Environmental Microbiology

3 Bacterial Metabolism
Textbook – Chapters 1 & 2

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The growth of microorganisms demands two things:

1.Energy sources :

- Solar energy
- Oxidation of organic molecules (stored chemical energy)
- Oxidation of inorganic molecules (storage chemical energy)

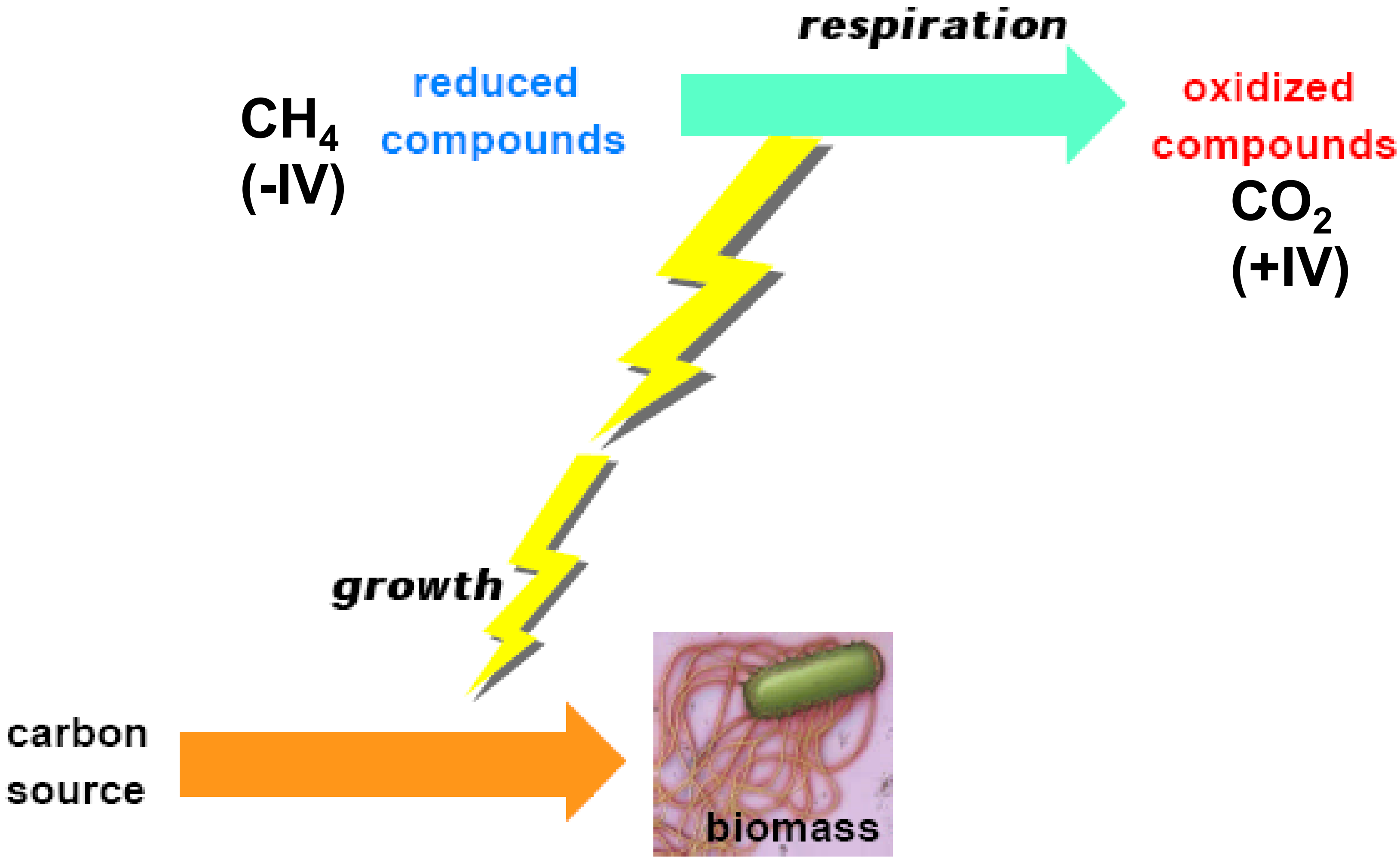
2.Carbon sources for synthesis of cells (with other nutrients)

- CO_2 - Autotrophic
- Organic molecules - Heterotrophic
- All required nutrients (N, P and other inorganic ions)

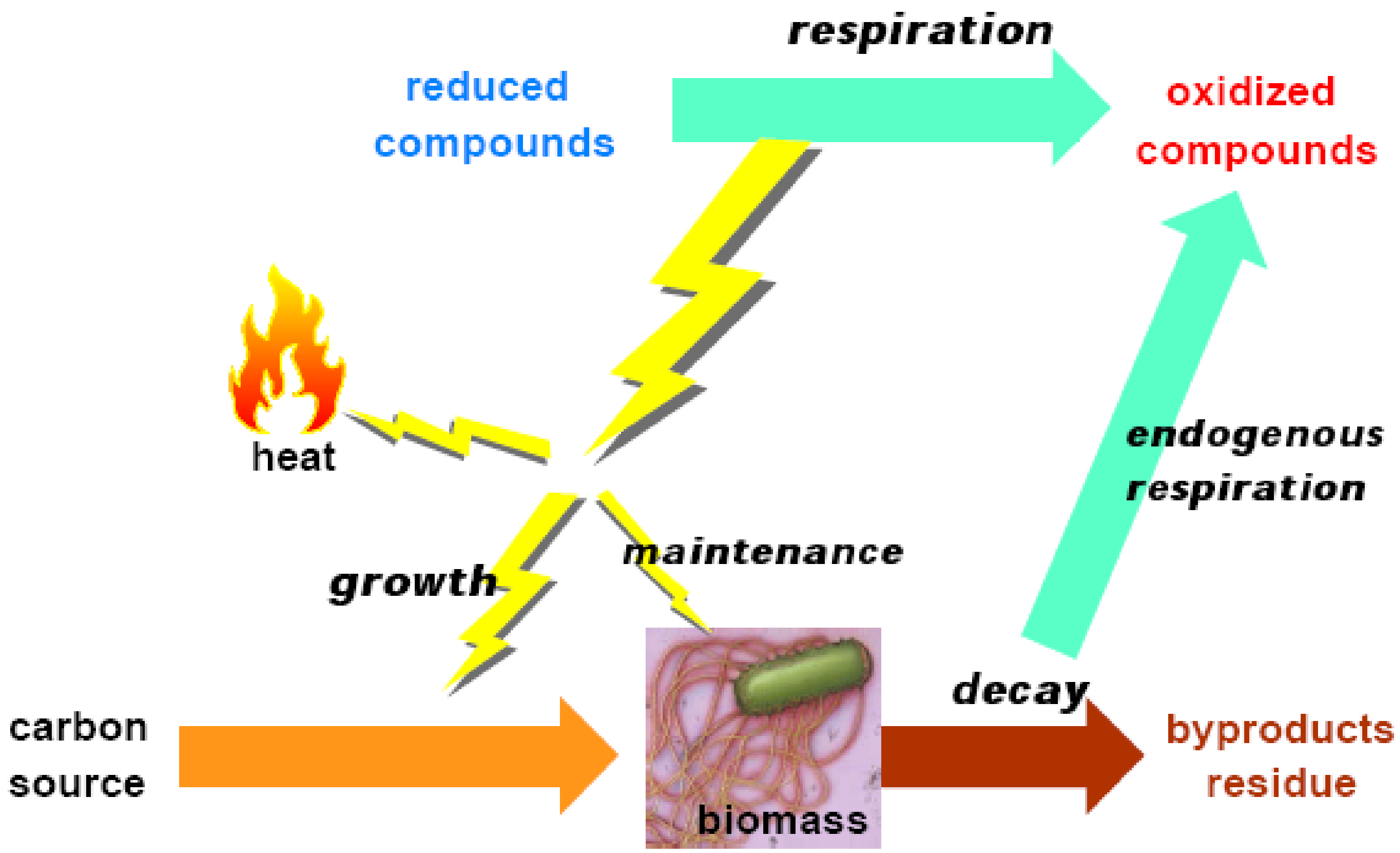
Microbial Metabolism

- energy production
  - oxidation of organic compounds
  - oxidation of inorganic compounds
  - solar energy
- energy consumption
  - synthesis and growth
  - motility
  - active transport
  - maintenance
  - heat

