

## CHAPTER 2

### AN OVERVIEW OF BIOLOGICAL BASICS – ENGINEER’S PERSPECTIVE

- I. 2.1 ARE ALL CELLS THE SAME?
- a. 2.2.1 - Microbial Diversity (p. 11-12)
- i. Temperature – psychrophiles (<20 C), mesophiles (20-50 C), thermophiles (>50 C)
  - ii. Oxygen (aerobic), anaerobic (obligate), facultative
  - iii. Nutrients – cyanobacteria are photosynthetic and also nitrogen fixers (convert N<sub>2</sub> into NH<sub>3</sub>)
  - iv. Shapes – spherical/elliptical (coccus, cocci), cylindrical/rod (bacillus, bacilli), spiral (spirillum, spirilla).
- b. 2.1.2 - Naming Cells (p. 12-14)
- i. Genus species strains substrains, e.g., Eschericia coli K12
    1. genus - group of related species
    2. species – organisms that are substantially alike
    3. strains/substrain – variation within species
  - ii. Two primary cell types – (1) prokaryotic and (2) eucaryotic
    1. prokaryotes – (Table 2.1, 2,2)–no nuclear membrane
      - a. eubacteria and archaeobacteria (Table2.2)
    2. eucaryotes – (Table 2.1, 2,2)–nuclear membrane, organelles
- c. 2.1.3 – Viruses (p. 14)
- i. Need host cell to be functionally active, are NOT free-living
  - ii. Size – 30 to 200 nm (nano-meters)
  - iii. DNA or RNA covered by a capsid (protein coat)
  - iv. Lytic cycle – host cells lyse to release phages
  - v. Lysogenic cycle - phase DNA is incorporated into the host DNA and the host continues to multiply in this state
  - vi. Phages - role in bioprocess technology
    1. phage attack on E. coli fermentation to make a recombinant protein product can be destructive
    2. phages can be used as agents to move genetic material into E. coli.
    3. Killed phage preparation has been used as a vaccine
    4. Production of virus-like particles that are empty shells (capsid) used a vaccines
    5. **Gene therapy** – replace virus genetic material with a desired gene; capsid acts as a Trojan Horse to protect the gene and to deliver it selectively to a cell type. In this case, the virus is a biotechnology tool.
- f. 2.1.4- Procaryotes (p.15)–0.5 to 3 microns (um) in radius, rapid growth (hrs)
1. Eubacteria- gram stain (Gm+ [B. subtilis], Gm– [E. coli])
    - a. Gm + better suited to excretion of proteins
    - b. Figure 2.2 (p. 16) Gm – bacterium with outer membrane

- c. Mycoplasma – (Not Gm + or Gm –), clinically important (primary atypical pneumonia) and common contamination of media used industrially for animal cell culture.
- d. Acinomyces (long and branched hyphae) –
- e. Antibiotics source
- f. Amylolytic and cellulolytic enzymes (some) for enzymatic hydrolysis of starch and cellulose (e.g., Actinomyces, Thermomonospora, and Streptomyces)
- g. Table 2.3 (p. 18) – Bacterial architecture
- 2. Archaeobacteria (p.17) – differ at the molecular level.
  - a. No peptidoglycan, different lipid composition of cytoplasmic membrane.
  - b. Methanogens, thermoacidophiles, halobacteria
  - c. Sources of active enzymes with novel properties.
- 3. Eucaryotes (p. 19) – yeast 5u, animal 10u, plant 20u
  - a. Figure 2.3 (p. 19) – Animal and Plant cells
  - b. Sterols in cytoplasmic membrane strengthen the structure and make membrane less flexible
  - c. Animal cells do NOT have cell wall, only cytoplasmic membrane → animal cells are shear sensitive, fragile, and **complicates the design of large-scale bioreactors for animal cells**
  - d. Reproduction and components – p. 20-22
  - e. Fungi - (1) yeasts (Fig. 2.6), (2) molds (Fig. 2.7)
    - i. Yeasts – Saccharomyces cerevisiae (anaerobic alcohol formation and aerobic baker's yeast production)
    - ii. Molds – **antibiotics**, citric acid (Aspergillus niger), make up a **large fraction of the fermentation industry** - submerged culture forms pellets (50 u to 1 mm) can **cause nutrient transfer problems (oxygen), but pellet formation reduced broth viscosity which can improve bulk oxygen transfer.**
    - iii. 4 classes: (1) phyco, (2) asco, (3) basidio, and (4) deuteromycetes (Trichophyton – **athlete's foot**)
  - f. Algae (p. 24) – diatoms (filter aids), Chlorella (wastewater treatment with single-cell protein production), gelling agents such as agar and alginic acid.

