I. Microbial Nutrition (Chapter 5)

A. Common nutrient requirements

1. **nutrients** = substances required for microbial growth
   a. biosynthesis
   b. energy production

2. analysis of cells shows that 95% of dry weight is made up of carbon, oxygen, hydrogen, nitrogen, sulfur, phosphorus, potassium, calcium, magnesium, and iron
   a. termed the **macroelements** or **macronutrients**
   b. COHNSP are components of carbohydrates, lipids, proteins, and nucleic acids
   c. cations have several roles, many of them enzyme related

3. **micronutrients** = **microelements** = **trace elements**
   a. manganese, zinc, cobalt, molybdenum, nickel, copper
   b. small amounts required (contaminants in water, glassware, media components often enough for growth)
   c. usually part of enzymes and cofactors

B. Requirements for carbon, hydrogen, and oxygen

1. usually all are provided by the same compound
2. in many cases the carbon source is also the energy source (needs to have electrons, *i.e.* hydrogens, that can be rearranged or transferred)

3. **autotrophs** = organisms that can use inorganic carbon (CO\(_2\)) as sole carbon source
   a. often use light for energy
   b. some use inorganic molecules as the energy source
   c. primary producers are autotrophs

4. **heterotrophs** = organisms that use preformed organic molecules as carbon source

5. microorganisms possess tremendous diversity in the types of organic molecules that can be degraded

C. Nutritional types of microorganisms

1. organisms can be classified on the basis of their sources of carbon, energy, and electrons (Table 5.1)
2. **phototrophs** use light energy
3. **chemotrophs** use chemical energy
   a. **lithotrophs** use inorganic molecules
b. **organotrophs** use organic molecules

4. Table 5.2 puts microbes into four nutritional classes

D. Requirements for nitrogen, phosphorus, and sulfur

1. sources may be organic or inorganic molecules

2. specific organisms have different capabilities for assimilation and may favor particular molecules

3. N primarily in amino acids and nucleotides

4. P primarily in nucleotides and phospholipids

5. S primarily in amino acids

E. growth factors

1. organic compounds that are essential cell components that cannot be synthesized by the cell

2. three major classes
   a. amino acids (required for protein synthesis)
   b. purines and pyrimidines (required for nucleic acid synthesis)
   c. vitamins
      (1) small organic molecules
      (2) all or part of enzyme cofactors

F. nutrient uptake

1. membrane-bound transport mechanisms required for uptake of nutrients by microbial cell
   a. mechanism must be specific (no use to transport unusable compound)
   b. membrane is selectively permeable
   c. transport of dilute solutes against a gradient

2. **passive diffusion** = molecules move from a region of higher concentration to one of lower concentration as a result of random thermal agitation
   a. rate is dependent on size of concentration gradient
   b. requires relatively high external concentration
   c. inefficient, not extensively employed

3. **facilitated diffusion** = diffusion process aided by a carrier
   a. **permeases** embedded in membrane function as carrier proteins
   b. more efficient than passive diffusion
      (1) more rapid at lower concentrations
      (2) saturatable (levels off, compared to linear increase with passive diffusion)
(3) no energy requirement, still gradient driven (still diffusion)
c. permeases may function by changing conformation, allowing buildup of internal concentration
  (1) allows lipid-insoluble molecules to enter cell
  (2) can work in reverse if internal concentration is high enough
d. does not appear to be very important in procaryotes (used with glycerol); larger role in eucaryotes (for sugar and amino acids)

4. **active transport** = energy-linked transport of solute molecules
   a. works against a concentration gradient
   b. employs carrier proteins
      (1) high specificity
      (2) saturatable
   c. can use ATP, other high energy phosphate compounds, or proton motive force
d. many compounds have multiple transport systems (may have selective advantage in different environments)
e. typically used with sugars and amino acids
f. **ATP-binding cassette transporters** (ABC transporters) = proteins carriers that span the membrane and require energy (usually ATP) for activity
   (1) specific for molecule to be transported
   (2) located in the periplasm of gram negative cells or on the outside of the cell membrane of gram positive cells
   (3) probably involved in chemotaxis
g. active transport can also be powered by a proton or sodium gradient
   (1) **symport** = linked transport of two substances in the same direction (H\(^+\) - linked uptake of amino and organic acids)
   (2) **antiport** = linked transport of two substances in opposite directions (Na\(^+\)/H\(^+\))
   (3) occurs in G- and G+

