2. Biology (from Engineering Perspective)

Read: Chapter 2
(pp. 10 - 55)

A. Cell types

1. Viruses

- obligate parasites
- 30 - 200 x 10^{-9} m diameter (0.03 - 0.2 μm)
- cause infections in other higher cells
- Bacteriophages are viruses which specifically infect bacteria.
- contain DNA or RNA enclosed in a protein coat called a capsid

- After infection:
  1) Lytic Cycle - host cells lyse (break apart) and release new viruses, which go on to infect new host cells.
  2) Lysogenic Cycle - viral DNA is incorporated into host DNA, and continues to multiply with host.
- Viruses are important because they can:
  1) infect bacteria used in a commercial process.
  2) cause disease in animals and plants.
  3) be used to incorporate foreign DNA into a host cell.

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2. Procaryotes

- 5 - 30 x 10^{-7} m diameter (0.5 - 3 μm)
- do not contain a membrane-enclosed nucleus
- usually do not associate with other cells
- often multiply quickly when nutrients are plentiful Some populations can double every 20 minutes.

a. Eubacteria

- categorized by their cell envelope

  Gram-negative

    8 nm cytoplasmic membrane
    7 nm periplasmic space
    2 nm peptidoglycan
    8 nm outer membrane

  Gram-positive

    8 nm cytoplasmic membrane
    80 nm peptidoglycan

Secretion of proteins is often more difficult for Gram-negative cells because of outer membrane.

Gram stain developed to distinguish these cell types, involves a dye which is retained by peptidoglycan.

- formation of spores in response to adverse conditions
- formation of inclusion bodies (can be large)

  examples: sulfur, proteins
- excretion of polysaccharides to adhere to surfaces

  (teeth, bioremediation)

b. Archaebacteria

- have no peptidoglycan
- have different lipid composition in cytoplasmic membrane
- live in extreme environments

  examples: 110ºC, dead sea water

3. Eucaryotes

- 1 - 20 x 10^{-6} m diameter (1 - 20 μm)
- contain a membrane-enclosed nucleus
- Cell membrane has sterols which provide rigidity.
- Plant cells contain cellulose fibers which make them strong. Animal cells do not have cellulose.
- have numerous complex cell organelles
  1) Mitochondria are site for respiration and oxidative phosphorylation. Cristae are their complex inner membrane folds.

     Have their own DNA

     Replicate independently
  2) Chloroplasts are site for photosynthesis.

Both eucaryotes and procaryotes contain:

Endoplasmic reticulum

- rough - Contain ribosomes and are site of protein synthesis
- smooth - site of lipid synthesis

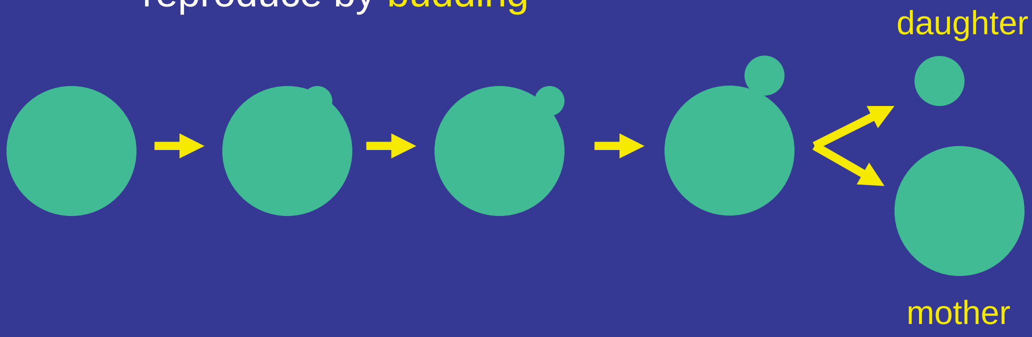
Both eucaryotes and procaryotes may contain:

Flagella and cilia are filamentous structures which permit cell to propel through fluid. Chemotaxis is the tendency of cells to move towards/away from chemicals.

a. Fungi

1) Yeasts

- spherical cells
- widespread in nature (soil)
- do not use sunlight for energy
- free-living
- reproduce by budding