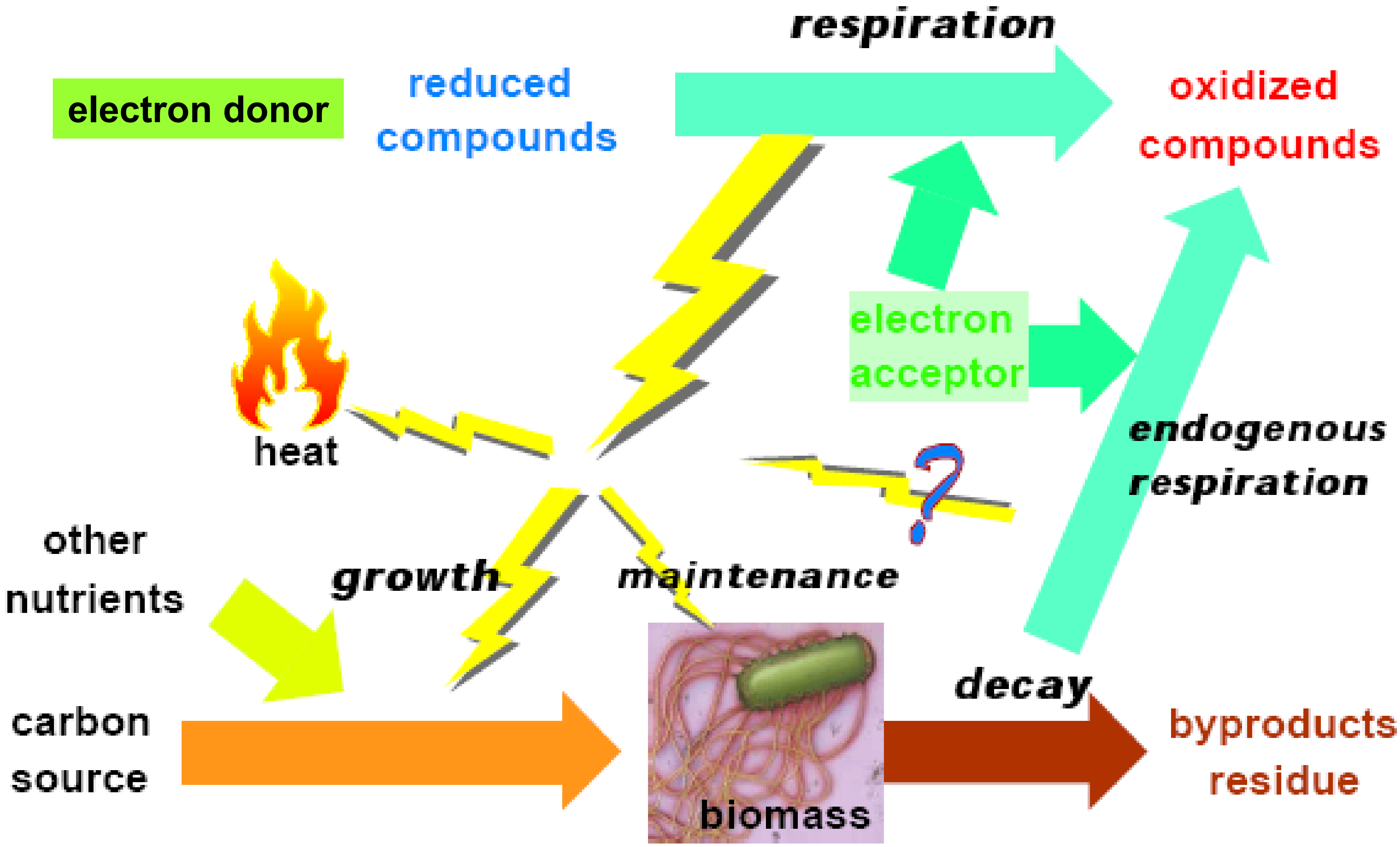
Microbial Metabolism - Energy



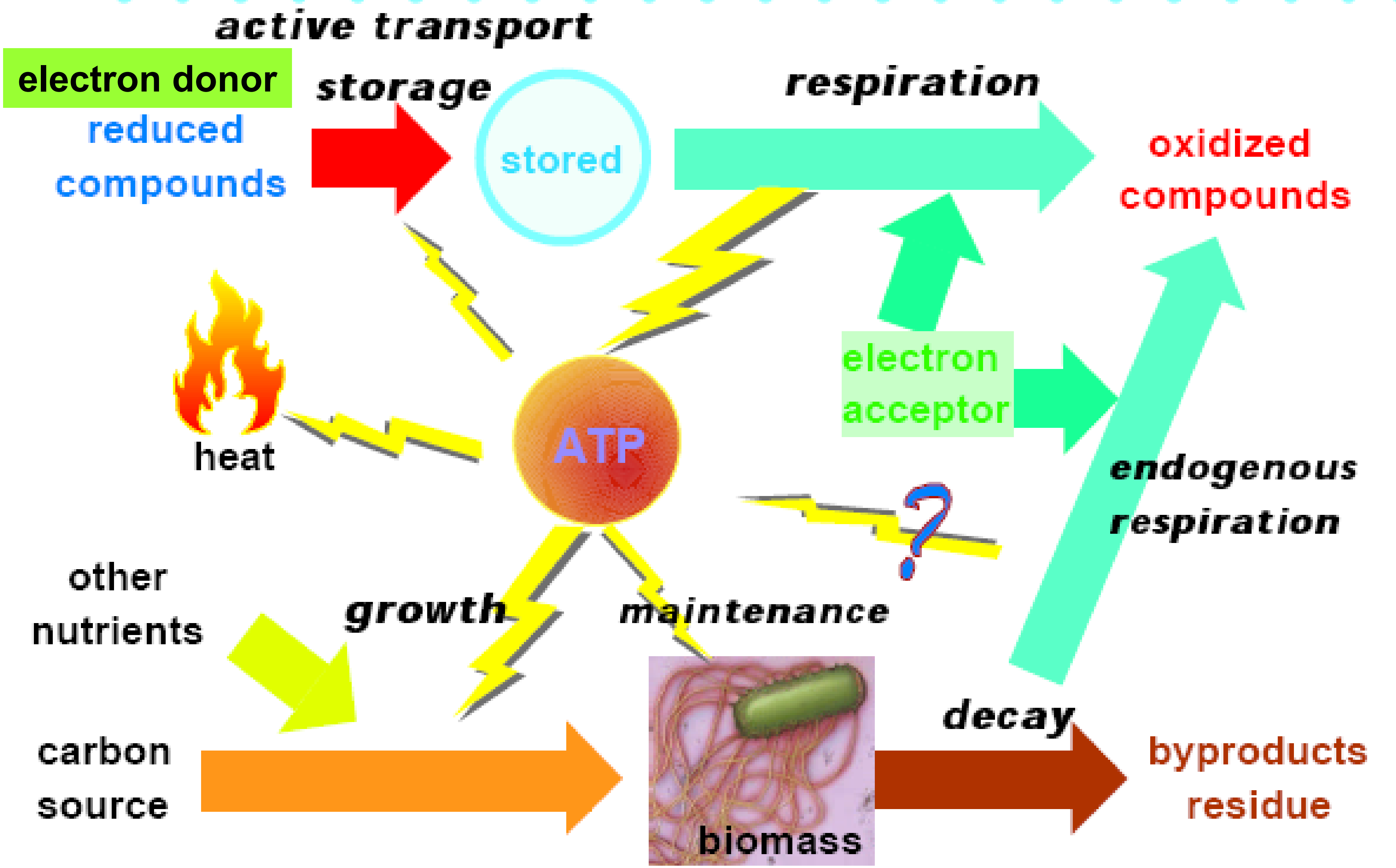
Microbial Metabolism - Energy



Microbial Metabolism - Energy



Microbial Metabolism - Energy



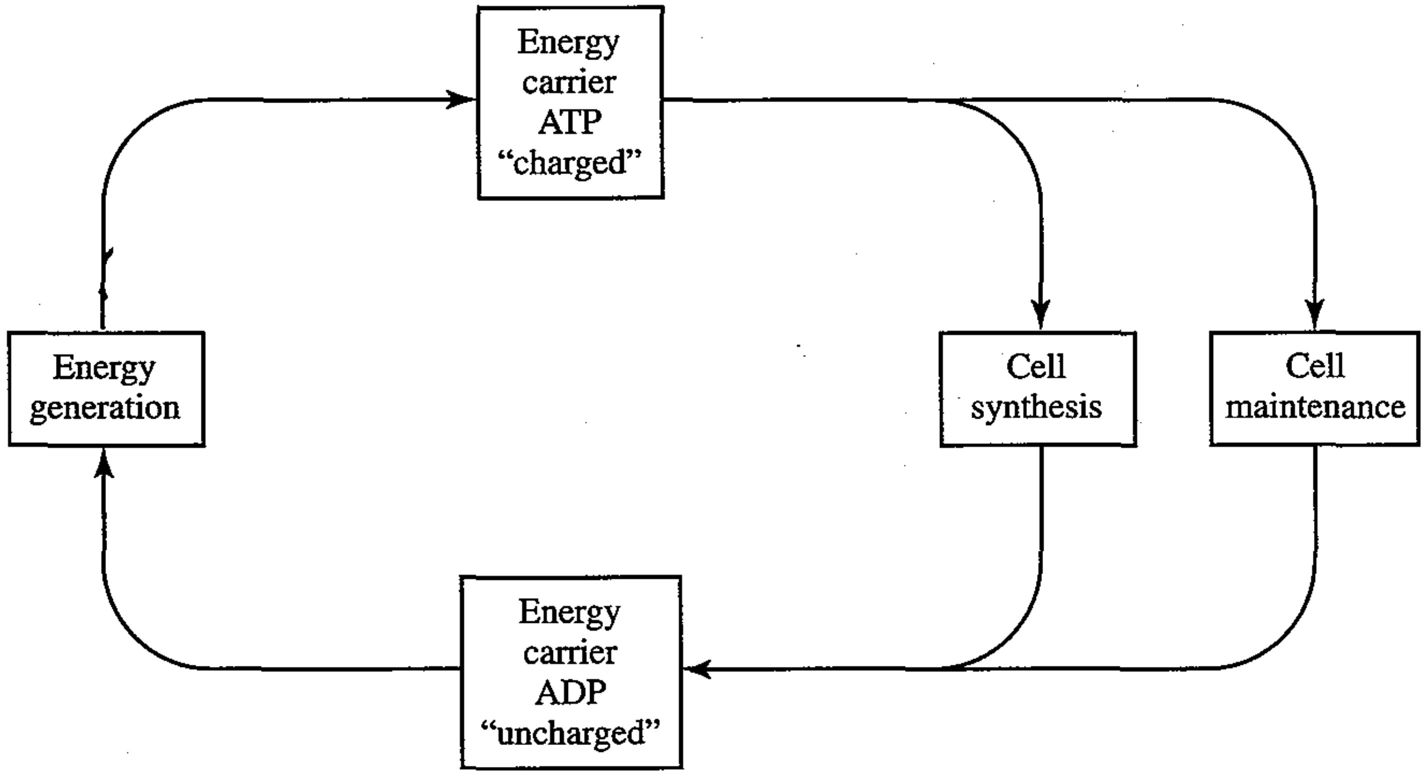


Figure 1.13 Transfer of energy from energy generation to cell synthesis or maintenance via an energy carrier, represented by ATP.

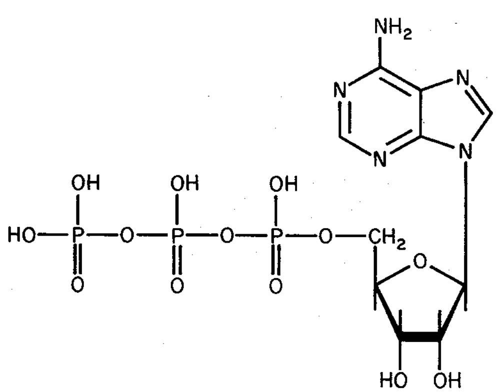
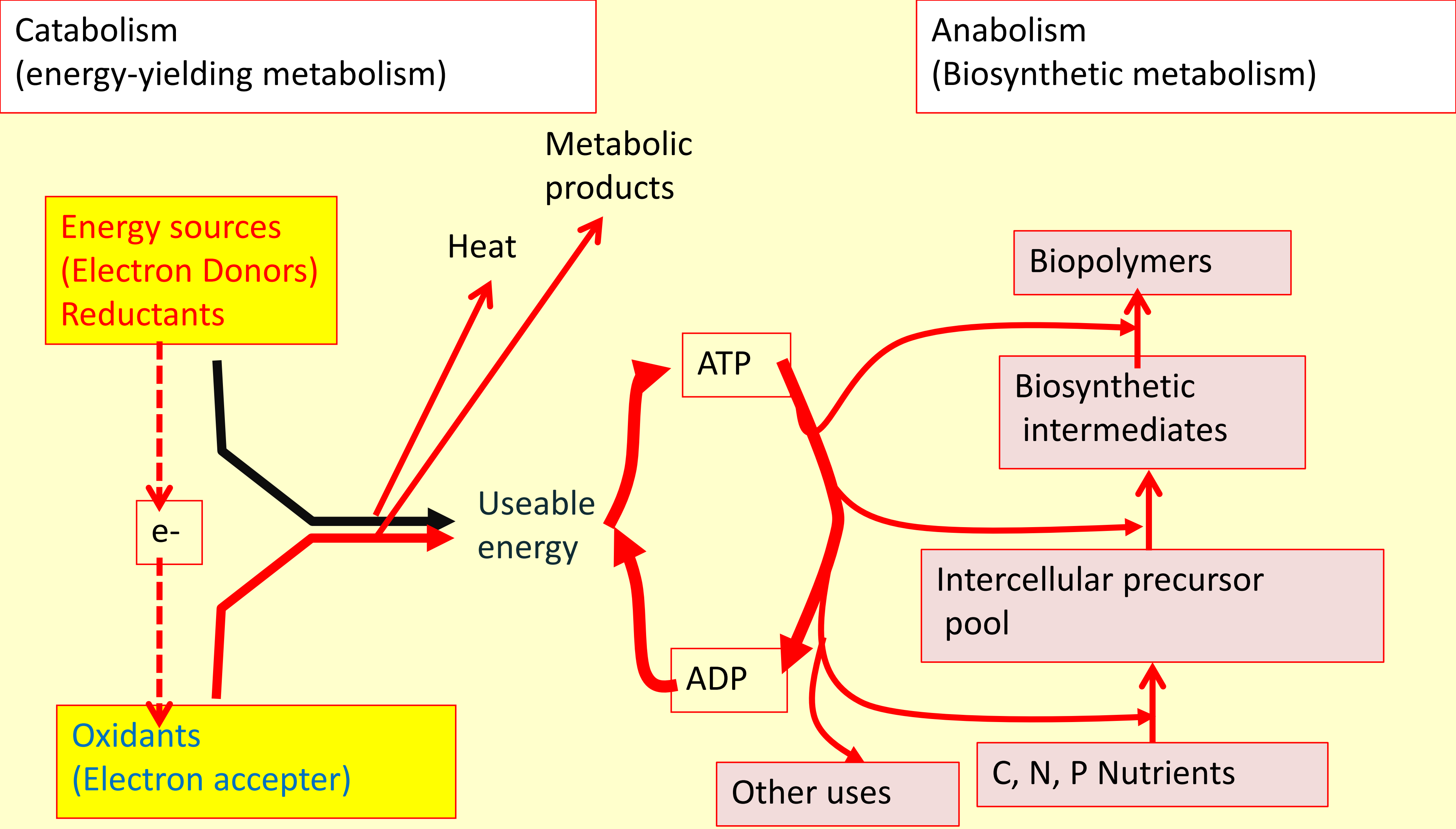


Fig. 7.3 Structure of ATP.

