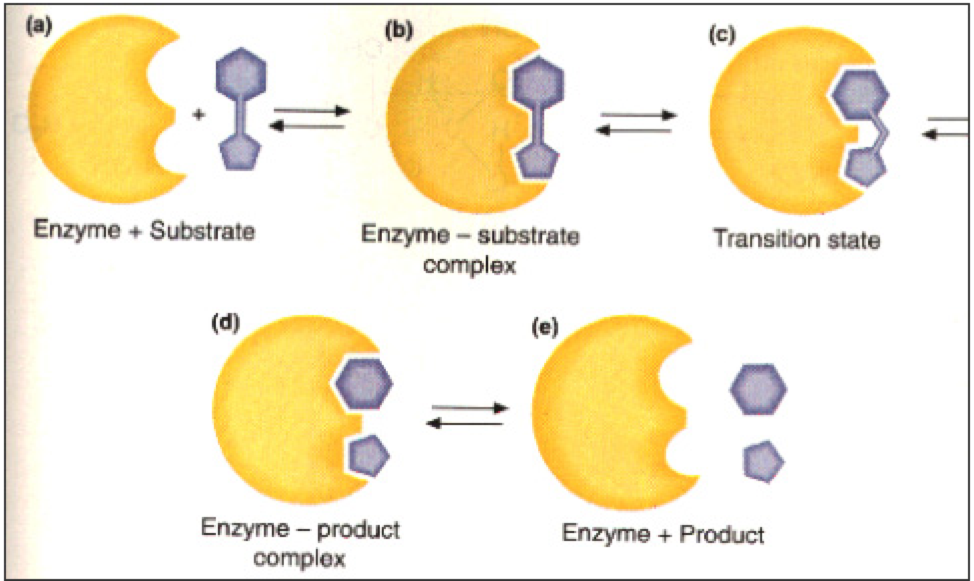


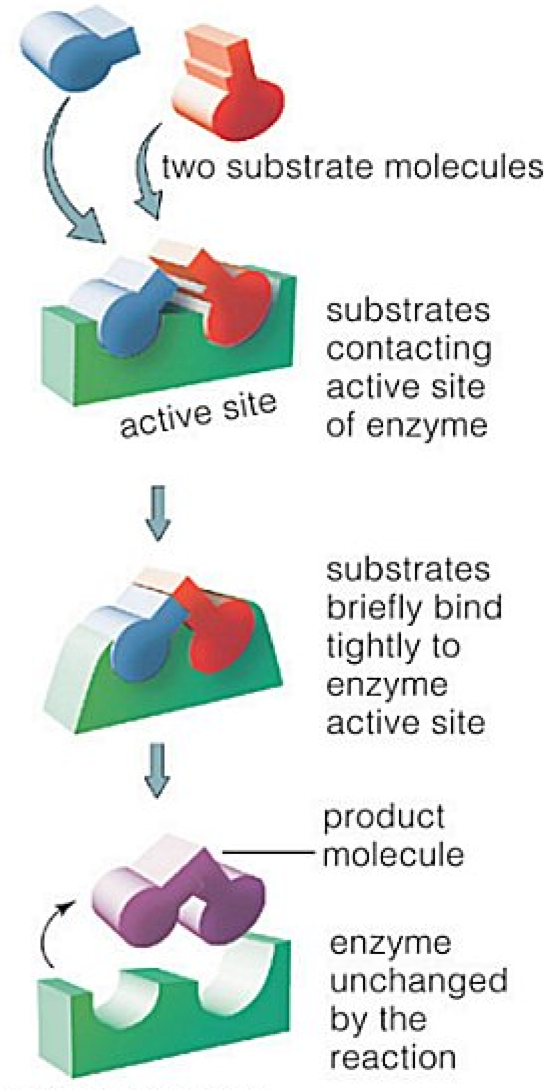


From: Sawyer, McCarty & Parkin, 1994;
also: Sawyer & McCarty, 1978

Enzymatic Reactions

- Many ways of illustrating the s
  - Substrate(s) bond to active site
  - Product(s) form via transition st
  - Product(s) are released





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Basic Enzyme Kinetics

Note that some references use k_2 for k_{-1}, and k_3 for k_2

- Irreversible

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} ES \xrightarrow{k_2} E + P$$