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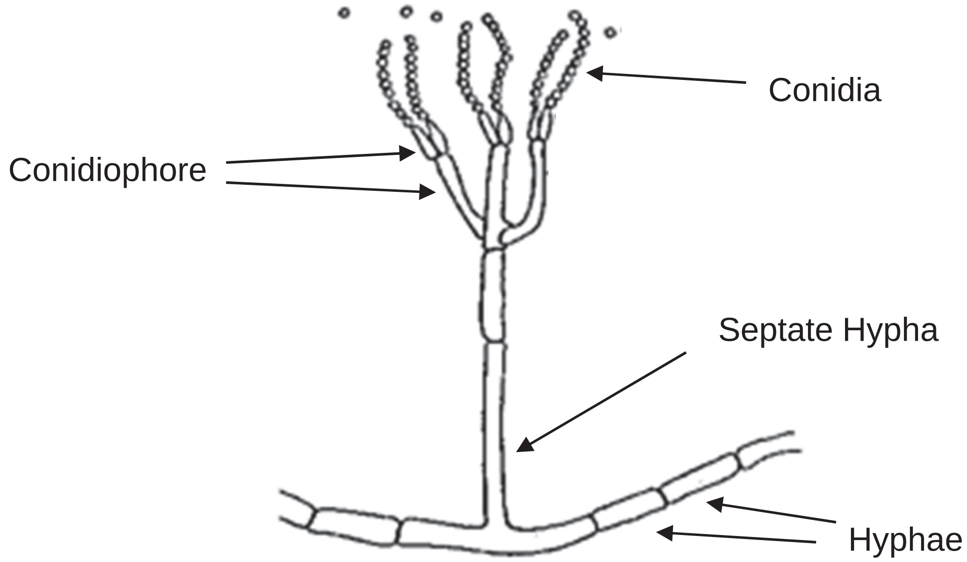
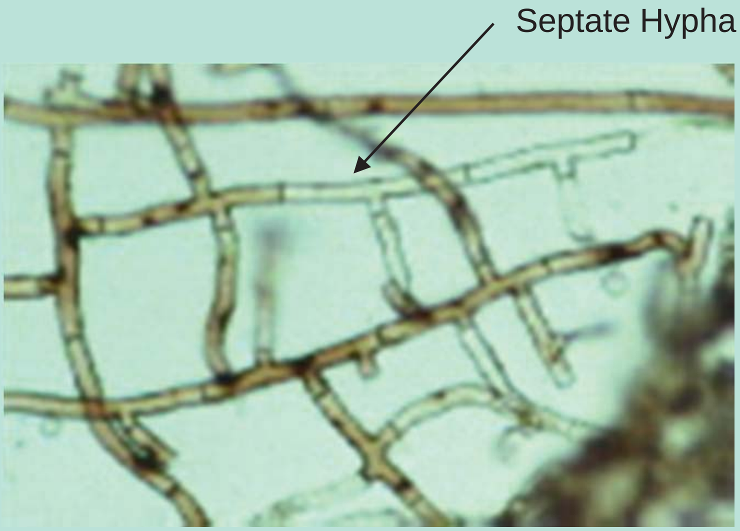
2) Molds

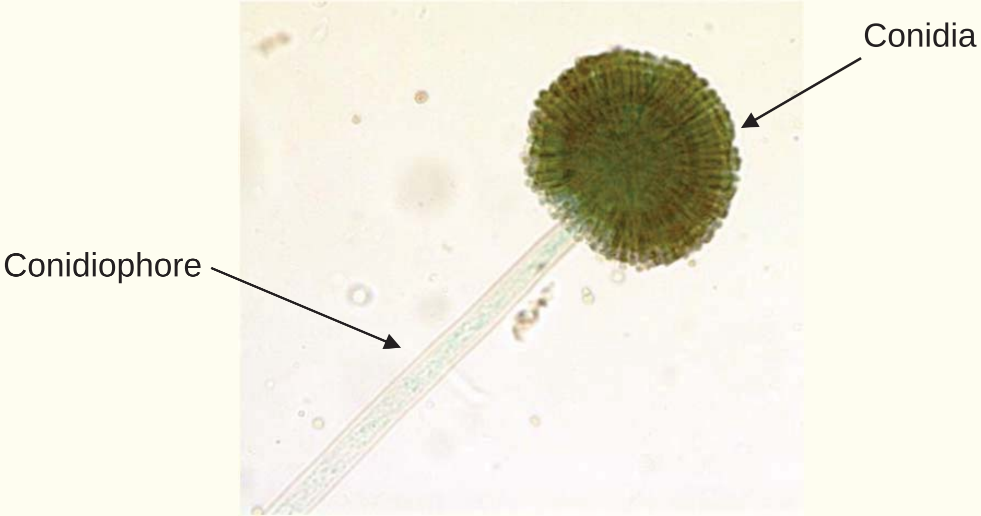
- distinctly non-spherical cells
- A Mycelium is a highly branched system of hyphae.
- Hyphae are thin filaments of cells.
- Conidia are asexual spores which form on hyphae.
- in submerged culture can form thick mats which result in complex rheological behavior