Metabolic Task	Function	Number of Reactions*
Bring nutrients into the cell	To transfer nutrients across the membrane and concentrate them in the cytoplasm	250
Catabolism	To process the major nutrients and produce the 12 precursor metabolites, ATP, and reducing power	406
Biosynthesis	To synthesize all necessary small molecules including building blocks for macromolecules from precursor metabolites	438
Polymerization	To link together building blocks forming macromolecules	482
Assembly	To assemble macromolecules into organelles	7



Different microbial groups use different ways of energy generation

Catabolic pathways are similar for all bacteria

Oxidation reduction (REDOX) reactions

Catabolism in chemoheterotrophs depends on the oxidation of organic chemicals in the organism's environment. Oxidation remove electrons and reduction adds electrons. Material that are oxidized are called electron donors and those being reduced are called electron accepters. An electron donor (food) and an electron accepter (respiration reductant) must be participated to complete an energy generating reaction.

In the environment, electron donors are organic compounds containing reduced carbons, reduced nitrogen compounds: ammonia, reduced sulfur compound:H_2S, reduced iron such as Fe^{++}. There are numerous electron donors in the environment.

The electron acceptor, by comparison, are few including primarily O_2, nitrate, nitrite, Fe(+3), sulfate, some organic compounds and CO_2. In recent years, one has found that perchlorate, chromate, selenate, chlorinated organics are capable of participating in biological redox reactions.

Table 2.3 Trophic classification of microorganisms (adapted from Rittmann and McCarty, 2001; Metcalf & Eddy, 2003)

		Energy source			Carbon source[1]
	Electron donor		Electron acceptor	Typical products[2]	
Trophic group	Microbial group	Type of e^- donor			
Chemotroph					
Organotroph	Aerobic heterotrophs	Organic	O_2	CO_2, H_2O	Organic
Heterotophs	Denitrifiers	Organic	NO_3^-, NO_2^-	N_2, CO_2, H_2O	Organic
	Fermenting organisms	Organic	Organic	Organic:VFAs[3]	Organic
	Iron reducers	Organic	Fe (III)	Fe (II)	Organic
	Sulfate reducers	Acetate	SO_4^{2-}	H_2S	Acetate
	Methanogens (acetoclastic)	Acetate	acetate	CH_4	Acetate
Lithotroph	Nitrifiers: AOB[4]	NH_4^+	O_2	NO_2^-	CO_2
Autotrophs	Nitrifiers: NOB[5]	NO_2^-	O_2	NO_3^-	CO_2
	Anammox[6] bacteria	NH_4^+	NO_2^-	N_2	CO_2
	Denitrifiers	H_2	NO_3^-, NO_2^-	N_2, H_2O	CO_2
	Denitrifiers	S	NO_3^-, NO_2^-	N_2, SO_4^{2-}, H_2O	CO_2
	Iron oxidizers	Fe (II)	O_2	Fe (III)	CO_2
	Sulphate reducers	H_2	SO_4^{2-}	H_2S, H_2O	CO_2
	Sulphate oxidizers	H_2S, S^0, $S_2O_3^{2-}$	O_2	SO_4^{2-}	CO_2
	Aerobic hydrogenotrophs	H_2	O_2	H_2O	CO_2
	Methanogens (hydrogenotrophic)	H_2	CO_2	CH_4	CO_2
Phototroph					
Autotrophs	Algae, plants	H_2O	CO_2	O_2	CO_2
	Photosynthetic bacteria	H_2S	CO_2	S (0)	CO_2

[1] Carbon source: organic for heterotrophs and inorganic (CO_2) for autotrophs; mixotrophs can use both. [2] Typical products: CO_2 and H_2O are products of catalysis (energy generation) by many micro-organisms. [3] VFAs: volatile fatty acids (typically acetate, propionate, butyrate).
[4] AOB: ammonia oxidizing bacteria. [5] NOB: nitrite oxidizing bacteria. [6] Anammox: anaerobic ammonia oxidizing bacteria.

II. THE MAJOR MODES OF NUTRITION – ORGANISMS CLASSIFIED ACCORDING TO THEIR:

A. CARBON SOURCE

autotrophs – organisms (microbes) w. CO_2 as 1° carbon source.

heterotrophs – organisms using organic carbon compounds as primary carbon source.

B. ENERGY SOURCE

phototrophs – organisms that use solar energy.

chemotrophs – organisms that oxidize/burn chemical compounds as their energy source.

C. ELECTRON SOURCE

lithotrophs – organisms that get their electrons from inorganic compounds.

organotrophs – organisms that get their electrons from organic compounds.

II. THE MAJOR MODES OF NUTRITION – ORGANISMS CLASSIFIED ACCORDING TO THEIR:

A. Carbon Source

B. Energy Source

TABLE 7.4

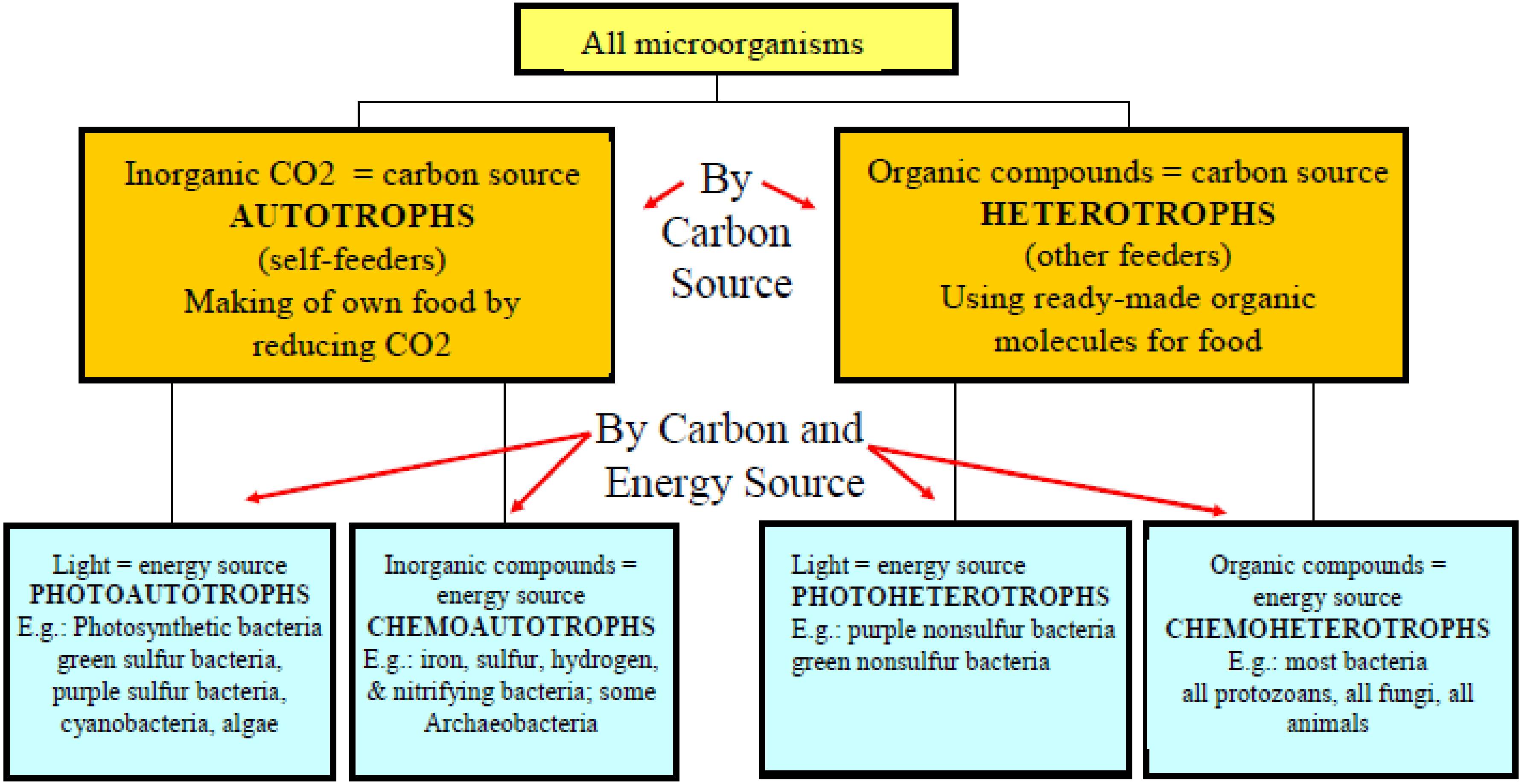
Nutritional Categories of Microbes by Carbon and Energy Source

Category	Carbon Source	Energy Source	Example
Autotroph	**CO_2**	**Nonliving Environment**	
Photoautotroph	CO_2	Sunlight	Photosynthetic organisms, such as algae, plants, cyanobacteria
Chemoautotroph	CO_2	Simple inorganic chemicals	Only certain bacteria, such as methanogens, vent bacteria
Heterotroph	**Organic**	**Other Organisms or Sunlight**	
Photoheterotroph	Organic	Sunlight	Nonsulfur bacteria
Chemoheterotroph	Organic	Metabolic conversion of the nutrients from other organisms	Protozoa, fungi, many bacteria, animals
Saprobe	Organic	Metabolizing the organic matter of dead organisms	Fungi, bacteria (decomposers)
Parasite	Organic	Utilizing the tissues, fluids of a live host	Various parasites and pathogens; can be bacteria, fungi, protozoa, animals

Table 1.6 Metals as electron acceptors for anaerobic respiration.

Reaction	*Microorganism*	*Reference*
$2\ Fe^{3+} + H_2 \leftrightarrow 2\ Fe^{2+} + 2\ H^+$	*Geobacter metallireducens* *Pelobacter carbinolicus*	Lovley and Lonergan (1990) Lovley et al. (1995)
$Mn^{4+} + H_2 \leftrightarrow Mn^{2+} + 2\ H^+$	*Geobacter metallireducens* mixed culture	Lovley (1991) Langenhoff et al. (1997)
$2\ Cr^{6+} + 3\ H_2 \leftrightarrow 2\ Cr^{3+} + 6\ H^+$	*Desulfovibrio vulgaris* *Bacillus* strain QC1-2	Lovley and Phillips (1994) Campos et al. (1995)
$Se^{6+} + H_2 \leftrightarrow Se^{4+} + 2\ H^+$	*Thauera selenatis* strains SES-1; SES-3	Macy et al. (1993)
$Se^{6+} + 3\ H_2 \leftrightarrow Se^0 + 6\ H^+$		Oremland et al. (1989)
$Te^{4+} + 2\ H_2 \leftrightarrow Te^0 + 4\ H^+$	*Schizosaccharomyces pombe*	Smith (1974)
$Pb^{2+} + H_2 \leftrightarrow Pb^0 + 2\ H^+$	*Pseudomonas maltophila*	Lovley (1995)
$As^{5+} + H_2 \leftrightarrow As^{3+} + 2\ H^+$	*Geospirillum arsenophilus*	Ahmann et al. (1994)
$Hg^{2+} + H_2 \leftrightarrow Hg^0 + 2\ H^+$	*Escherichia coli* *Thiobacillus ferrooxidans*	Robinson and Tuovinen (1984)
$U^{6+} + H_2 \leftrightarrow U^{4+} + 2\ H^+$	*Shewanella putrefaciens*	Lovley et al. (1991)

The Main Nutritional Types – a Summary



Energy produced from an oxidization reduction chemical reaction

Acetate and Oxygen (Aerobic Oxidation of Acetate)