  - Single intermediate
    - The overall rate is determined by the RLS, k_2

$$r \equiv -\frac{d[S]}{dt} = \frac{d[P]}{dt} = k_2[ES]$$

    - But we don't know [ES], so we can get it by the SS mass balance

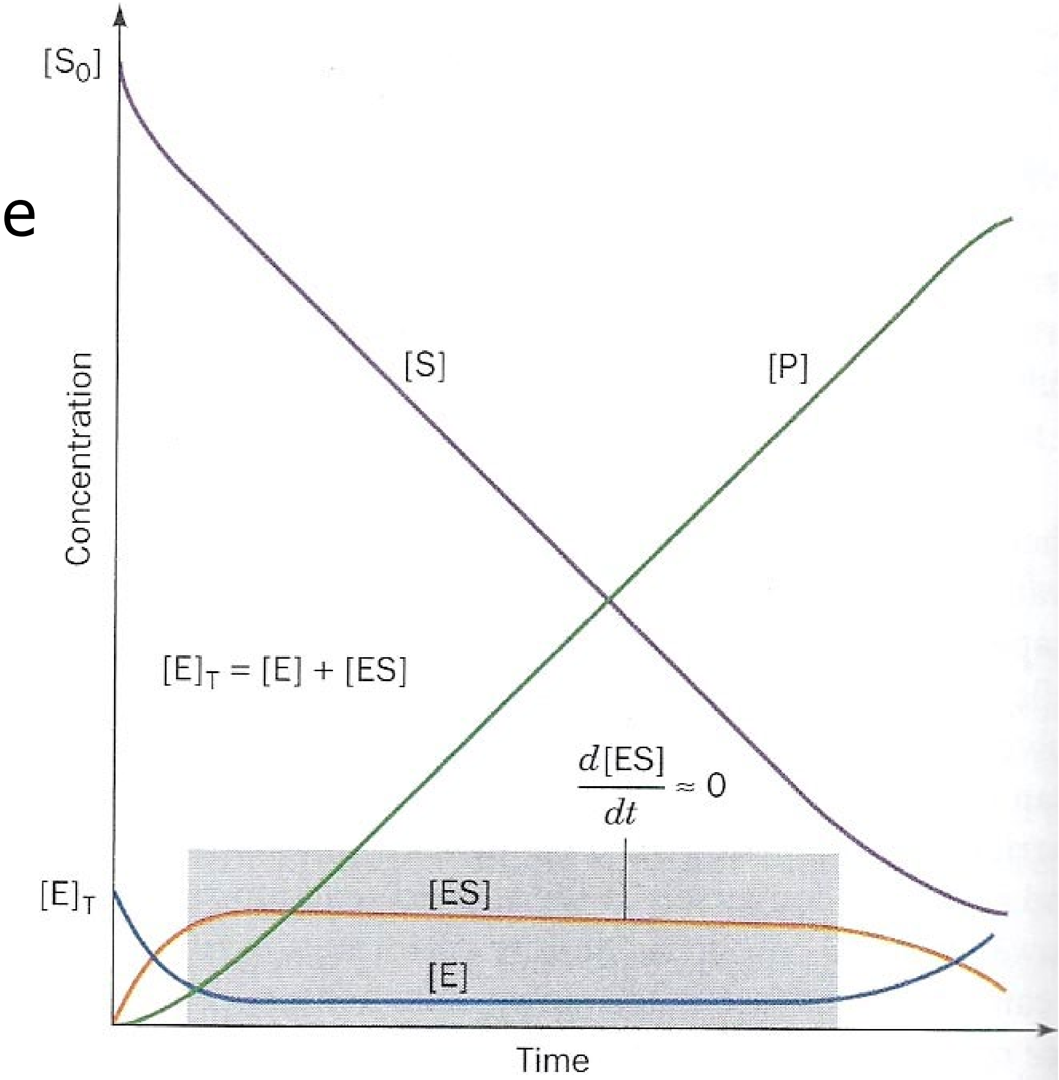
$$\frac{d[ES]}{dt} = 0 = k_1[E][S] - k_{-1}[ES] - k_2[ES]$$

    - Again, we only know $[E_o]$ or $[E_{tot}]$, not free [E], so:

$$0 = k_1\left([E_o] - [ES]\right)[S] - k_{-1}[ES] - k_2[ES]$$

Reactants, products and Intermediates

- Simple Progression of components for simple single intermediate enzyme reaction
  - Shaded block shows steady state intermediates
  - Assumes [S]>>[E]$_t$
  - From Segel, 1975; Enzyme Kinetics



Basic Enzyme Kinetics II

- And solving for [ES],

$$k_1[ES][S] + k_{-1}[ES] + k_2[ES] = k_1[E_o][S]$$

$$[ES] = \frac{k_1[E_o][S]}{k_1[S] + k_{-1} + k_2}$$

$$[ES] = \frac{[E_o][S]}{[S] + \frac{k_{-1}+k_2}{k_1}}$$

Michaelis-Menten

- Irreversible
  - Single intermediate

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} ES \xrightarrow{k_2} E + P$$