Penicillium



Rhizoctania



Aspergillus

Chrysosporium

2) Molds

- distinctly non-spherical cells
- Mycelium are a highly branched system of tubes.
- Hyphae are thin filaments of cells.
- Conidia are asexual spores which form on hyphae.
- in submerged culture can form thick mats which result in complex rheological behavior
- have high oxygen requirement
- do not use sunlight for energy
- free-living
- spores

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b. Algae

- photosynthetic
- often, but not always, unicellular
- some contain silica or calcium carbonate in structure
- principal products:
  - diatomaceous earth
  - agar
  - foodstuffs

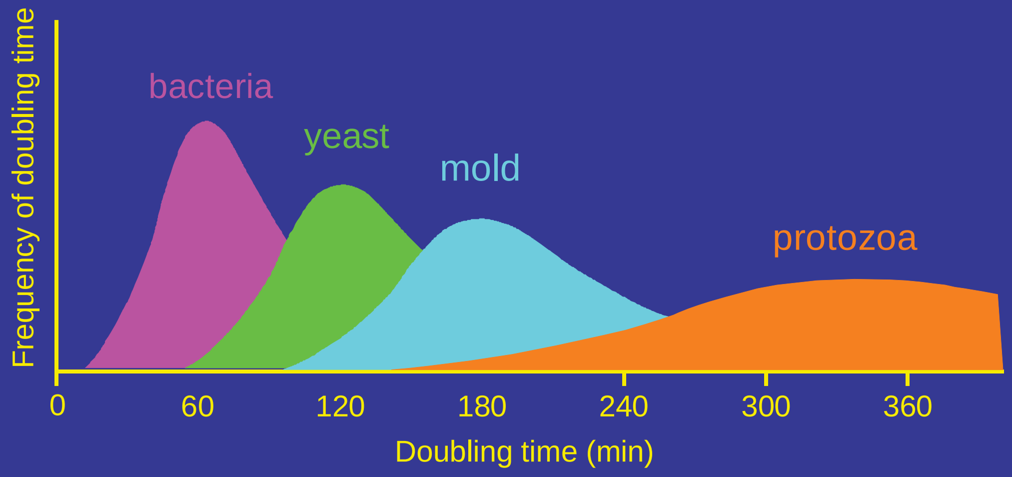
c. Protozoa

- large unicellular cells that lack cell walls
- ingest other smaller organisms
- move to seek prey
  - amebae - cytoplasm forms pseudopodium
  - flagellates - have one flagellum
  - ciliates - have many cilia
  - sporozoa - usually have aid of host

d. Animal and Plant Cells

- used for many vaccines (animal cells)
- therapeutic proteins (animal cells)
- colors, flavors, insecticides (plant cells)

Comparison of cell growth rates



B. Cell construction

1. Proteins

- composed of 20 amino acids covalently linked
- have diverse functions:

Class	Example	Function
enzymes	alcohol dehydrogenase	oxidizes alcohols to aldehydes
regulatory	insulin	regulates glucose metabolism
transport	hemoglobin	transport of oxygen and carbon dioxide in blood
protection	IgG antibody	forms complex with foreign protein and initiates immune response
storage	albumin	egg-white
structure	collagen	cartilage and tendons

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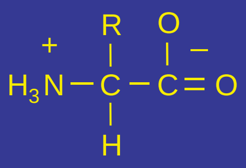
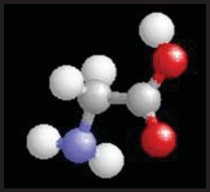
2. Amino acids

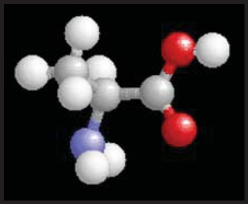


$$H_3\overset{+}{N}-\underset{H}{\overset{R}{C}}-\overset{O^-}{C}=O$$

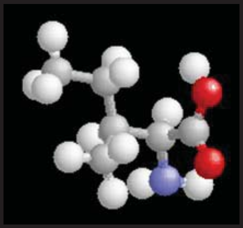
- zwitterion - doubly charged
- chiral - L-form found in proteins
- Isoelectric point is pH at which amino acid has no net charge.
- R = side chain
  The R-group distinguishes amino acids.
- Proteins are formed from amino acids by peptide bonds



