Neidhardt Chapter 5: Biosynthesis and Fueling

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How do bacteria create themselves from components in the medium?

- Several hundred enzyme-catalyzed reactions. Can be subdivided into:
  - **Biosynthetic Pathways** - from 12 precursor metabolites to building blocks
  - **Fueling Pathways** - from ingredients of the medium to metabolic needs

The 12 precursor metabolites

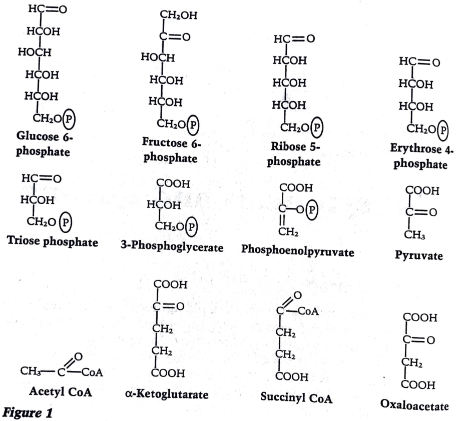


Figure 1

Structures of the 12 precursor metabolites.

Table 1 - Basic Building Blocks by mol

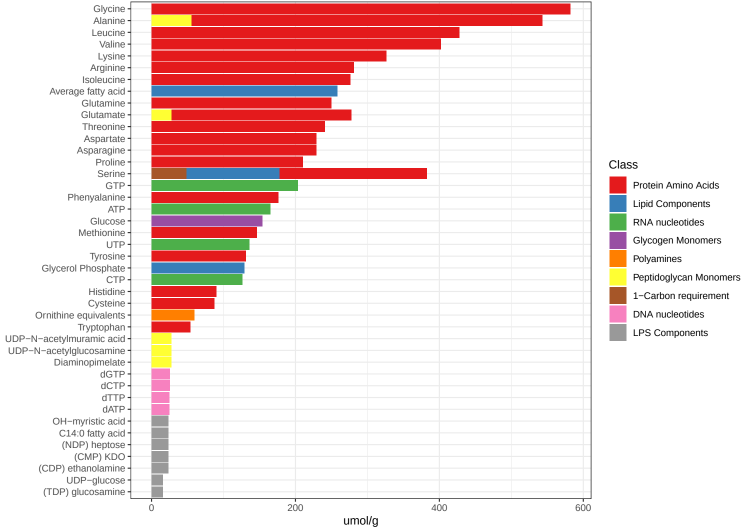


Table 2 - Basic building block requirements

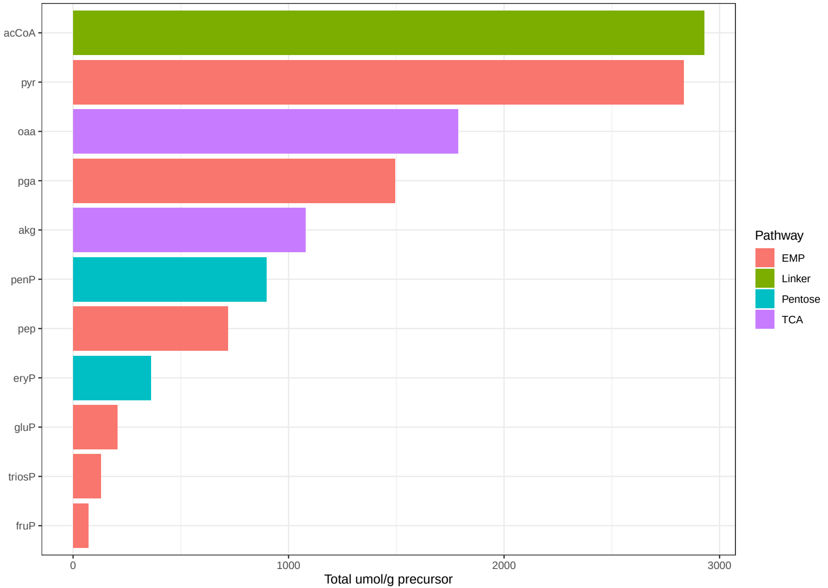


Table 1 - Basic building blocks by precursor

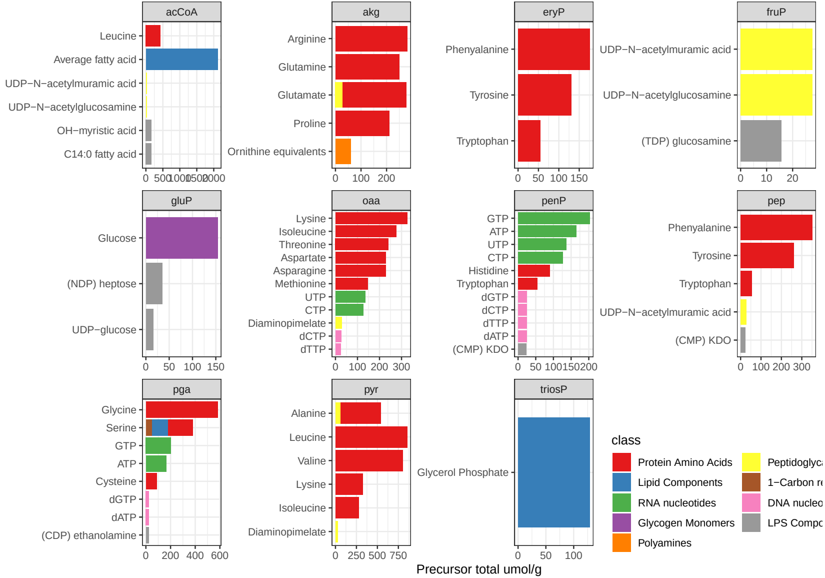


Table 1 - Basic building blocks by required ATP

Table 1 - Basic building blocks by required NADPH

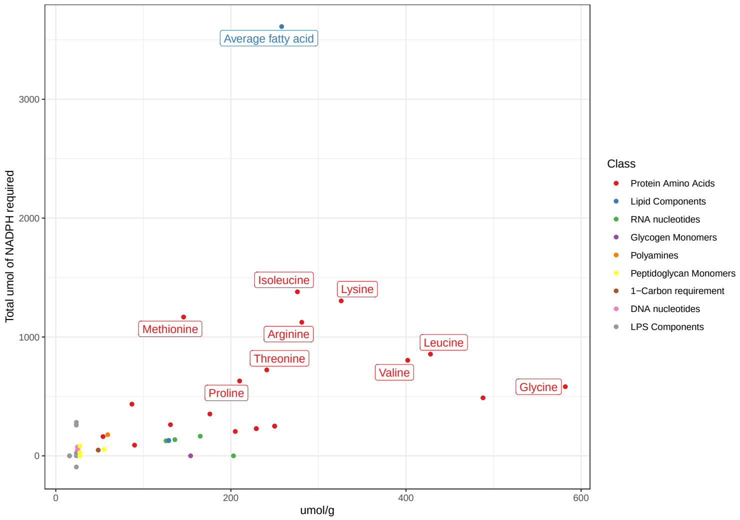


Table 1 - Basic building blocks by required NH_4^+

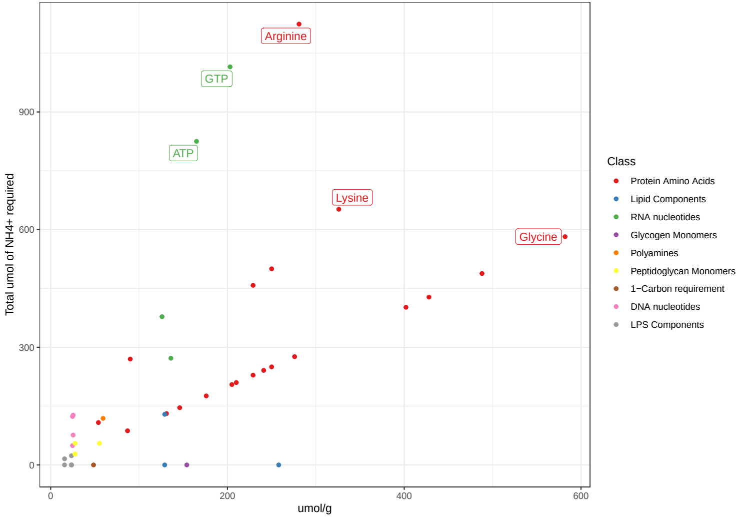


Table 1 - Basic building blocks by required S

Assimilation of Nitrogen - organic sources

- None of the 12 precursor metabolites contain nitrogen or sulfur