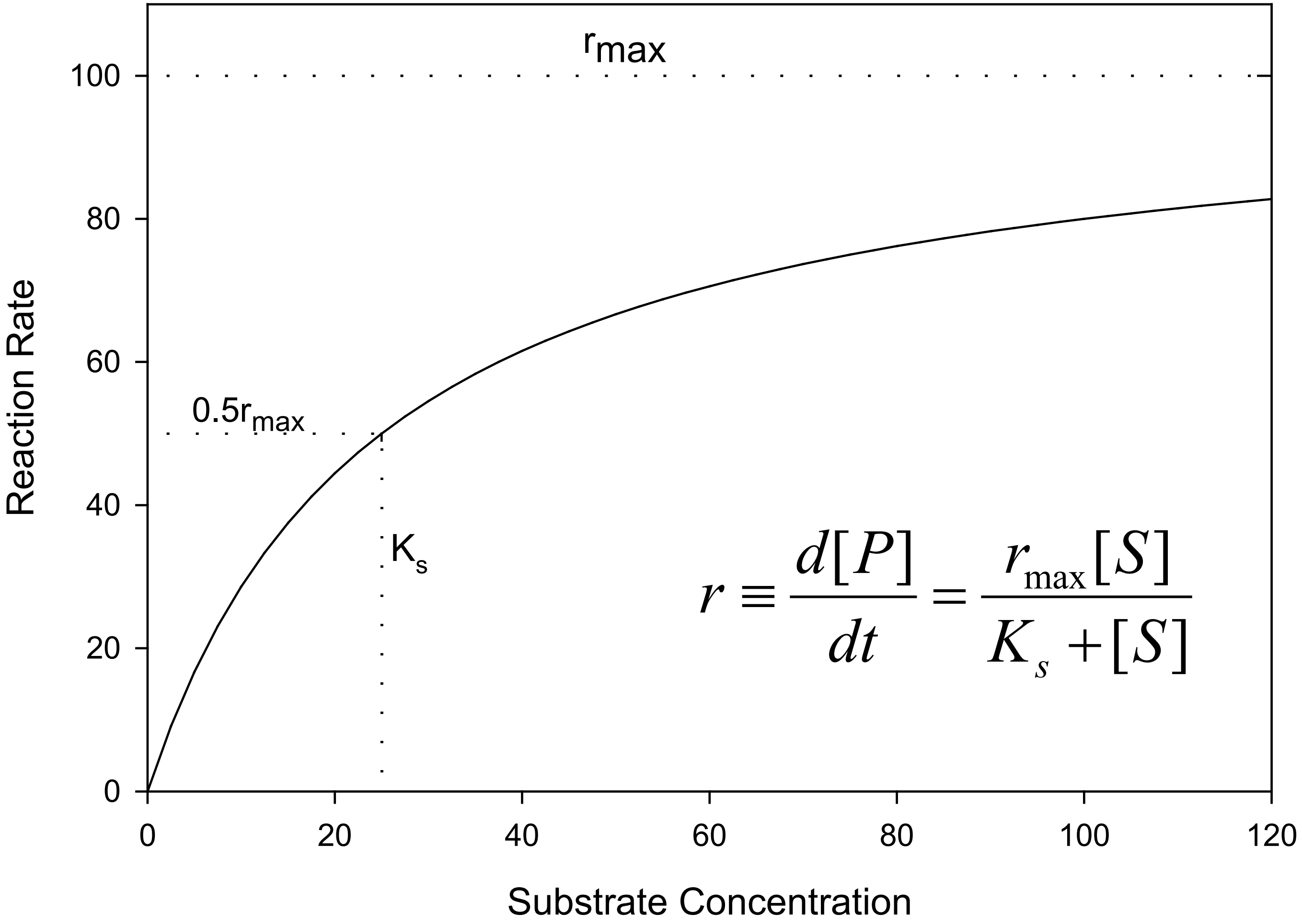
$$r \equiv \frac{d[P]}{dt} = k_2[ES]$$

$$[ES] = \boxed{\frac{[E_o][S]}{[S] + \frac{k_{-1}+k_2}{k_1}}}$$

$$r \equiv \frac{d[P]}{dt} = \frac{k_2[E_o][S]}{\frac{k_{-1}+k_2}{k_1} + [S]} = \frac{r_{\max}[S]}{K_s + [S]}$$

Michaelis Menten Kinetics

- Classical substrate plot



$$r \equiv \frac{d[P]}{dt} = \frac{r_{\max}[S]}{K_s + [S]}$$

David

Maud Menten

Maud Menten

Maud Leonora Menten (March 20, 1879 – July 17, 1960)

- One of the first women to receive an advanced medical degree in Canada
- Completed some of the earliest research in treating cancer with radiation
- Completed early research on the benefit of vaccines on treating infectious disease in animals
- Published 100s of research papers
- Was among the first female faculty at Pitt

Menten as a petite dynamo of a woman who wore "Paris hats, blue dresses with stained-glass hues, and Buster Brown shoes."