		$\Delta G^{0'}$, kJ
Donor:	$\frac{1}{8} CH_3COO^- + \frac{3}{8} H_2O = \frac{1}{8} CO_2 + \frac{1}{8} HCO_3^- + H^+ + e^-$	-27.40
Acceptor:	$\frac{1}{4} O_2 + H^+ + e^- = \frac{1}{2} H_2O$	-78.72
Net:	$\frac{1}{8} CH_3COO^- + \frac{1}{4} O_2 = \frac{1}{8} CO_2 + \frac{1}{8} HCO_3^- + \frac{1}{8} H_2O$	-106.12

Acetate and Carbon Dioxide (Methanogenesis of Acetate)

		$\Delta G^{0'}$, kJ
Donor:	$\frac{1}{8} CH_3COO^- + \frac{3}{8} H_2O = \frac{1}{8} CO_2 + \frac{1}{8} HCO_3^- + H^+ + e^-$	-27.40
Acceptor:	$\frac{1}{8} CO_2 + H^+ + e^- = \frac{1}{8} CH_4 + \frac{1}{4} H_2O$	23.53
Net:	$\frac{1}{8} CH_3COO^- + \frac{1}{8} H_2O = \frac{1}{8} CH_4 + \frac{1}{8} HCO_3^-$	-3.87

Glucose and Carbon Dioxide (Methanogenesis from Glucose)

		$\Delta G^{0'}$, kJ
Donor:	$\frac{1}{24} C_6H_{12}O_6 + \frac{1}{4} H_20 = \frac{1}{4} CO_2 + H^+ + e^-$	-41.35
Acceptor:	$\frac{1}{8} CO_2 + H^+ + e^- = \frac{1}{8} CH_4 + \frac{1}{4} H_2O$	23.53
Net:	$\frac{1}{24} C_6H_{12}O_6 = \frac{1}{8} CH_4 + \frac{1}{8} CO_2$	-17.82

Hydrogen and Oxygen (Aerobic Oxidation of Hydrogen)

		$\Delta G^{0'}$, kJ
Donor:	$\frac{1}{2} H_2 = H^+ + e^-$	-39.87
Acceptor:	$\frac{1}{4} O_2 + H^+ + e^- = \frac{1}{2} H_2O$	-78.72
Net:	$\frac{1}{2} H_2 + \frac{1}{4} O_2 = \frac{1}{2} H_2O$	-118.59

Aerobic oxidation of ammonia

		ΔG^0 Kj / e- eq
Acceptor	$0.25\ O_2 + H^+ + e^- = 0.5\ H_2O$	- 78.72
Donor	$0.125\ NH_4^+ + 0.375\ H_2O = 0.125\ NO_3^- + 1.25\ H^+ + e^-$	+35.11
Re:	$0.125\ NH_4^+ + 0.25\ O_2 = 0.125\ H_2O + 0.125\ NO_3^- + 0.25\ H^+$	- 43.61

5 Anaerobic sulfate reduction with ammonia

		ΔG^0 Kj / e- eq
Acceptor	$0.125\ SO_4^= + 1.188\ H^+ + e^- = 0.062\ HS^- + 0.062 H_2S + 0.5\ H_2O$	20.85
Donor	$0.125\ NH_4^+ + 0.375\ H_2O = 0.125\ NO_3^- + 1.25\ H^+ + e^-$	35.11
Re:	$0.125\ NH_4^+ + 0.125\ SO_4^= = 0.125\ H_2O + 0.0625\ H_2S + 0.0625 HS^- + 0.0625\ H^+$	~~+55.96~~

Redox reaction with positive free energy production is thermodynamically impossible. Anaerobic degradation of ammonia using $SO_4^=$ as acceptor is not possible

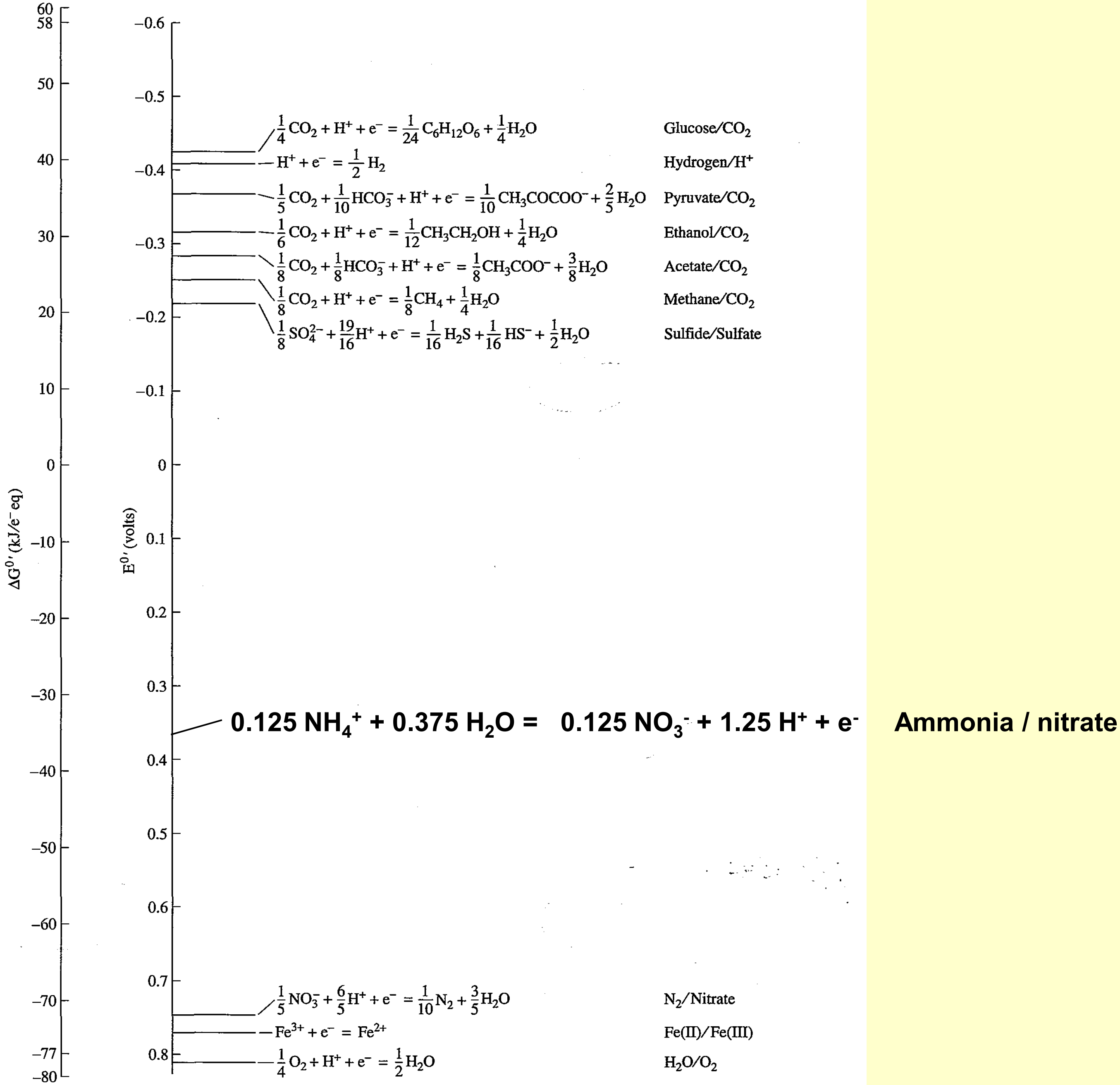


Figure 1.15 Energy scale for various oxidation/reduction couples. Energy scales can be expressed as kJ/e⁻ eq or volts, where the relationship between the two is given as one volt equals −96.485 kJ.