- Most organic sources have N in the -3 oxidation state
  - Amino groups
  - Imino groups
  - heterocyclic nitrogen atoms
- These get metabolized by bacteria into NH_4^+
- Enzymes that catalyze pathways leading to NH_4^+ are usually genetically controlled
  - They are off when plenty of NH_4^+ is around

Assimilation of Nitrogen - inorganic sources

- Most significant inorganic sources are:
  - NH_4^+
  - NO_3^- (*E. coli* only anaerobically)
  - N_2 (*E. coli* cannot use this)
- Utilization of NO_3^- uses two reactions
  - $NO_3^- \rightarrow NO_2^-$ by Assimilatory Nitr**a**te Reductase
  - $NO_2^- \rightarrow NH_4^+$ by Assimilatory Nitr**i**te Reductase
- NO_3^- can also be used as the terminal electron acceptor in respiration
  - Anaerobic respiration
  - *E. coli* can do this
- Nitrogen fixation ($N_2 \rightarrow NH_4^+$)
  - Nitrogenase - combination of two iron-sulfur proteins
  - Azoferredoxin
  - Molybdoferredoxin (has two molybdenum atoms as well)

Assimilation of Nitrogen - $NH_4^+ \rightarrow$ organic compounds

- Large diversity in obtaining NH_4^+ but only two ways to utilize it
- Directly into glutamate by L-Glutamate Dehydrogenase (GDH)
- Indirectly by a cycle of reactions (GS-GOGAT)
  - Glutamine synthetase (GS)
  - Glutamate Synthase (glutamate-α-oxoglutarate aminotransferase, GOGAT)
- Reaction is basically the same though (α-ketoglutarate + $NH_4^+ \rightarrow$ glutamate)
- Major differences between GDH and GS
  - GS has higher affinity for ammonia than GDH
  - GS-catalyzed reaction needs ATP whereas GDH doesn't
- Organisms that have both (like *E. coli*) can balance energy with nitrogen needs
  - GS is turned off if ammonia is high
  - GS is also inhibited by eight compounds derived from glutamine
  - GS is also post-translationally modified by adenylation (on/off controlled by one enzyme)
    - Controlled by ratio of glutamine to α-ketoglutarate

Assimilation of Sulfur - $H_2S \rightarrow$ cysteine

- Major reaction is O-acetylserine sulfohydrolase
  - H_2S + O-acetylserine + $\rightarrow$ cysteine + acetate + water
- Any other source of sulfur must be converted to H_2S to be used
- H_2S is only really in anaerobic environments
- Sulfate is the most common source in the lab (probably not in environment though)
- Bulk of S probably comes in through C-bonded S in organic compounds
- Enzymes that can use sulfates are turned on by organic sulfates