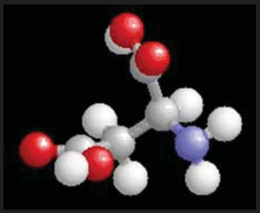
Glycine



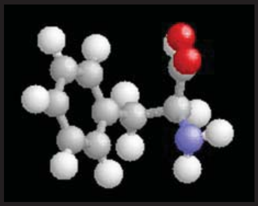
Alanine



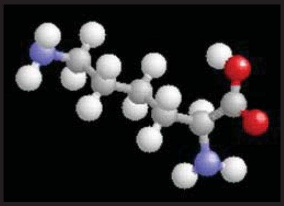
Isoleucine



Aspartate



Phenylalanine



Lysine

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3. Protein structure

- Primary structure is merely the sequence of amino acids
- Secondary structure is the structural conformation of amino acids that are close neighbors. The peptide bond restricts flexibility.
- Tertiary structure is the three dimensional structure arising from hydrogen bonds, disulfide bridges, hydrophobic and ionic interactions between amino acids which are widely separated in linear sequence but are spatially proximate.
- Quarternary structure is the interactions between protein subunits.

4. Carbohydrates

- General formula is $(CH_2O)_N$.
- Involved in structure, storage and signaling
- Analogous to amino acids, monomeric units can combined to form polymeric molecules called polysaccharides

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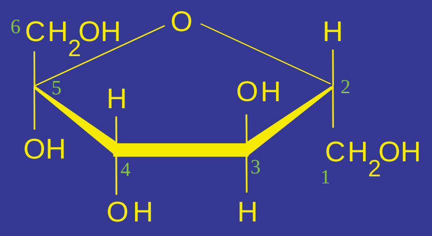
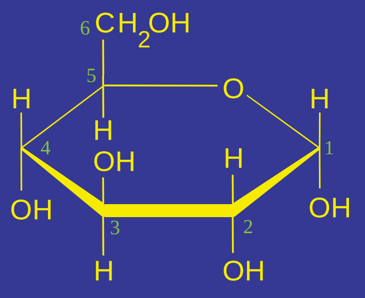
a. Monosaccharides

- 3-9 carbon atoms
- 5 or 6 carbon atoms most common, which often form stable furanose (5-membered) or pyranose (6-membered) ring
- examples:

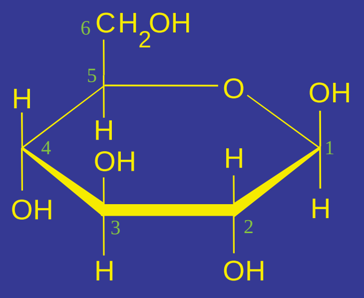


β-D-fructose

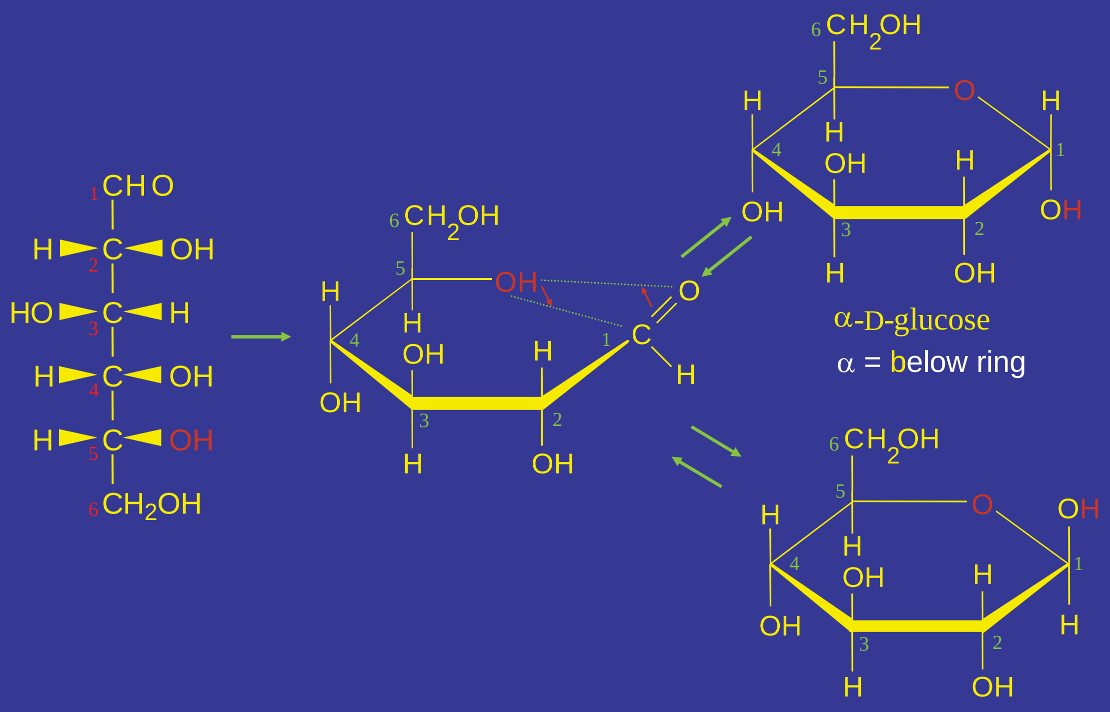
Haworth projection



α-D-glucose



β-D-glucose

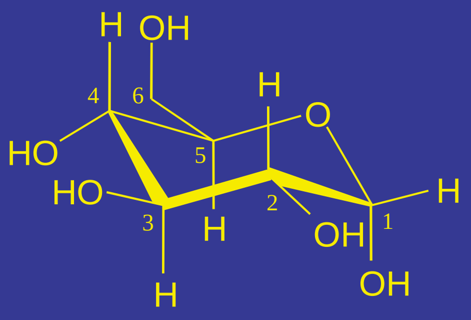


α-D-glucose

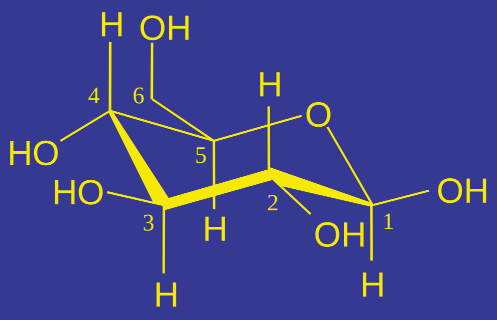
α = below ring

β-D-glucose

β = above ring



α-D-glucopyranose



β-D-glucopyranose

35% α
64% β
1% furanose
<0.03% linear

b. Disaccharides

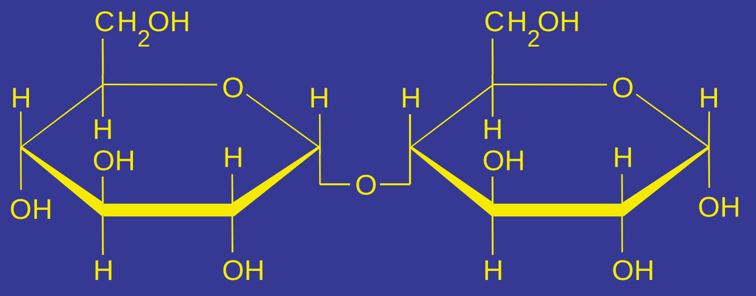
- formed by the condensation of two monosaccharides

  (while amino acids only form one type of covalent bond - peptide bond - saccharides can form several different linkages)

- examples:

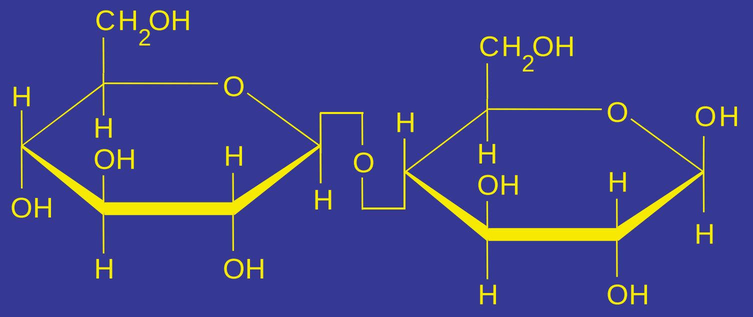
α-1,4 linkage

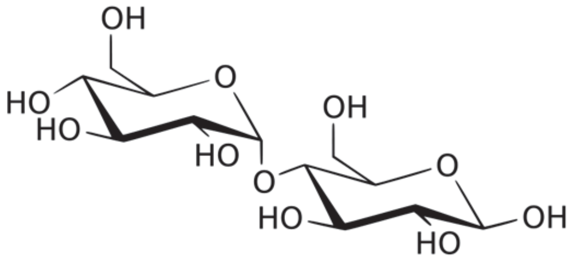
maltose



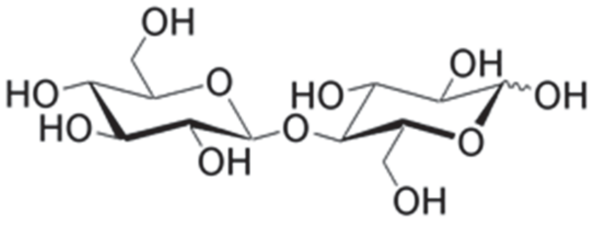
β-1,4 linkage

cellobiose

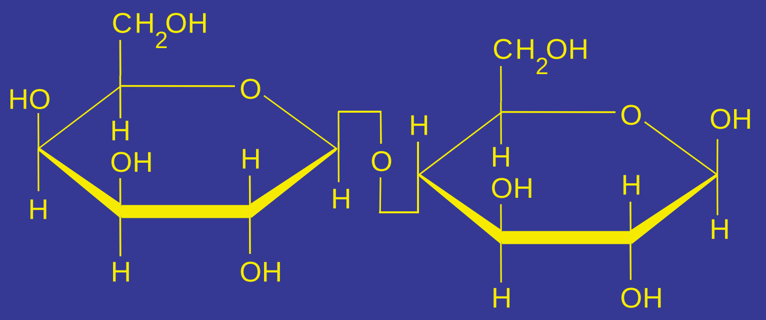




Maltose



Cellobiose



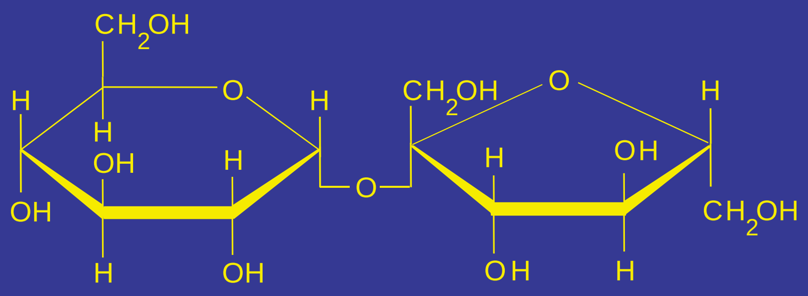
β-D-galactose

+

β-D-glucose

with β-1,4 linkage yields lactose

β (1→4) galacto-glucopyranose



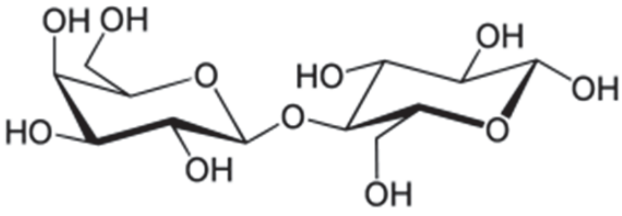
α-D-glucose

+

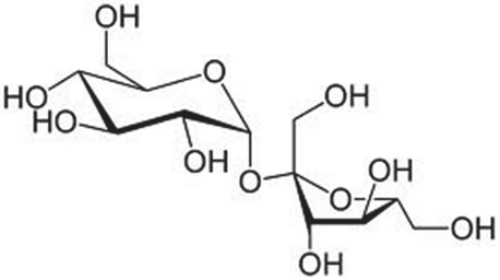
β-D-fructose

with α,β-1,2 linkage yields sucrose

α,β (1→2) gluco-fructofuranose



Lactose



Sucrose

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c. Polysaccharides

- formed by the condensation of more than two monosaccharides
- examples:

Amylose	Straight chain of 15 - 3,000 glucose units linked by α-1,4 glycosidic bonds. Insoluble in water
Cellulose	Straight chain of 300 - 6,000 glucose units linked by β-1,4 glycosidic bonds. Insoluble in water

c. Polysaccharides (cont’d)

Amylopectin	Branched chain of 5,000 - 10,000 glucose units linked by α-1,4 and α-1,6 glycosidic bonds. Branching occurs via α-1,6 bonds after an average of 25 units. More exposed monomers available for enzymatic cleavage. Soluble in water (forms gels)
Glycogen	Like amylopectin but smaller and more frequent branching.