- II. 2.2 – CELL CONSTRUCTION (p. 25)
- a. 2.2.1 - Introduction – living cell structural elements include: proteins, nucleic acids, polysaccharides, lipids, and the storage materials including fats, polyhydroxybutyrate, and glycogen.
  - b. Biological system – levels of understanding:
    - i. Molecular (molecular biology, biochemistry)
    - ii. Cellular (cell biology, microbiology)
    - iii. Population (microbiology, ecology)
    - iv. Production (bioprocess engineering)
  - c. 2.2.2 – Amino Acids and Proteins (p. 26)
    - i.  $\alpha$ -Amino Acids – monomers of proteins, form peptide bonds
      1. Table 2.4 (p. 29) Amino acid structure
      2. Isoelectric point – protein purification processes
    - ii. **Proteins –**
      1. **5 categories**
        - a. structural: glycoproteins, collagen, keratin
        - b. catalytic: enzymes (> 2,000 known)
        - c. transport: hemoglobin, serum albumin
        - d. regulatory: hormones (insulin, growth hormones)
        - e. protective: antibodies, thrombin
      2. 3-D structures described at 4-levels of structure (p. 29)
        - a. primary–linear sequence of amino acids (1-d length)
        - b. secondary-hydrogen bonding: (1) helixes, (2) sheets
        - c. tertiary-R-group interactions (covalent, disulfide, H)
        - d. quaternary-polypeptide chains interacting
      3. Antibodies or immunoglobulins (p. 31)
        - a. immune (Ab-Ag reactions), diagnostic kits, protein separation schemes, delivery of anticancer drugs
        - b. one of most important products of biotechnology**
        - c. abzymes – impart catalytic activity to antibodies.**  
Couple **protein engineering** to antibodies promises development of specific catalytic agents.
      4. Carbohydrates – 2.2.3 (p. 34)
        - a. Modulate some chemical signaling-animals/plants
        - b. Monosaccharides: 3-9 C-atoms. Tale 2.5 (p. 35)
          - i. D-ribose, deoxyribose in RNA, DNA
          - ii. glucose, sucrose (plant sugar), lactose (milk and whey)
        - c. polysaccharides:  $\geq 2$  monosaccharides (p. 37)
          - i. biochemical engg-> polysaccharide industry (dextrins – branched sections of amylopectin are used as thickeners)
          - ii. cellulose = chain of D-glucose monomers linked by  $\beta$ -1,4 glycosidic bonds. Resistant to enzymatic hydrolysis. Interest in

converting cellulose wastes into fuels or chemicals.

5. Lipids, Fats, and Steroids – 2.2.2 (p. 38)
  - a. Lipids – present in nonaqueous biological phases (plasma membranes); fatty acid composition
    - i. Fats – lipids that serve as biological fuel storage
    - ii. Phospholipids – key membrane component
    - iii. Polyhydroxyalkanoates (PHA), e.g., polyhydroxybutyrate (PHB), a storage product in some cells. (p. 40)
    - iv. Steroids - hormones ( $10^{-8}\text{M}$ ), cholesterol, cortisone, estrogen, progesterone
      1. Commercial production depends on microbial conversion, because the large number of asymmetric centers makes total synthesis difficult (p.40)
6. Nucleic Acids, RNA, DNA – 2.2.5 (p. 40)
  - a. Nucleic Acids – reproduction of living cells
  - b. DNA – stores & preserves genetic information
  - c. RNA – central role in protein synthesis
    - i. m-RNA (p. 46), short half-life
    - ii. t-RNA, stable
    - iii. r-RNA, major component of ribosomes
  - d. Nucleotides –
    - i. Building blocks of DNA and RNA
    - ii. Store Energy – ATP, ADP
    - iii. Reducing power - NAD, NADP
  - e. Plasmids – circular DNA in cytoplasm, nonchromosomal, autonomous, self-replicating.
  - f. Easily moved in and out of cells, and used for genetic engineering**

### III. 2.3 - CELL NUTRIENTS (p. 46)

- a. Introduction – 2.3.1 (p. 46)
  - i. Cell composition different from its environment (due to semipermeable membrane and energy expenditure to keep it away from thermodynamic equilibrium.
  - ii. Table 2.7 (p. 48) – 80% water
  - iii. Macronutrients – required at  $>10^{-4}\text{M}$  (C,N,O,H,S,P, Mg, K)
  - iv. Micronutrients – required at  $< 10^{-4}\text{M}$  (Mo, Zn, Cu, Mn, Ca, Na, vitamins, growth hormones, metabolic precursors)
- b. Macronutrients – 2.3.2 (p. 49), see Tables 2.8 (p. 49) and 2.9 (p.50)
  - i. Carbon Source – (1) Heterotrophs use organic compounds ; (2) Autotrophs use  $\text{CO}_2$ . See Table 2.8 (p. 49)

1. Most common for industrial fermentations – molasses (sucrose), starch (glucose, dextrin), corn syrup, and waste sulfite liquor (glucose). (methanol, ethanol, and methane are also cheap carbon sources for some fermentations)
- ii. Energy Sources – (1) Chemo (reduced inorganic chemicals), (2) photo (light energy)
- c. Micronutrients – 2.3.3 (p. 50)
  - i. Iron – plays a regulatory role in some fermentation processes
    1. deficiency required for excretion of riboflavin by Ashbya gossypii
    2. conc. Regulates penicillin production by P. chrysogenum
    3. Mn – enzyme co-factor, plays role in regulation of secondary metabolism and excretion of primary metabolites
  - ii. Copper – in certain respiratory-chain components & enzymes
    1. deficiency stimulates penicillin and citric acid production
  - iii. Cobalt – vitamin B<sub>12</sub>. Required in propionic bacteria & methanogens
  - iv. Mo- nitrate reductase and nitrogenase & for growth on NO<sub>3</sub> and N<sub>2</sub> as sole source of nitrogen
  - v. Ca – co-factor for amylases and some proteases
  - vi. Vitamins – required at concs. of 10<sup>-6</sup> to 10<sup>-12</sup> M
- d. Growth Media – 2.3.4 (p. 52) See Table 2.10 (p. 52)
  - i. Defined media - specific amounts of pure chemical compounds with known chemical compositions.
  - ii. Complex media – contain natural compounds whose chemical composition is not exactly known (e.g., yeast extract, peptone, molasses, or corn steep liquor). Usually provides growth factors, vitamins, hormones, and trace elements often resulting in higher cell yields.