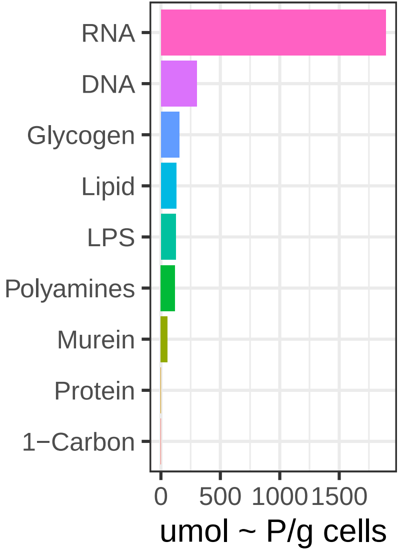
Assimilation of Phosphorus - phosphate $\rightarrow$ ATP

- Differs from nitrogen and sulfur in two key aspects
  - Is not oxidized or reduced
  - Assimilated through fueling pathways instead of biosynthesis
- Organic phosphate compounds don't usually penetrate the cell membrane
- Hydrolysis of organic phosphates is driven by akaline phosphatase in the periplasm
- Akaline phosphatase is repressed when phosphate is in the medium
- *E. coli* also has a $5'$ nucleotidase that acts in the periplasm
- Free phosphate is taken up into ATP

Nutritional diversity - How specialized is a bug?

- Bacteria can take advantage of preformed building blocks
- **Auxotrophs** lack one or more biosynthetic pathways
- **Prototrophs** are "complete biochemists" (like *E. coli*)
- Reciprocal relationship between biosynthetic capability and nutritional requirements
- Those with rich growth environments tend to do away with unnecessary pathways

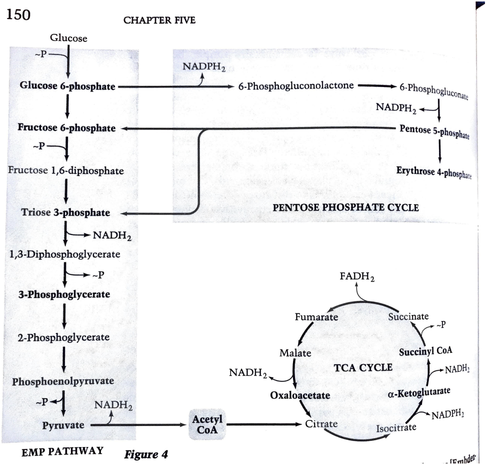
Table 4 - Biosynthesis costs in rich medium



- Medium has
  - glucose and inorganic salts
  - 21 amino acids, ribonucleosides, deoxyribonucleosides
  - glycerol phosphate, fatty acids, ornithine, glucosamine, ethanolamine, heptose, KDO
- 85% of the energy and 100% of reducing power is saved by harvesting from media
- These costs swing even more wildly depending on carbon source

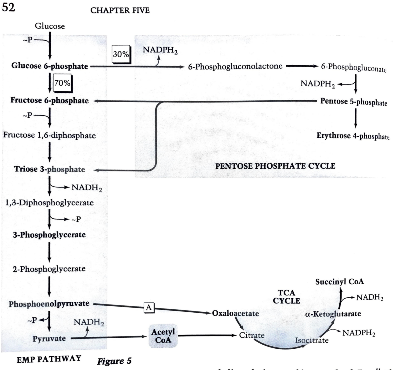
Fueling reactions - where bacteria show their diversity

- Biosynthesis is basically the same in all bacteria
- Goal is to create the 12 precursor metabolites/energy
- These reactions are typically called central metabolism