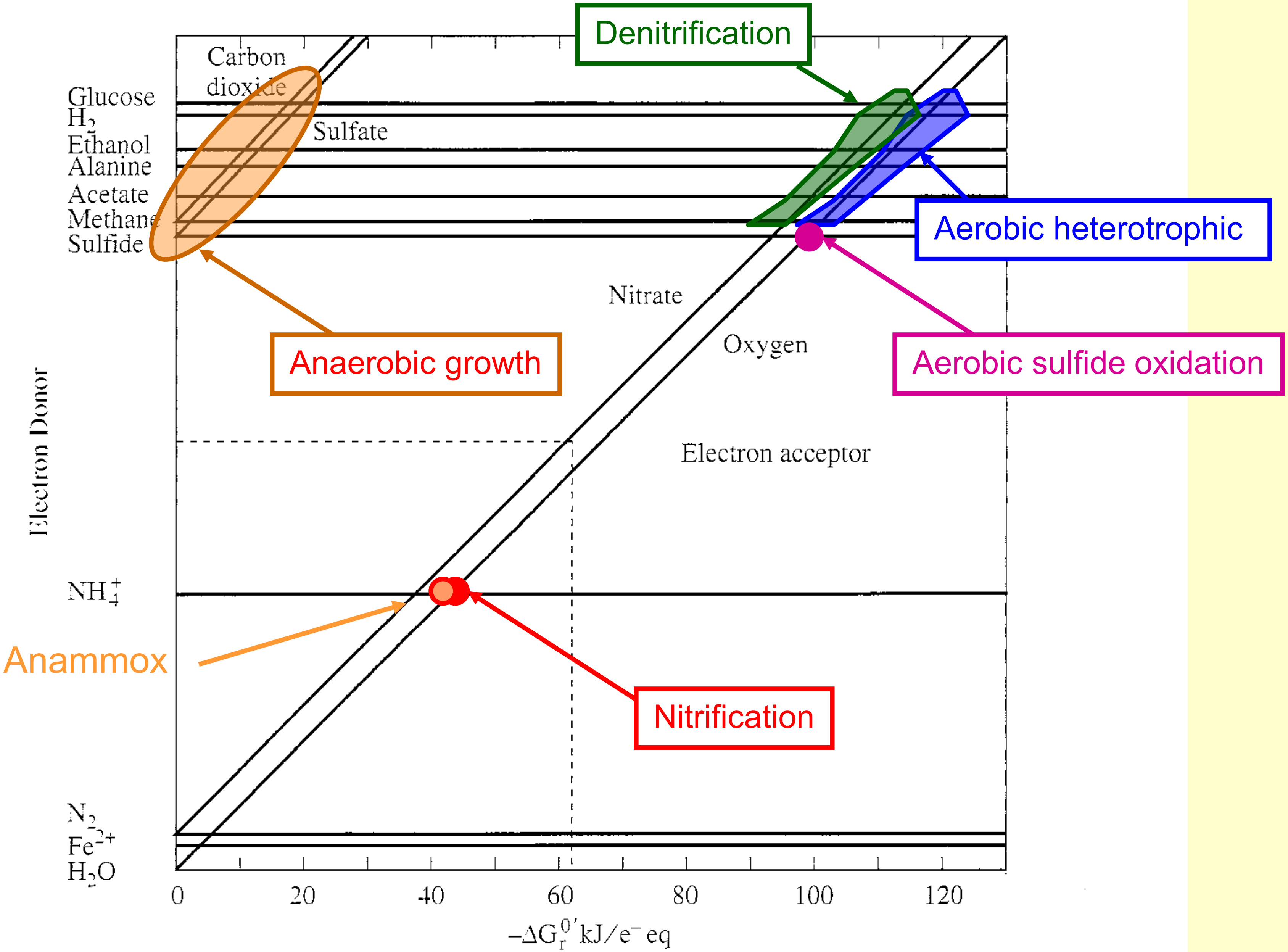
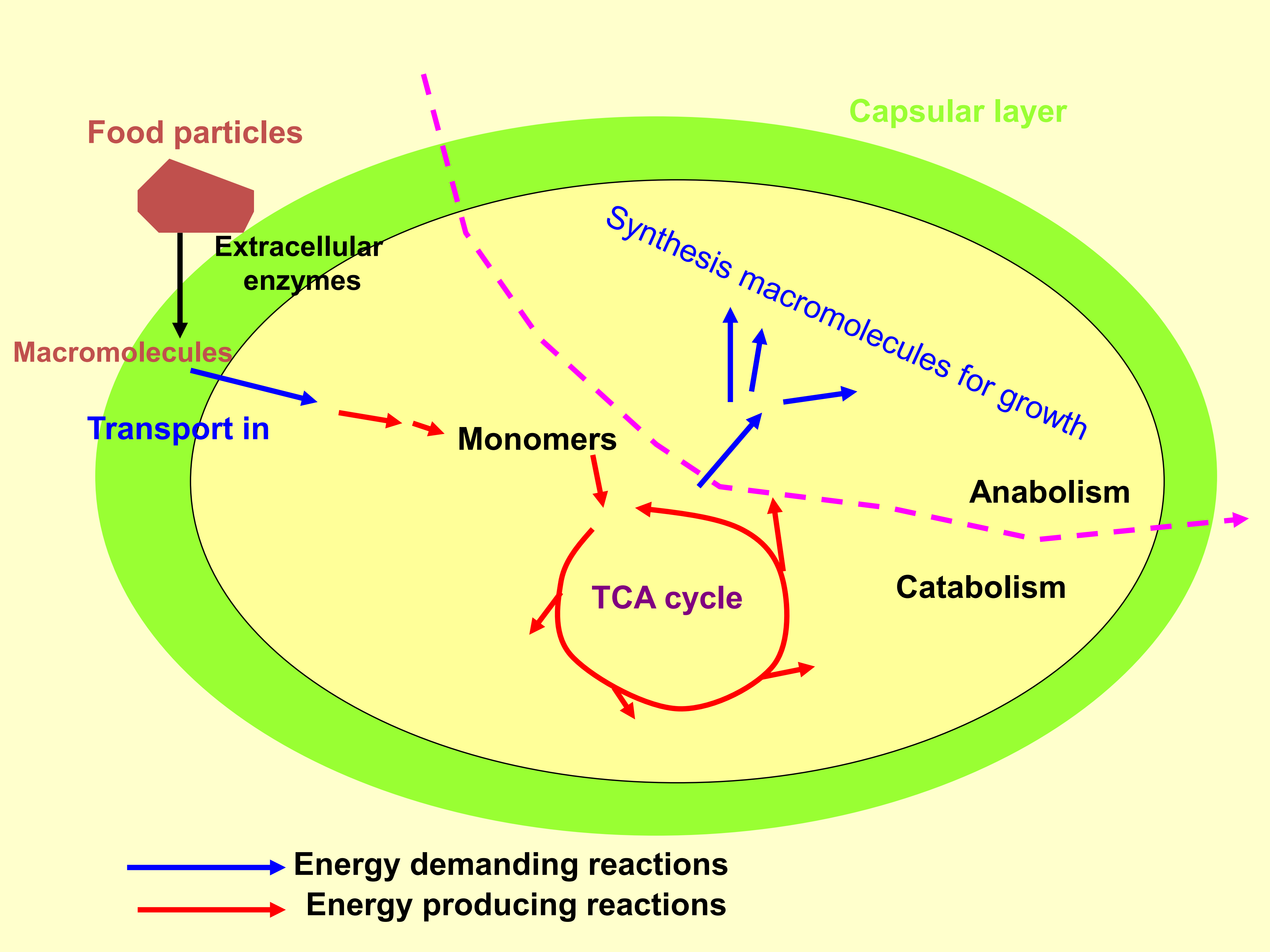
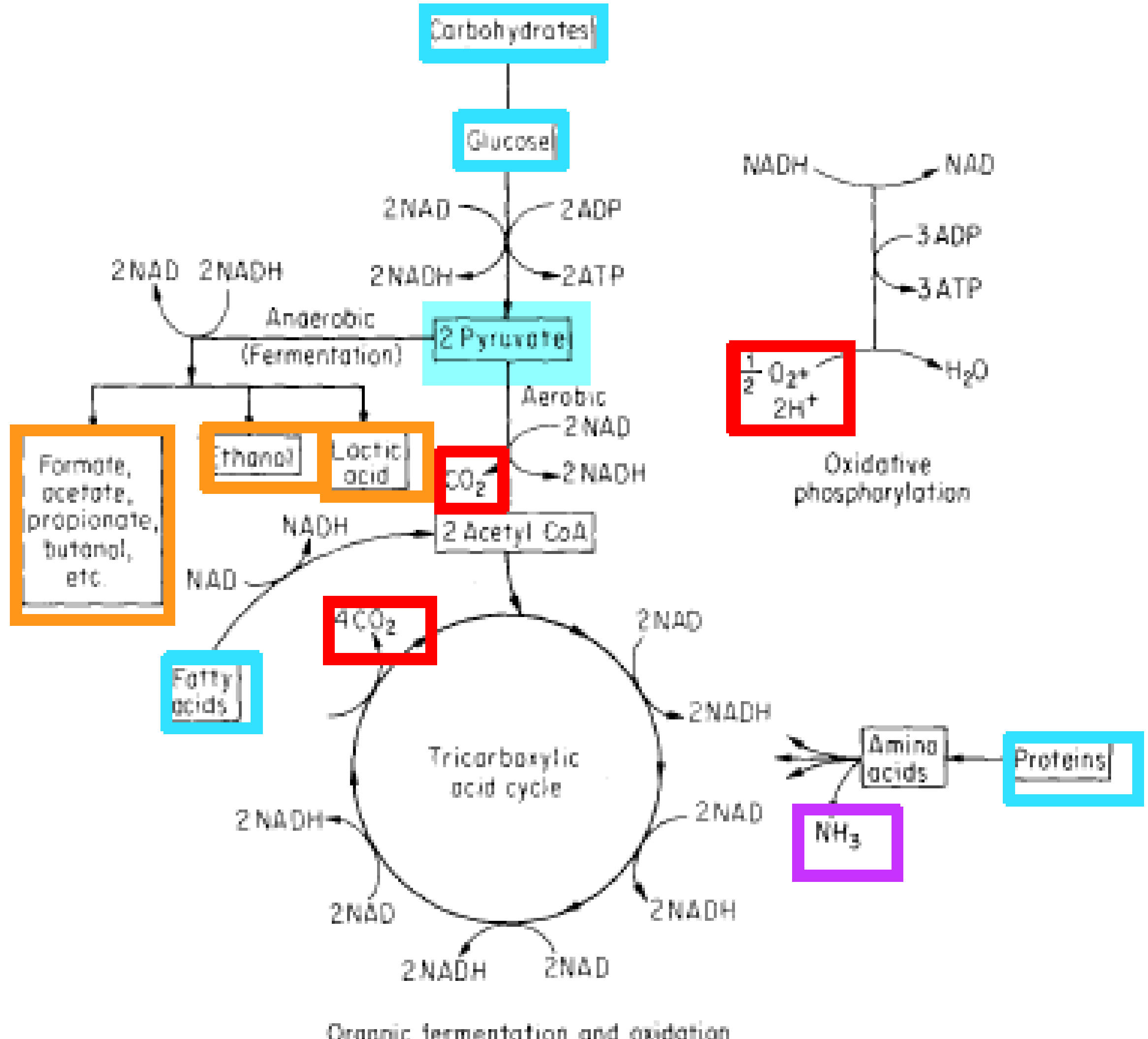


Figure 2.2 Relationship between various electron donors and acceptors and resulting reaction free energy.


Capsular layer
Food particles
Extracellular enzymes
Macromolecules
Transport in
Monomers
Synthesis macromolecules for growth
Anabolism
Catabolism
TCA cycle
Energy demanding reactions
Energy producing reactions

Aerobic Metabolism



from Sawyer and McCarty, Chemistry for Environmental Engineers

The catabolic pathways are in step wide manner, so that energy carrier ATP can be synthesized from ADP.

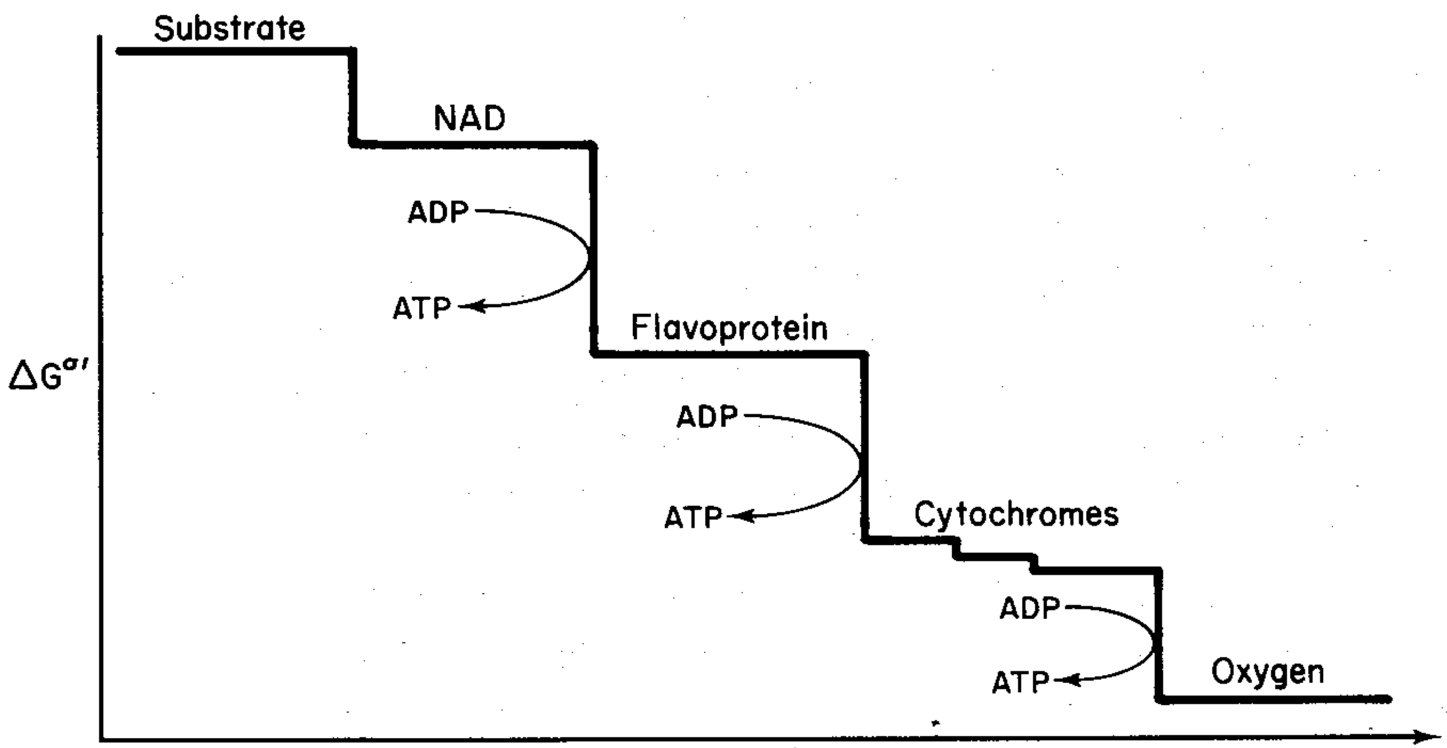


Fig. 7.8 Free energy changes during electron transport.