Substrate and growth

- If we consider Y

$$r \equiv \frac{d[P]}{dt} = -\frac{d[S]}{dt} = \frac{1}{Y}\frac{dX}{dt}$$

- We can define a microorganism-specific substrate utilization rate, U

$$U \equiv \frac{r}{X} = \left.\frac{dX}{dt}\middle/ YX\right. \equiv \frac{\mu}{Y}$$

- And the maximum rates are then

$$U_{\max} \equiv k \equiv \frac{\mu_{\max}}{Y}$$

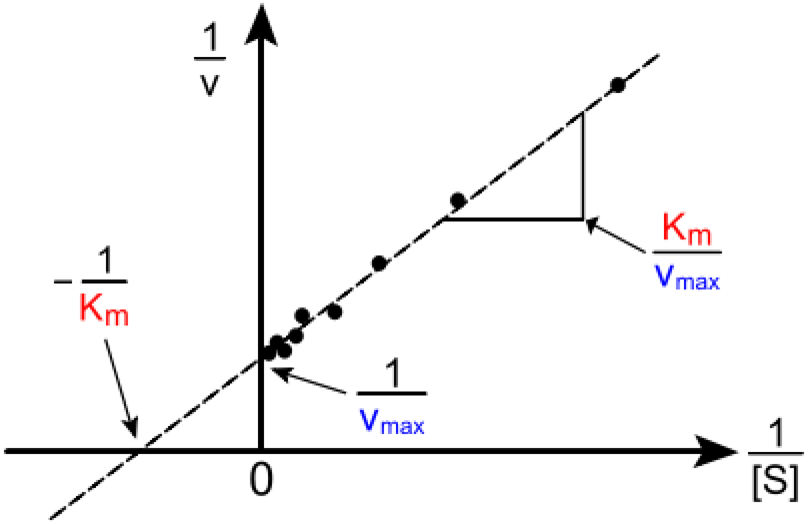
$$U \equiv \frac{1}{X}\frac{d[S]}{dt} = \frac{k[S]}{K_s + [S]}$$

and

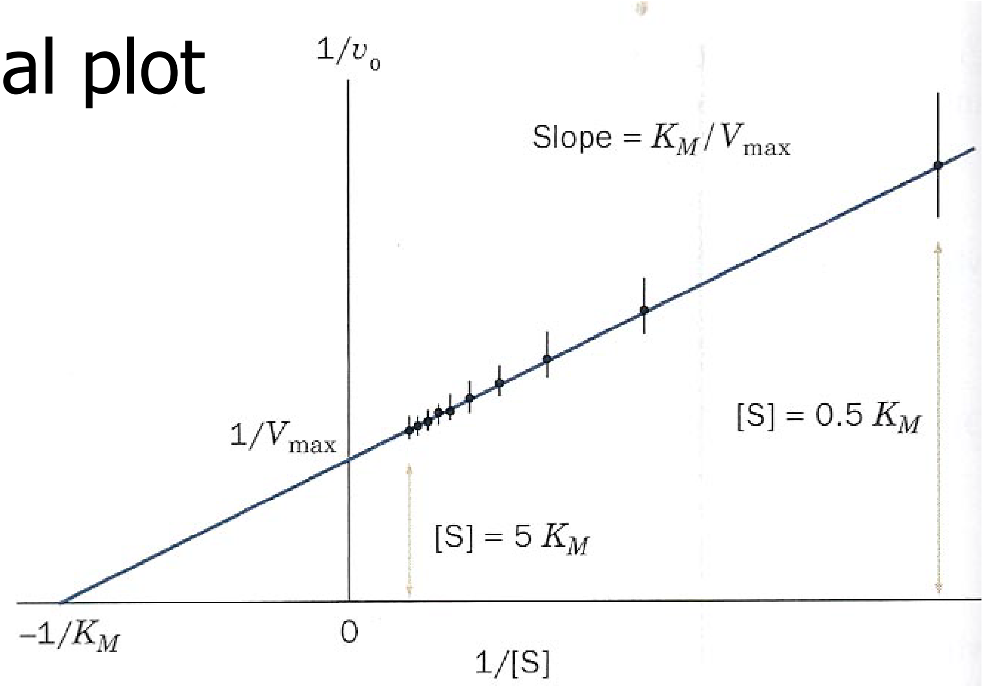
$$\mu \equiv \frac{1}{X}\frac{d[X]}{dt} = \frac{\mu_{\max}[S]}{K_s + [S]}$$

Linearizations

- Lineweaver-Burke
  - Double reciprocal plot



Wikipedia version



Voet & Voet version

- To next lecture