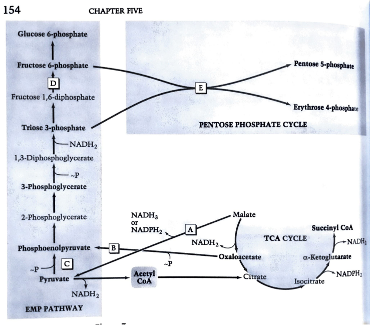


Fueling reactions - *E. coli* central metabolism by carbon source

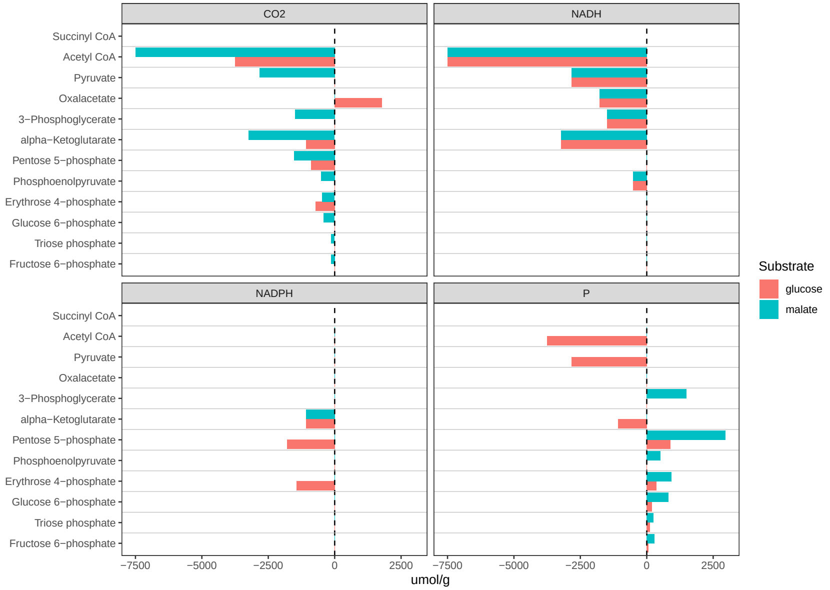
Glucose



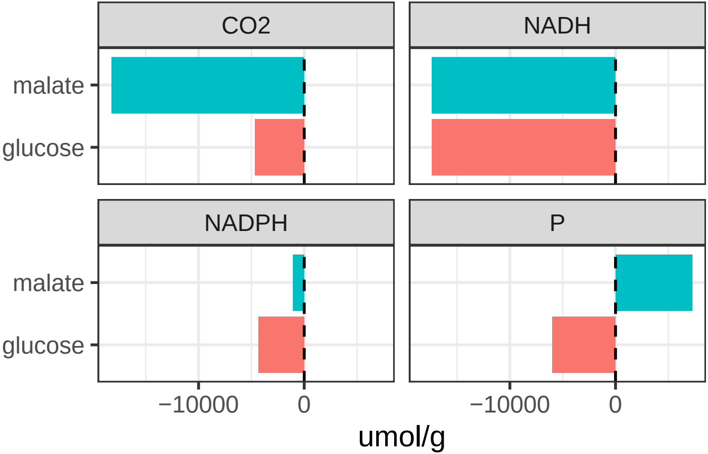
Malate



Fueling reactions - Energy cost by carbon source



Fueling strictly for precursors is not enough



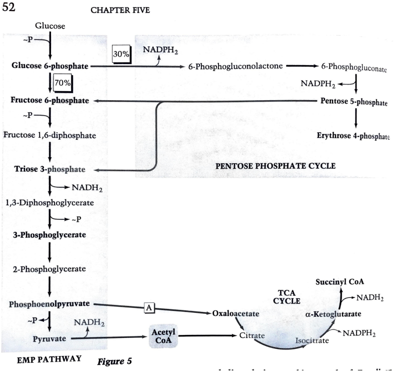
- Malate: net energy needed, Glucose: net energy produced
- Need 42,703 μmol of ~P
  - 24,218 for polymerization (Chapter 4)
  - 18,485 for biosynthesis (Chapter 5)

Fermentation vs. Respiration - what do I do with all these reduced pyridine nucleotides?