Heterotrophic metabolism pathways – stepwise transformation

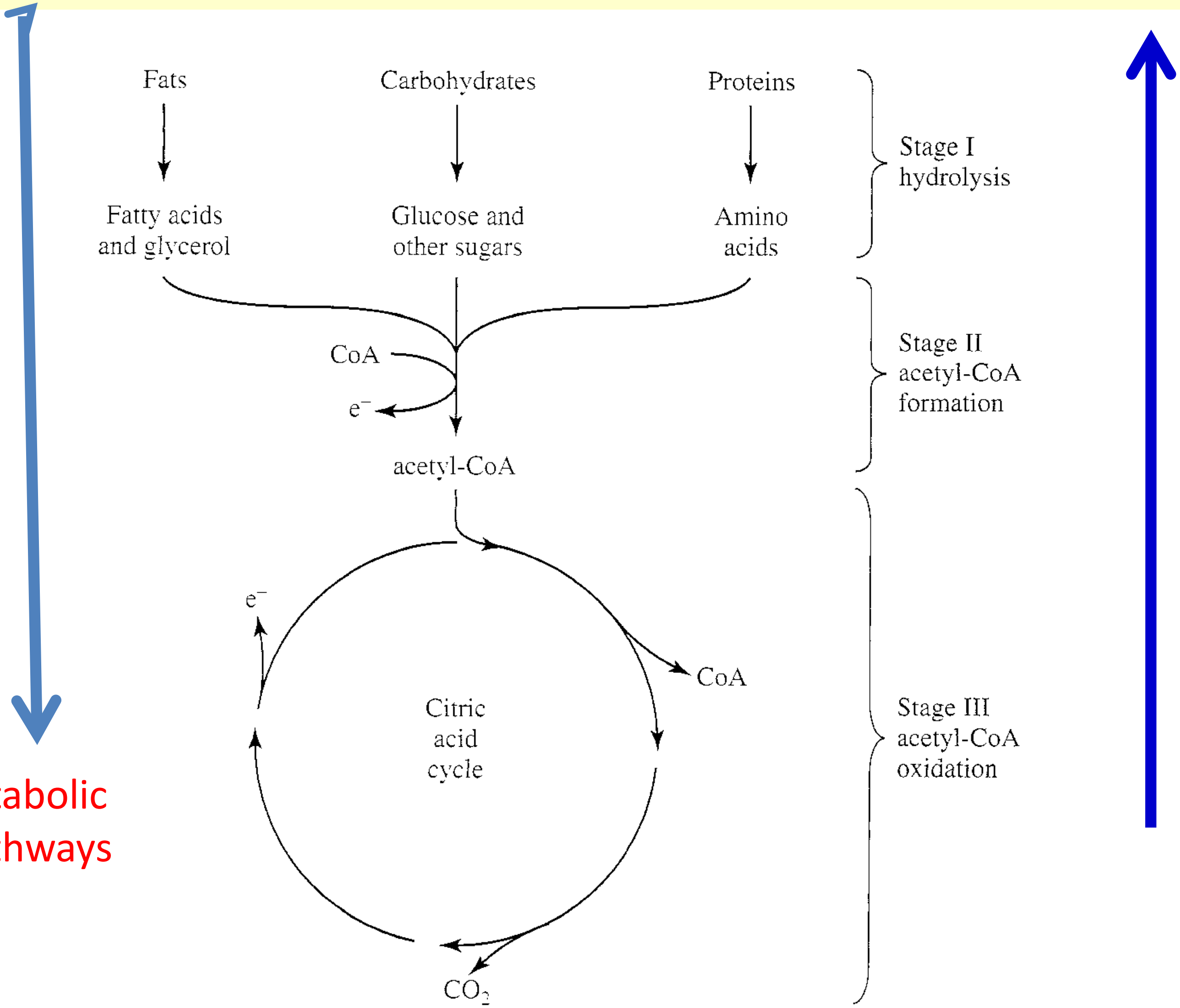


Figure 1.14 The three general stages of catabolism of fats, carbohydrates, and proteins under aerobic conditions. Reversing the processes gives anabolism.

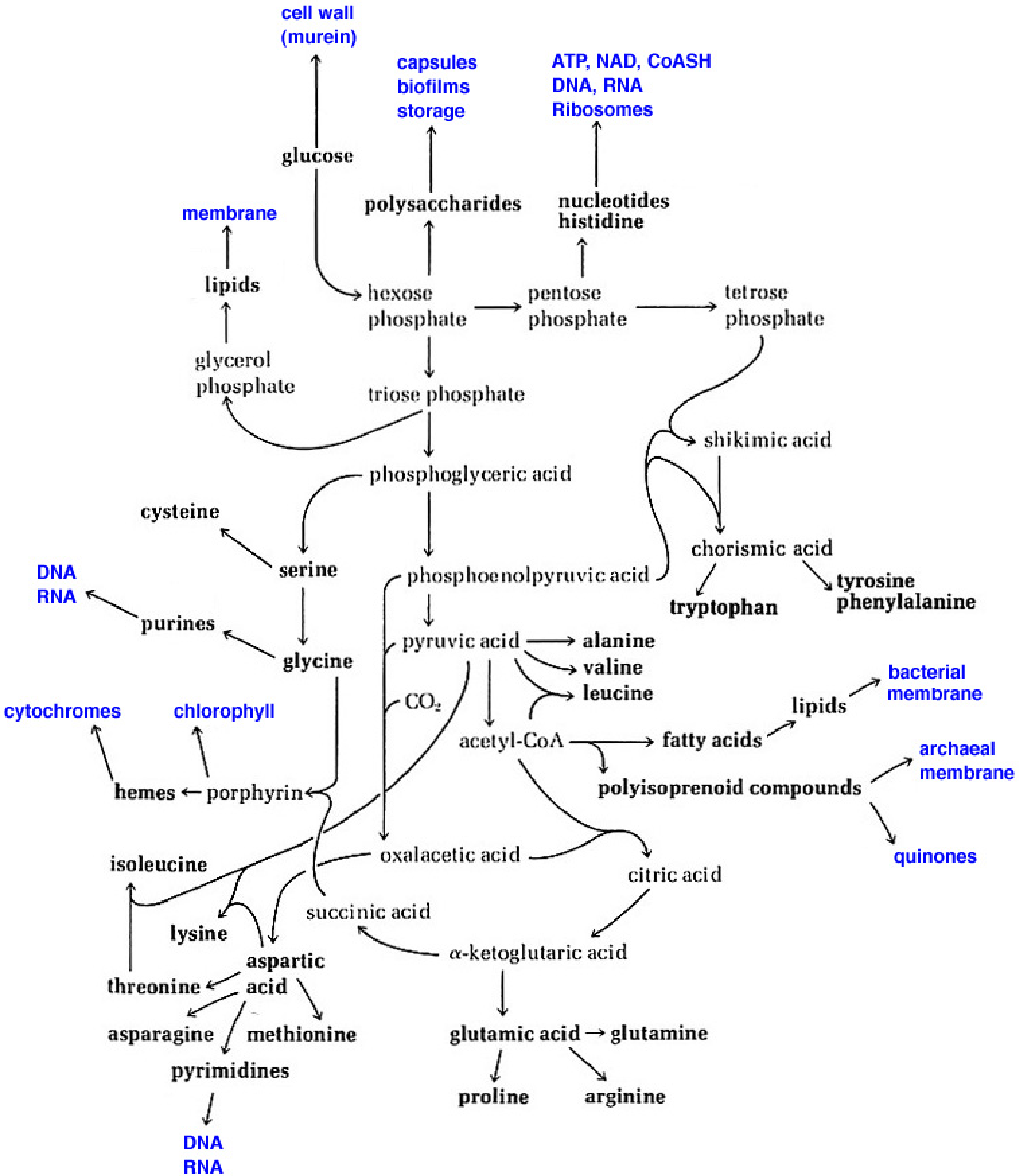
Catabolic pathways

Anabolic Pathways

Stepwise

Parallel but Reversed

The main pathways of biosynthesis in procaryotic cells



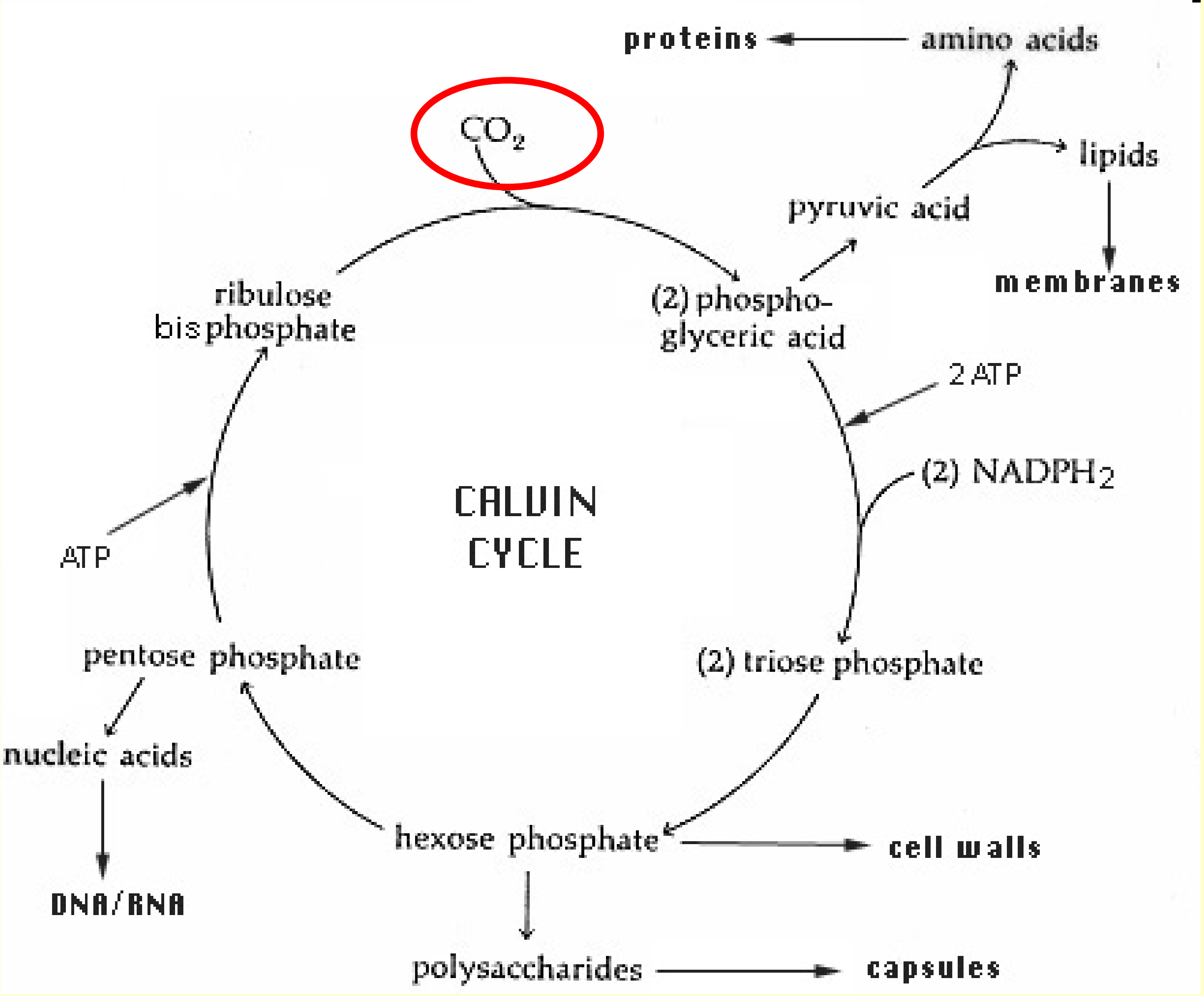
There are also significant difference in energy required for bio-synthesis between heterotrophs and autotrophs

For heterotrophs, the biosynthesis starting point is organic carbons and it takes little energy to elevate to the biomass level :

$$3\,CH_3COO^- + 3\,H^+ \rightarrow C_6H_{12}O_6 \quad \Delta G^{0'} = 335\text{ kJ} \qquad \textbf{[1.50]}$$

For autotrophs, the starting point is inorganic CO_2 and it will take significantly higher amount of energy to synthesis biomass:

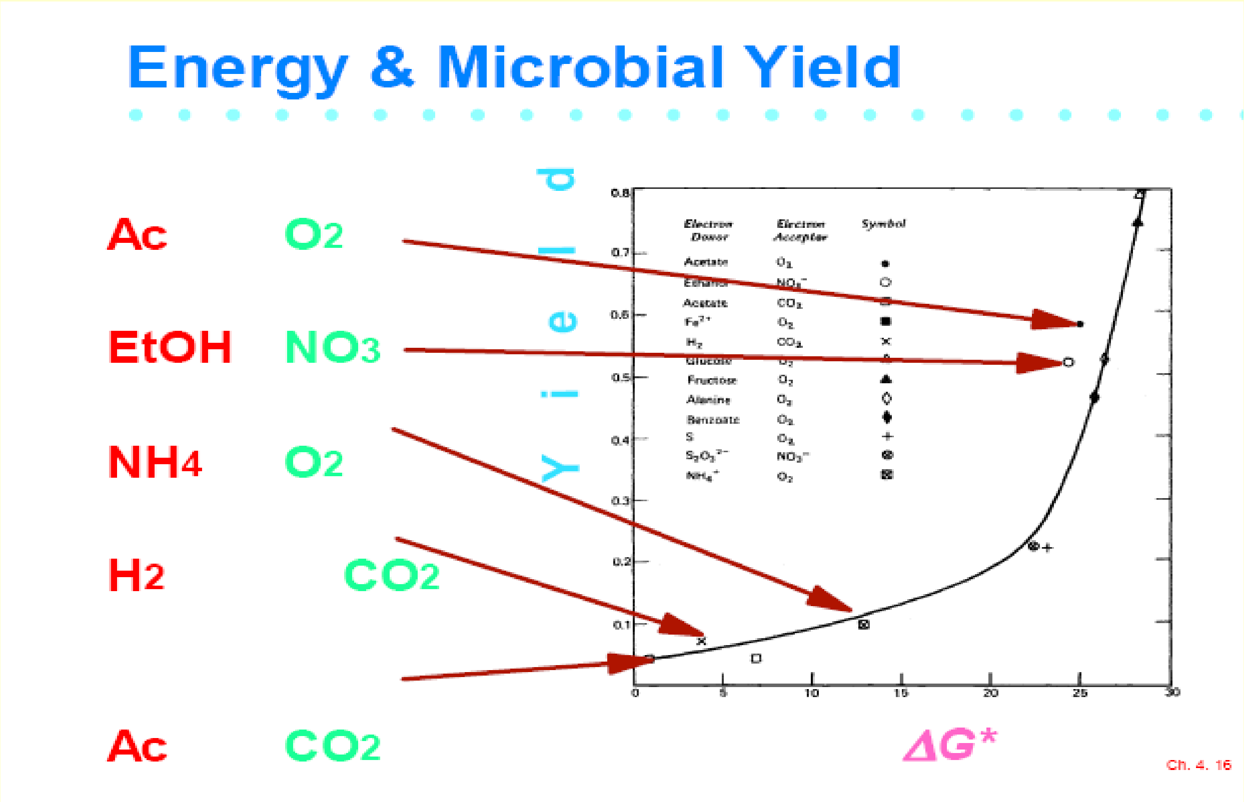
$$6\,CO_2 + 6\,H_2O \rightarrow C_6H_{12}O_6 + 6\,O_2 \quad \Delta G^{0'} = 2{,}880\text{ kJ}$$



The Calvin cycle and its relationship to the synthesis of autotrophic cell materials. **The fixation of CO2 to the level of glucose ($C_6H_{12}O_6$) requires 18 ATP and 12 $NADPH_2$.**

Catabolic energy generation: The higher -ΔG is produced, the more energy is available for growth and maintenance. Aerobic oxidation > anoxic nitrate reduction > anaerobic sulfate reduction > fermentation > anaerobic methane production.

Anabolic energy utilization: The higher the energy level of the carbon source with relation to the biomass, the less energy it takes to synthesis. The autotrophic growth demands the most amount of energy to synthesis biomass.



Cell Yield (Y) for heterotrophy

- Not all of the carbon added as the carbon source is converted to cell biomass
- A fraction is respired as CO_2 during the transformation of the organics to energy (ATP)
- Cell yield coefficient is defined as the amount of biomass produced per unit substrate consumed

Stoichiometry of aerobic bacterial growth

Bacterial Growth: biomass is in both side of the growth equation

substrate + biomass + oxygen + nutrients $\longrightarrow$ CO_2 + H_2O + more bacteria + residue

We can simplify the growth equation by treating biomass as a catalyst

substrate + oxygen + nutrients $\xrightarrow{\text{Bacteria as catalyst}}$ CO_2 + H_2O + bacteria + residue

Bacterial Decay or maintenance :

biomass + oxygen $\longrightarrow$ CO_2 + H_2O + nutrients+ residue

The concept of Yield:

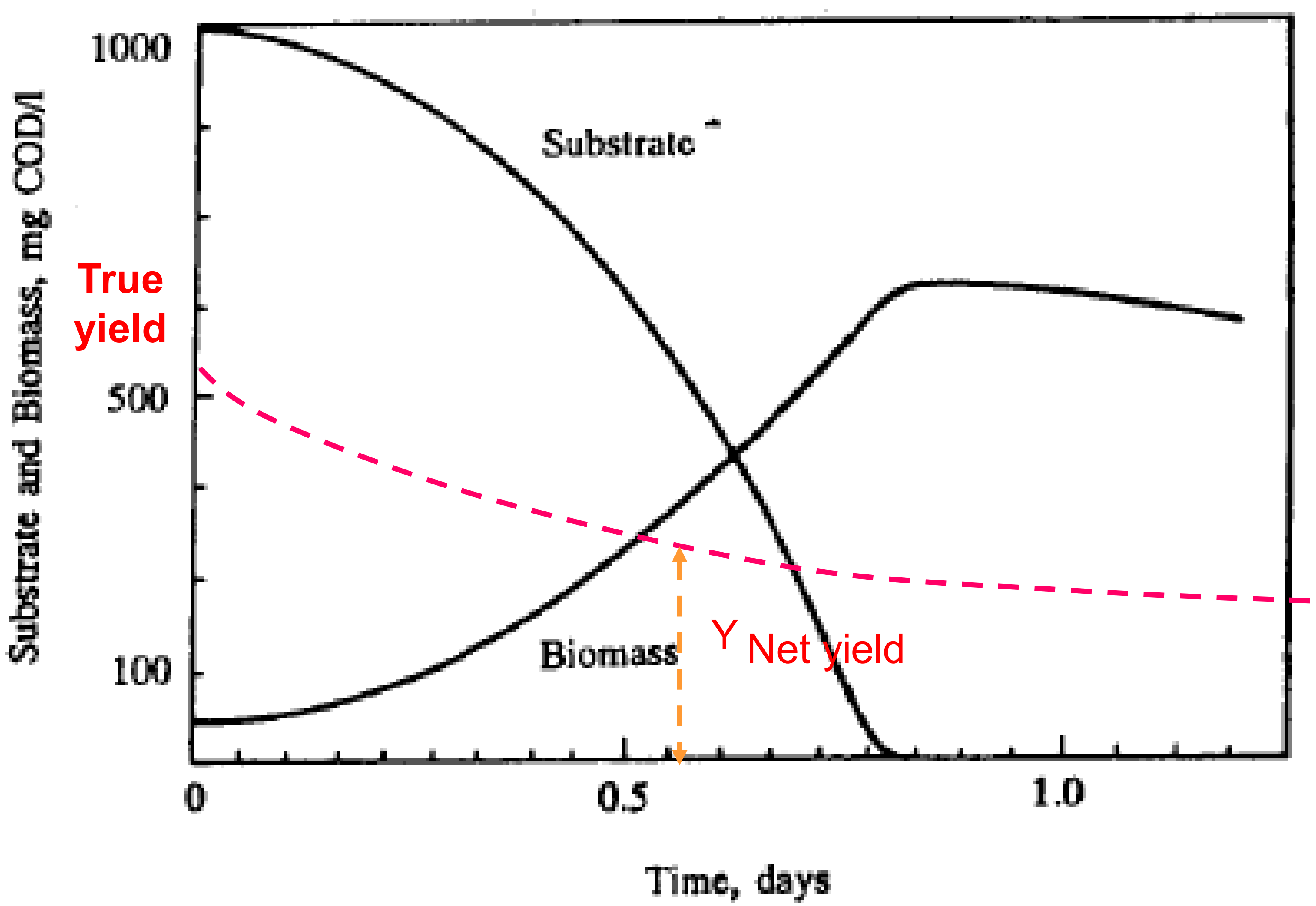
Mathematically, the growth rate of microbial cells is frequently expressed as

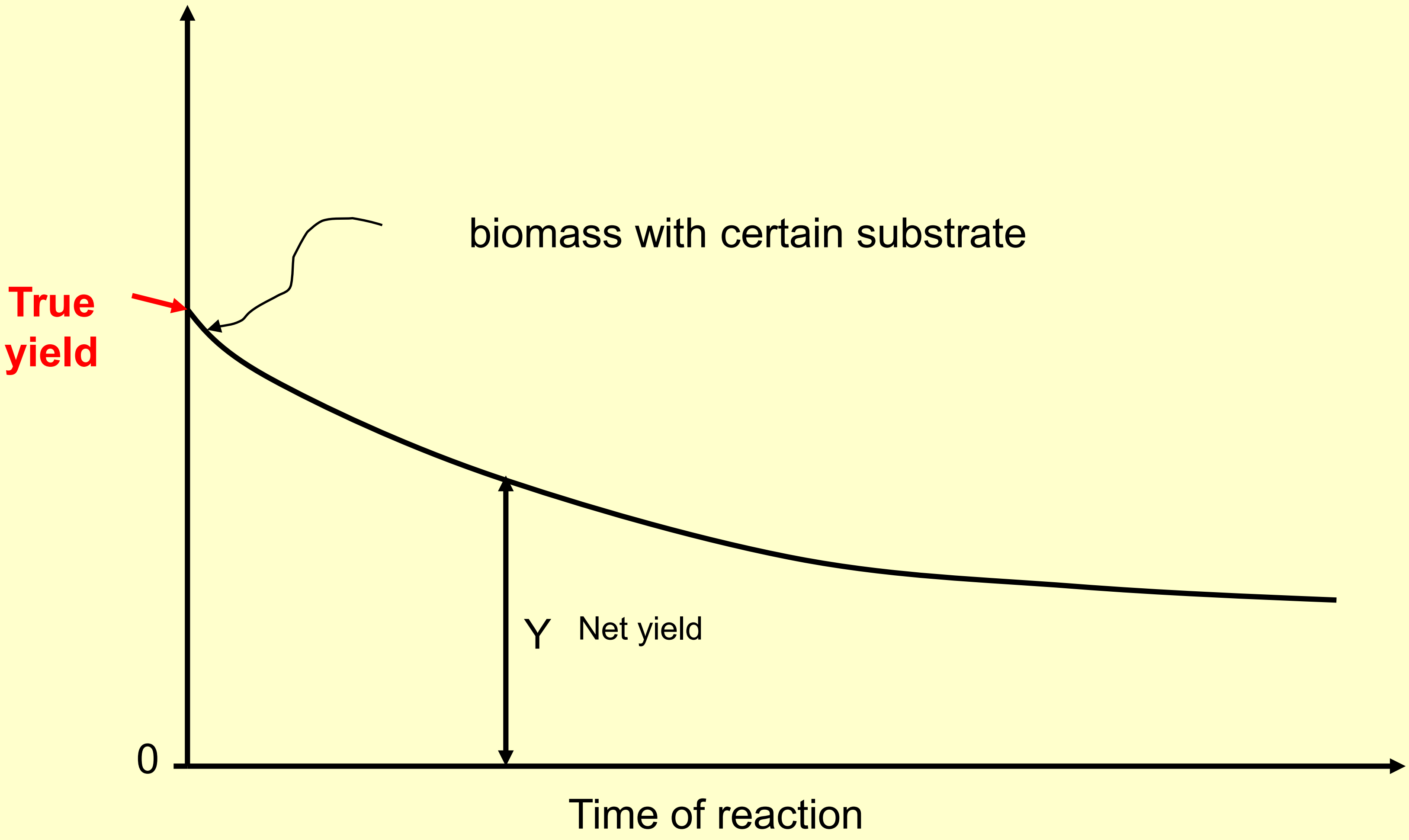
$$\frac{dX_a}{dt} = Y\left(\frac{-dS}{dt}\right) - bX_a \qquad \textbf{[2.5]}$$