5. **group translocation** = molecule is transported and chemically altered at the same time
   a. phophenolpyruvate:sugar phosphotransferase system (PTS) best studied
      (1) transports sugars into procaryotes
      (2) phosphorylates sugar using phosphoenol pyruvate (PEP) as phosphate donor
   b. systems are fairly complex, with cytoplasmic and membrane enzymes involved
c. present in facultative anaerobes and anaerobes (rare in aerobes, except for some *Bacillus* species)

6. **siderophores** = low molecular weight molecules that complex with ferric iron
   a. hydroxamates or phenolates-catecholates
   b. complex binds to cell surface receptor protein and iron or complex is transported inside
   c. iron reduced to ferrous form inside cell

G. media composition (enrichment, pure culture)

1. **culture medium** = nutrient mixture prepared for the growth of microorganisms
   a. **culture** = microbes that grow in or on a culture medium
   b. many different types of media, depending on the organism of interest or the application
      (1) no single medium to grow all organisms
      (2) some organisms have specific requirements
      (3) estimated only 1-10% of bacteria are culturable
      (4) different media used to test anti-microbials, water qualities, vitamin concentrations, etc.
      (5) enrichment media

2. **defined medium** = **synthetic medium** = a medium in which all components are known
   a. allows accurate assessment of nutrient utilization
   b. can limit contamination
   c. "clean" medium often useful in physiology/ecology studies

3. **complex medium** = medium that contains some ingredients of unknown chemical composition
   a. extracts from meat, yeasts, plants, soil, water, rumen, etc.
   b. digests of proteins
   c. very rich
   d. good for growth of a wide variety of bacteria

4. **solid medium**
   a. useful for isolation, enumeration
   b. **agar** (gelling agent made from seaweed derivative) most common agent
      (1) generally non-bacterially degraded
      (2) solid below 100°C
      (3) suggested for use by Fanny Angelina Hesse
c. gelatin can be used but is degraded by many bacteria and liquifies above 28° C
d. gel-rite, a gellan gum, also in use

5. **general purpose media** support a wide range of organisms

6. **selective media** favor the growth of particular organisms, often inhibiting the growth of others
   a. addition of basic fuschin or crystal violet inhibits G+  
   b. used to **enrich** for bacteria with specific traits

7. **differential media** distinguish between different groups based on identifiable reactions

8. selective and differential media can be combined
   a. MacConkey agar contains bile salts and crystal violet that inhibit G+, and lactose identify lactose fermentors
   b. Mannitol salts agar contains 7.5% NaCl to select for *Staphylococcus* and a pH indicator to differentiate between mannitol fermentors and non-fermentors

II. Growth (Chapter 6)

A. General growth

1. **growth** = increase in cell number or cell mass (usually refers to number in case of bacteria)

2. most bacteria reproduce by **binary fission**
   a. cell numbers increase by powers of 2
   b. **generation time** = amount of time required for population to double (reproduce)
   c. Let \( N_o \) = initial cell number
      \( N_t = \text{cell number at time } t \)
      \( n = \text{number of generations in time } t \)
      \( N_t = N_o \times 2^n \)
      \( \log N_t = \log N_o + n(\log 2) \)
      \( n = (\log N_t - \log N_o)/\log 2 = (\log N_t - \log N_o)/0.301 \)
   d. cell numbers increase logarithmically, so numbers are usually plotted on a semi-log scale to give a straight line

B. **bacterial growth curve** = plot of cell growth over time, usually in a **batch culture** or closed system

1. **lag phase** = period of little or no cell division
   a. "gearing up" phase
   b. intense metabolic activity, particularly DNA and enzyme synthesis
2. **log or exponential phase** = period of most rapid growth, where cells are in continual state of cell division
   a. generation time reaches a constant minimum based on genetic potential and medium limitations
   b. cells most active metabolically
   c. microorganisms particularly sensitive to adverse conditions
   d. single bacterium weighing $9.5 \times 10^{-13}$ g with doubling time of 20 minutes could, theoretically, result in 80,000 tons of cell material

3. **stationary phase** = period of equilibrium between growth and death of cells
   a. depletion of nutrients and accumulation of waste products
   b. changes in pH