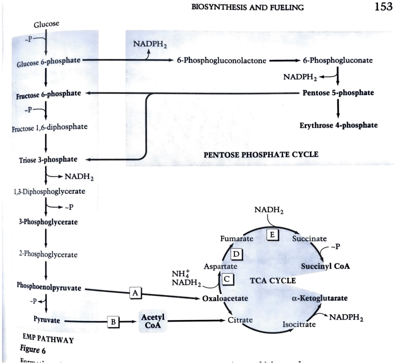
- **Fermentation** - reoxidation through reducing organic metabolites
- **Respiration** - reoxidation through electron transport
  - Does **not** neccesarily need oxygen (anaerobic respiration)
- Oxygen does play into what pathways are used by a cell
  - **Strict Aerobes** - only grow in oxygen
  - **Anaerobes** - only grow without oxygen
  - **Faculative Anaerobes** - can grow in +/- oxygen
  - **Indifferent Anaerobes** - make ATP by fermentation regardless of oxygen

E. coli - oxygen presence changes flow through central metabolism

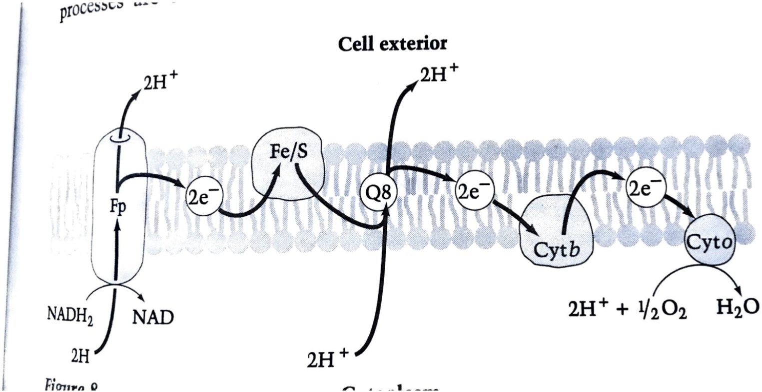
Aerobic



Anaerobic



E. coli - Electron transport chain



- Major energy production with O_2 as the acceptor

Electron transport chain in other bacteria

- **Chemolithotrophs** - generate ATP but uncoupled from NAD reduction
- Use reverse electron transport to reduce NAD
- Hydrogen bacteria couple substrate oxidation directly to reduction of NAD

Table 7. Redox potentials of the primary energy-yielding reactions of chemolithotrophs

Class of chemolithotroph	Half-reaction of substrate (oxidation)[a]	E'_0 (volts)
Hydrogen bacteria	$H_2 \rightarrow 2H^+ + 2e^-$	−0.41
Sulfur bacteria	$H_2S \rightarrow S + 2H^+ + 2e^-$	−0.25
	$S + 3H_2O \rightarrow SO_3^{2-} + 6H^+ + 4e^-$	+0.005
	$SO_3^{2-} + H_2O \rightarrow SO_4^{2-} + 2H^+ + 2e^-$	−0.28
Iron bacteria	$Fe^{2+} \rightarrow Fe^{3+} + e^-$	+0.79
Ammonia oxidizers	$NH_4^+ + 2H_2O \rightarrow NO_2^- + 8H^+ + 6e^-$	+0.44
Nitrite oxidizers	$NO_2^- + H_2O \rightarrow NO_3^- + 2H^+ + 2e^-$	+0.35

[a]The oxidation of NADH, (1/2 NADH + H^+ → NAD^+ + 2...

Utilization of oxygen - what is needed?

- A lack of enzymes that are intrinisically sensitive to oxygen
- The ability to break down hydrogen peroxide (H_2O_2) and superoxide($O\cdot_2^-$)
- Some aerobes have enzymes that are sensitive to O_2
  - *Azotobacter* respires fast enough to protect its nitrogenase
  - Cyanobacteria have heterocysts to contain nitrogenase in an anaerobic pocket
- Catalase - $2H_2O_2 \rightarrow 2H_2O + O_2$
- Superoxide dismutase - $2O\cdot_2^- + 2H^+ \rightarrow O_2 + H_2O_2$