where dX_a/dt represents the net growth rate (M/L^3T) of active organism (X_a, M/L^3), $-dS/dt$ represents the rate of disappearance (M/L^3T) of substrate (S, M/L^3), b is the decay rate (T^{-1}) of the organisms, and Y is the true yield of microorganisms (M/M). (A full development of Equation 2.5 and the equations that follow is given in Chapter 3. A short development is used here to support the concept of yield.) The net growth rate is equal the difference between the growth from substrate consumption minus decay due to self (endogenous) respiration or predation. The net yield (Y_n, M/M) can be found by dividing Equation 2.5 by the rate of substrate utilization:

$$Y_n = \frac{dX_a/dt}{-dS/dt} = Y - b\frac{X_a}{-dS/dt} \qquad \textbf{[2.6]}$$

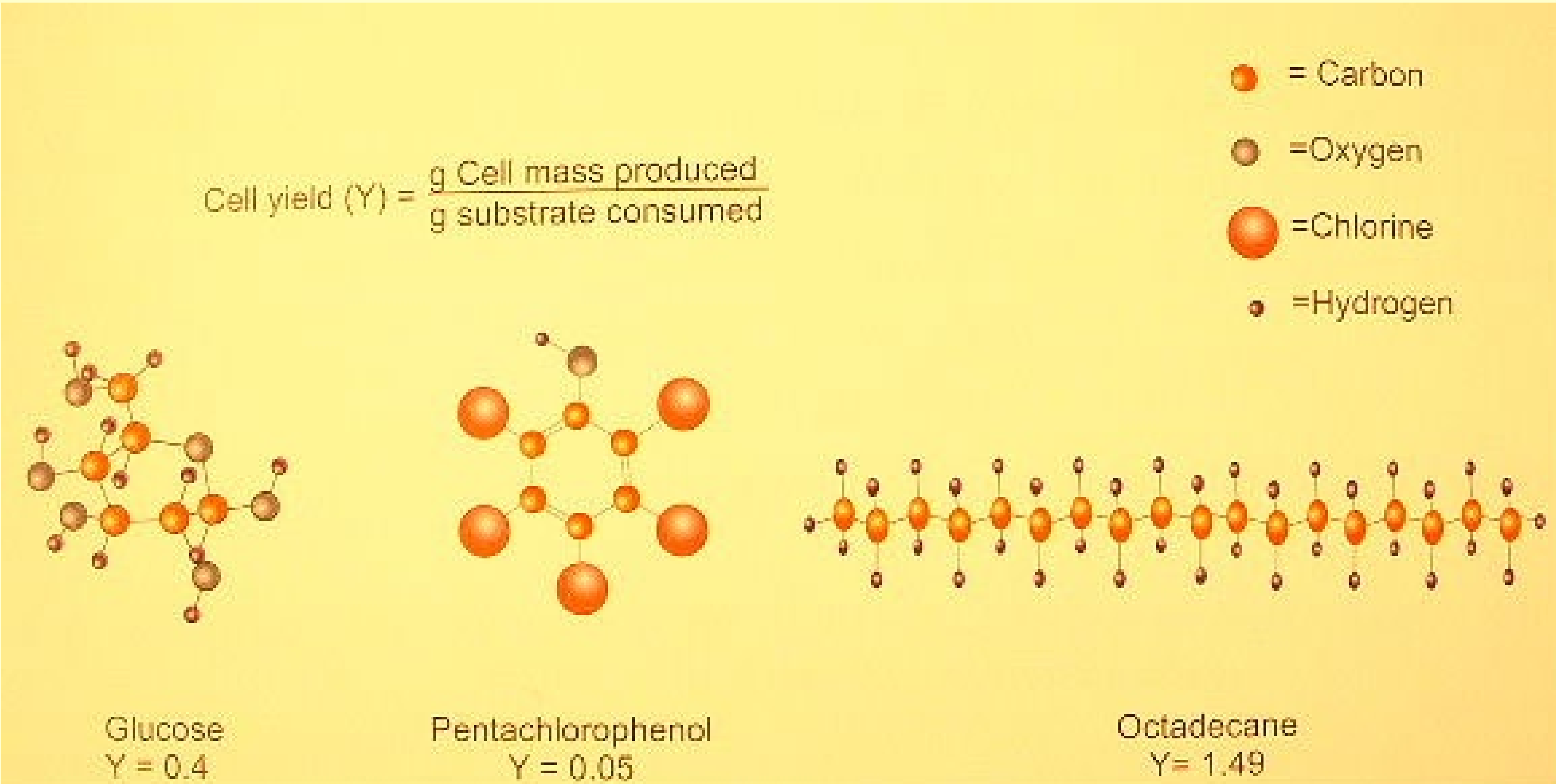
True Yield is the theoretical biogrowth per unit of substrate consumed without considering decay or maintenance




biomass with certain substrate
True yield
Y
Net yield
0
Time of reaction

Cell Yield

Carbon source	Yield coefficient G of cell per g of chemical
Glucose	0.4
Pentachlorophenol (PCP)	0.05
octadecane	1.49



Cell yield (Y) = g Cell mass produced / g substrate consumed
= Carbon
=Oxygen
=Chlorine
=Hydrogen
Glucose
Y = 0.4
Pentachlorophenol
Y = 0.05
Octadecane
Y= 1.49

Biochemical basis of cell yield

- In case of PCP, it is a new chemical that microbes have only encountered since its initial production in 1936
- There is not much chemical energy stored in PCP due to the C-Cl bonds.
- Energy is required to break the C-Cl bonds-energy not available for biomass production.

Biochemical basis of cell yield

- In case of octadecane, it is a component of crude oil that microbes have encountered for millions of years

- Consequently, microbes have had time to evolve efficient enzyme reactions and metabolic pathways to convert it to biomass.

- Octadecane is a highly reduced form of carbon (contains only C-H bonds) and can thus store more energy than compounds that are less reduced or have more oxygen atoms such as carbohydrates (CH_2O)

The COD of a compound is very nearly proportional to its heat of formation, and a crude value of its energy of formation can often be obtained. Thus a rough estimate of the potential energy for the metabolic needs of organism can be made from COD data.

$$C_nH_aO_bN_c + \left(\frac{2n + 0.5a - 1.5c - b}{2}\right) O_2 \rightarrow nCO_2 + cNH_3 + \frac{a - 3c}{2} H_2O \quad \textbf{[2.2]}$$

and

$$\text{COD}'/\text{Weight} = \frac{(2n + 0.5a - 1.5c - b)16}{12n + a + 16b + 14c} \quad \textbf{[2.3]}$$

(B) The COD of the organic matter consumed is calculated from the oxidation equation below:

$$8CH_2O + 8O_2 \rightarrow 8CO_2 + 8H_2O$$

$$f_{COD} = \frac{8O_2}{8CH_2O} = \frac{8 \times 32}{8 \times 30} = 1.06 \text{ g COD/g substrate}$$

$$Y_H' = \frac{Y}{f_{COD}} = \frac{0.47 \text{ g VSS/g substrate}}{1.06 \text{ g COD/g substrate}} = 0.44 \text{ g VSS/g COD}$$

(C) The following redox reactions are used to calculate the COD of the biomass generated:

$$C_5H_7NO_2 + 4H_2O \rightarrow 5CO_2 + NH_3 + 20H^+ + 20e^-$$

$$5(4e^- + 4H^+ + O_2 \rightarrow H_2O)$$

$$C_5H_7NO_2 + 5O_2 \rightarrow 5CO_2 + NH_3 + 2H_2O$$

$$f_X = \frac{5O_2}{C_5H_7NO_2} = \frac{5 \times 32}{113} = 1.42 \text{ g cell COD/g biomass}$$

Chemical Oxygen Demand (COD)

(D) Benzene $C_6H_6 + 7½\ O_2 \longrightarrow 6\ CO_2 + 3\ H_2O$

MW=78 MW=32

$$\text{Th COD} = \frac{7\,{}^{1}\!/_{2} * 32}{78} = 3.08\ \text{mg COD/mg benzene}$$

Chemical compounds	**Theoretical COD Values** **mg of COD/ mg of compound**
Chlorinated organics	0.3 - 1
carbohydrates	1 - 1.2
bacterial cell	1.42
proteins	1.8
short chain carboxylic acids	2
Oil molecules	3
Methane	4

Yield values based on COD are closer together

Organic source	**Cell yield g cell per g of compound**	**Cell yield g cell per g of COD compound**
Glucose	0.4	0.40
Pentachlorophenol	0.05	0.15
octadecane	1.45	0.44

Organisms	Yield, g of COD / g of Feed COD
Bacteria with substrate to grow, in one day	0.6
Bacteria with much substrate and extensive storage, in one hour	0.95
Fish (one year to 0.5 kg)	0.45
Hen	0.32
Pig (to 65 kg)	0.23
Cow (no milk)	0.18
Human (0 to 16 years)	0.01
Human (0 to 70 years)	0.002

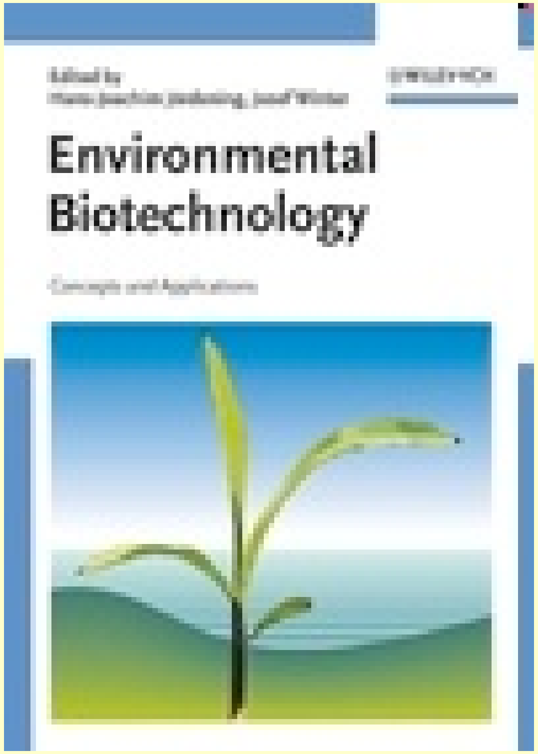
http://bioinfo.bact.wisc.edu/themicrobialworld/homepage.html

The mutation rate for most procaryotic genes is in the neighborhood of 10^{-8}

http://bioinfo.bact.wisc.edu/themicrobialworld/homepage.html

The Microbial World - a website devoted to microbial sciences

University of Wisconsin – Madison
Department of Bacteriology
[Kenneth Todar]



Environmental Biotechnology: Concepts and Applications
Edited by Hans-Joachim Jördening and Josef Winter
Wiley December 2004

Table of content

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Bacterial Metabolism in Wastewater Treatment Systems

Claudia Gallert and Josef Winter

1.1
Introduction

Water that has been used by people and is disposed into a receiving water body with altered physical and/or chemical parameters is defined as wastewater. If only the physical parameters of the water were changed, e.g., resulting in an elevated temperature after use as a coolant, treatment before final disposal into a surface water may require only cooling close to its initial temperature. If the water, however, has been contaminated with soluble or insoluble organic or inorganic material, a combination of mechanical, chemical, and/or biological purification procedures may be required to protect the environment from periodic or permanent pollution or damage. For this reason, legislation in industrialized and in many developing countries has reinforced environmental laws that regulate the maximum allowed residual concentrations of carbon, nitrogen, and phosphorous compounds in purified wastewater, before it is disposed into a river or into any other receiving water body However, enforcement of these laws is not always very strict. Enforcement seems to be related to the economy of the country and thus differs significantly between wealthy industrialized and poor developing countries. In this chapter basic processes for biological treatment of waste or wastewater to eliminate organic and inorganic pollutants are summarized.

1.2
Decomposition of Organic Carbon Compounds in Natural and Manmade Ecosystems