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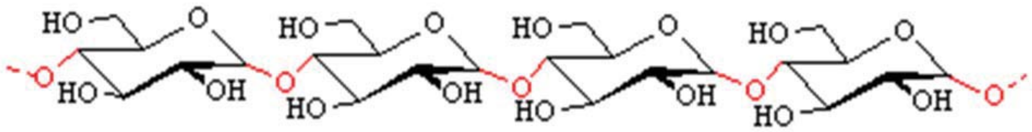
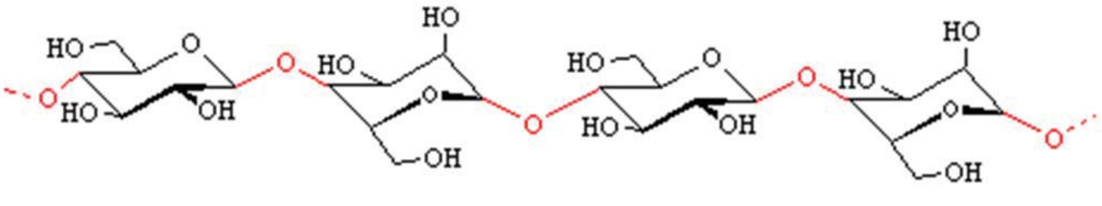
c. Polysaccharides (cont’d)

Starch	20% amylose 80% amylopectin

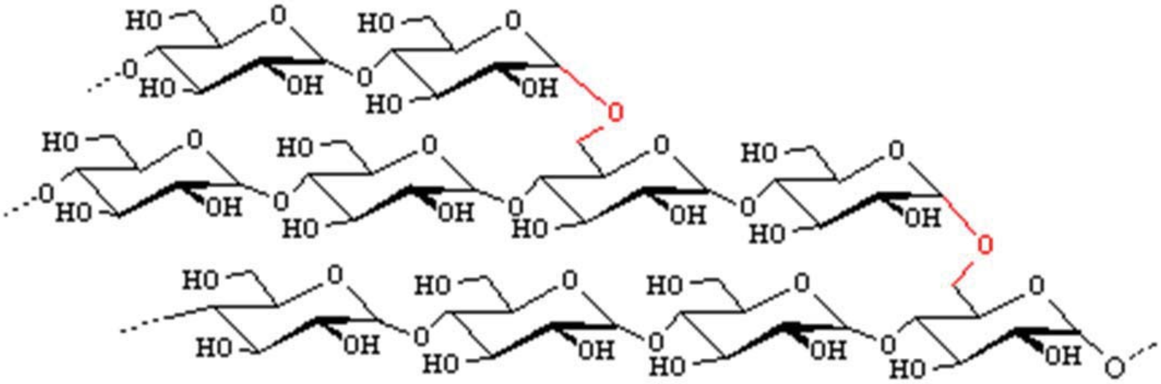
Cellulose is more difficult to decompose in nature than amylose and amylopectin because of *interchain* hydrogen bond between C3 hydroxyl and pyranose oxygen leading to crystalline structure.



amylose



cellulose



amylopectin

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c. Polysaccharides (cont'd)

Production of Sweeteners

Corn Starch → Corn Syrup

partial hydrolysis

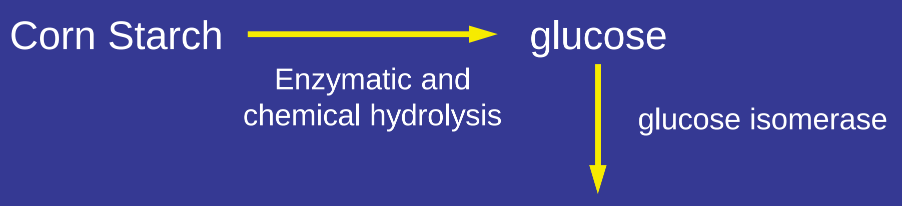
glucose, maltose, etc.

Relative Sweetness

glucose	0.7
sucrose	1.0
fructose	2.0

How to obtain more fructose?

c. Polysaccharides (cont’d)



Corn Starch → glucose

Enzymatic and chemical hydrolysis

glucose isomerase

glucose/fructose

“high fructose corn syrup”

annual U.S. consumption 50 - 70 kg/person

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5. DNA

- long threadlike molecule which carries genetic information in its sequence of purine and pyrimidine bases
  - transcription: DNA template to messenger RNA
  - codon on mRNA interacts with anticodon on transfer RNA
  - translation: RNA code converted to amino acid sequence

5. DNA (cont’d)

- plasmids are nonchromosomal self-replicating segments of DNA floating around in cytoplasm
  - plasmids are a means for cells to transfer DNA quickly between cells and between species (e.g., give resistance to cells against antibiotics).
  - plasmids can be inserted into cells to get them to overproduce a desired protein.
  - copy number is the number of desired plasmids in a cell.