Fermentation - making ATP and oxidizing NADH → NAD+

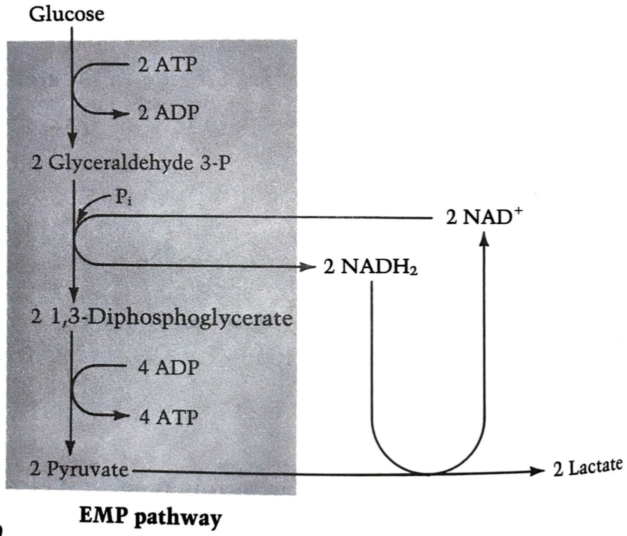
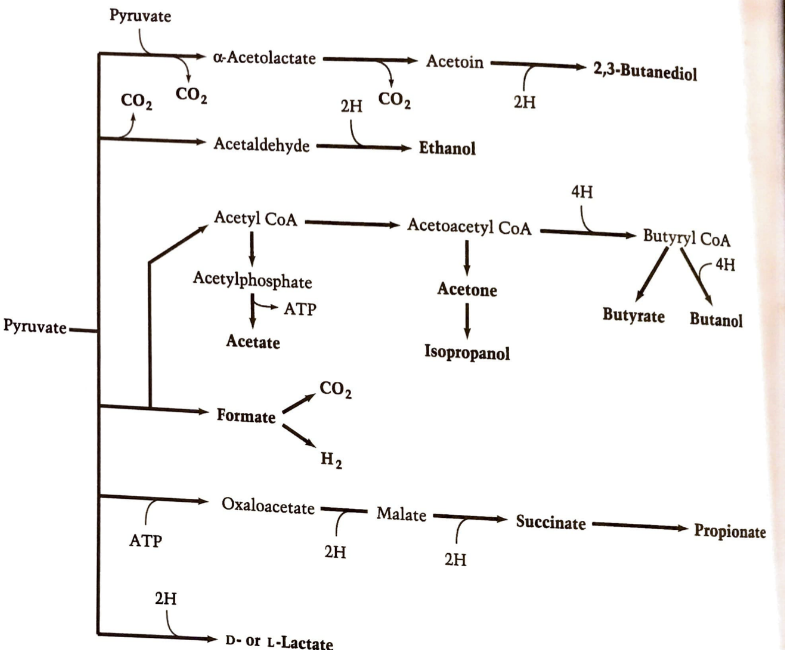


Figure 10

Homolactic fermentation.