C. Cell Nutritional Requirements

1. Energy

- phototrophs utilize light energy
- chemotrophs utilize chemical energy
  - lithotrophs utilize inorganic chemicals
  - organotrophs utilize organic chemicals

2. Carbon

- autotrophs utilize inorganic carbon (CO_2)
- heterotrophs utilize organic carbon

Industrial fermentations use cheap carbon sources such as molasses, malt extract, corn steep liquor, sulfite waste liquor

“complex” media

Nature of carbon source has great impact on growth of cells:

Example (with *E. coli*)

J. Bacteriol. (1971) 105:20+

	Doubling time	
complex media	35 min	
glucose + salts	50 min	“defined” media
acetate + salts	80 min	
succinate + salts	300 min	

Advantages of using complex media instead of defined media:

1) Cheap
2) Readily available
3) Best cell growth
4) Have all nutritional requirements

Disadvantages of using complex media instead of defined media:

1) Considerable variation in media composition
2) Processes less reliable and reproducible
3) Complicates separation and purification

3. Nitrogen

12% of bacterial dry mass
7.5% of yeast dry mass

- complex media
  - yeast extract
    (Baker's yeast lysed at 55ºC)
  - peptone (protein hydrolysate)
    meat, cottonseeds, soy
- defined media
  - ammonia
  - ammonium salts
  - urea

Prices for Defined Nitrogen Sources (Dec 2002)

Substance	%N	$ per lb	$ per lb N	
NH_3	0.823	0.073	0.088	
NH_4Cl	0.262	0.21	0.802	
$(NH_4)_2SO_4$	0.212	0.053	0.250	
$NH_4H_2PO_4$	0.122	0.082	0.672	1)
$(NH_4)_2HPO_4$	0.212	0.062	0.292	2)
NH_4NO_3	0.350	0.069	0.197	
urea	0.46	0.054	0.117	

1) 27% P - $0.30/lb P
2) 24% P - $0.26/lb P

Are materials pure enough for biological processes?

4. Phosphorus

3% of bacterial dry mass
1.5% of yeast dry mass

- phosphate salts added if more P is necessary
  - 25% as much P is needed as N
  - 180% as much P is supplied as N
- in some fermentations, P can inhibit production of desired product

5. Sulfur

1% of bacterial and yeast dry mass

- sulfate salts added if more S is necessary
  - 10% as much S is needed as N
  - 60% as much S is supplied as N

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6. Micronutrients

a) trace elements:
K^+, Mg^{++}, Ca^{++}, Fe^{++}, Mn^{++}, Co^{++}, etc.

- supplied as necessary (in excess?) by addition of salts
- very important in regulating enzymes (both presence and absence)
- carefully controlled (example: citric acid fermentation)

b) vitamins

- expensive

Elemental Composition of *E. coli*

(mg / g dry cell weight)

Element		growth		osmol,	
		early	late	low	high
C	---	393	---	---	---
N	---	140	---	---	---
P	---	41	55	---	---
S	---	10	13	---	---
Mg	2.8	6.4	8.7	---	---
K	12	41	22	18	40
Na	---	23	53	3.9	3.2
ref	(1)	(2)		(3)	

Elemental Composition of *E. coli*

(mg / g dry cell weight)

Element		growth	
		early	late
Ca	2.3	2.1	0.5
Cl	---	23	25
Zn	0.15	41	55
ref	(1)	(2)	

(1) Kung et al. *J. Bacteriol.* 1976, 126(3):1089

(2) Heldal et al. *Appl. Environ. Microbiol.* 1985 50(5):1251

(3) Epstein & Schultz *J. Gen. Physiol.* 1965 49:221

Comments

- composition of cells is very flexible…cells have ability to adapt to environment.
- composition of medium can strongly impact composition of cells, which in turn can profoundly impact cellular metabolism (and product formation).
- ~20-30% of carbon consumed by an organoheterotroph ends up as cell material. Thus, 3-5 times as much carbon needs to be added as calculated merely from cell composition.