Fermentation - the diversity of end products



Fermentation - *E. coli* and mixed acid fermentation

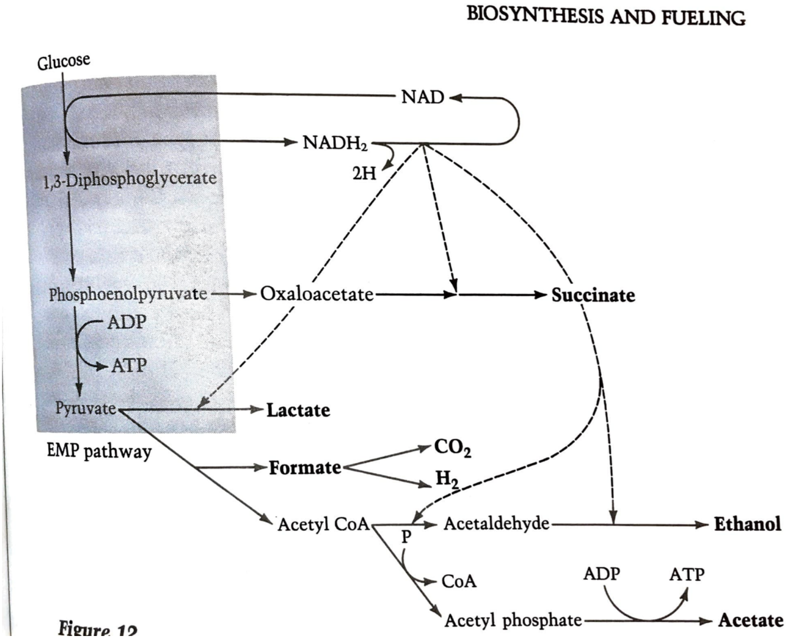
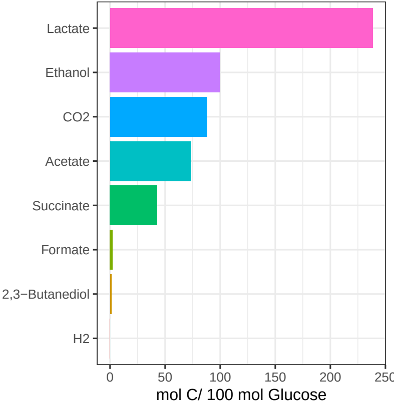
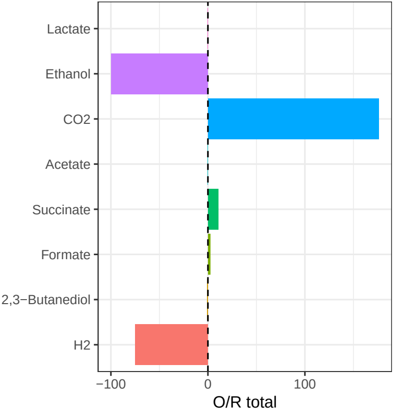


Table8 - Carbon flux and redox balance during *E. coli* fermentation in glucose

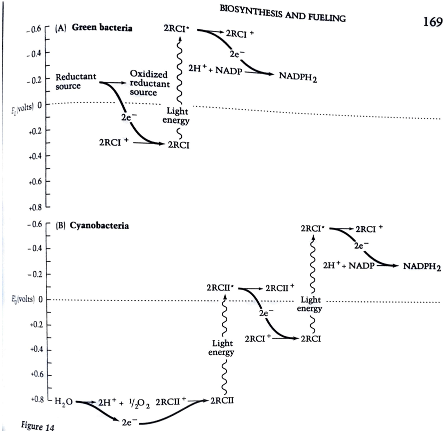




Phototrophs and their fueling reactions

- **Oxygenic** photosynthesis - cyanobacteria and blue-green bacteria
- **Anoxygenic** photosynthesis - purple bacteria, purple-sulfur, green bacteria
- Cyclic photophosphorylation - basically ETC but using light as the energy source
  - Chlorophyll is the electron donor and acceptor
  - Membrane-bound so have increased cytoplasmic membrane
- Photosynthetic aparatus
  - light-harvesting antenna
  - reaction center
  - ETC

Sources of reducing power in phototrophs



- Since NADH/NADPH is not used, phototrophs reduce NADP in different ways
  - Purple nonsulfur use organic compounds or H_2
  - Purple sulfur and green use reduced sulfur
  - oxygenic cyanobacteria use water and produce O_2

Phototrophs - Formation of precursors

- Synthesize precursors through CO_2
- Green bacteria fix CO_2 by reversing the TCA cycle
- Other phototrophs fix CO_2 by the Calvin-Benson pathway
  - Uses ribulosebisphosphate carboxylase (may be most abundant enzyme on earth)
  - Utilizes ATP and reduced NADP rather than generating them
- Fueling is done through photosynthesis
- Fueling for chemolithotrophs is done through aerobic respiration of inorganic substrate