4. **death phase** = period when more cells are dying than dividing
   a. **logarithmic decline phase** (constant proportion dies with time)
   b. population can diminish or become extinct

C. measurement of growth

1. growth can be measured in terms of cell numbers, cell components (e.g., protein or DNA concentration), or cell mass
2. direct, viable counts
   a. can take some time for results
   b. **pour plates** and **spread plates**
      (1) assumes that inoculum is homogeneous and cells are not clumped
      (2) culture is usually serially diluted to provide inoculum with 30 - 300 cells
3. direct, total counts
   a. counting chamber
   b. counts viable and nonviable cells
4. indirect methods
   a. turbidity
      (1) absorbance is proportional to cell density
      (2) requires cell densities above $10^6$ cells/ml
      (3) can be standardized with viable counts or total counts
   b. most probable number (MPN)
      (1) statistical estimation based on number of cultures showing growth in serially diluted
series
(2) useful with cells that won't grow on solid media
c. metabolic activity
   (1) accumulation of product
   (2) depletion of substrate
   (3) useful for ecological studies
d. dry weight
   (1) can be nearly direct, in the case of unicellular organisms (standardized with direct method)
   (2) especially useful for filamentous fungi
   (3) can be extended to cellular components
      (a) protein
      (b) DNA
      (c) membrane lipids
   (4) cell yields can be determined for a limiting nutrient
      \[ Y = \frac{\text{mass of cells}}{\text{mass of substrate consumed}} \]
      (a) basis of microbial assays for vitamins and other growth factors
      (b) can be extended to \( Y_{\text{ATP}} \) if pathways known

D. continuous culture
1. continuous culture = open system (continual introduction of nutrients and removal of wastes)
   a. bacteria maintained in exponential phase
   b. growth rate dependent on dilution rate (rate of introduction of fresh medium)
   c. cell density dependent on concentration of limiting nutrient
   d. if dilution rate is greater than the growth rate, cells are washed out
2. more similar to natural systems than batch culture
3. many systems are combinations of batch and continuous culture
   a. semi-continuous
   b. fed-batch

E. Influence of environmental factors (role of physical parameters on bacterial growth) (Table 6.3)
1. water activity
   a. describes the availability of water to the organism
   b. \( a_w \) = ratio of vapor pressure of air over a substance or solution divided by vapor pressure
of water at the same temperature
c. high water activity = dilute environment
d. low water activity inhibits bacterial growth
   (1) high solute concentration
   (2) method of preservation
e. **halotolerant** = able to survive with salt, but less than optimal growth
f. **halophiles** = require salt for growth
g. **osmophiles** = live in environments with low water activity (could be due to non-salt solutes)
h. **xerophiles** = live in dry environments

2. **pH**
a. each organism has a pH range where growth is possible, along with an optimum pH
b. most life occurs around neutrality, but ranges from 2 - 10
c. **acidophiles** live at low pH
d. **alkalinophiles** live at high pH
e. **neutraphiles** live around pH 7
f. most cells maintain internal pH around 7
   (1) K⁺/H⁺ or Na⁺/H⁺ antiporters
   (2) H⁺ ATPase
   (3) heat-shock and chaperone proteins are synthesized under severe conditions
g. buffers are used to maintain pH
   (1) maintain a constant [H⁺]
   (2) composed of a weak acid and its conjugate base
      (a) added protons react with salt to give weak acid
      (b) added hydroxyl ions are neutralized by weak acid donating protons

3. **temperature**
a. every organism has a minimum temperature, a maximum temperature, and an optimum temperature which comprise the **cardinal temperatures**
b. **psychrophile** opt. ≤ 15° C
   (1) oceans (avg temp 5° C)
   (2) organisms growing under ice in polar regions (snow alga)
   (3) membranes rich in unsaturated fatty acids
c. **mesophile** opt about 35°C
   (1) best studied group
   (2) corresponds to pathogens

d. **thermophile** opt about 60°C
   (1) hot springs
   (2) water heaters
   (3) compost heaps and digestors
   (4) membranes rich in saturated fatty acids
   (5) changes in enzyme structures

e. **extreme thermophile** opt ≥ 85°C
   (1) thermal vents
   (2) steam vents

4. oxygen concentration
   a. bacteria vary in their need for and tolerance
   b. **obligate anaerobes** are killed by oxygen
   c. **aerotolerant anaerobes** can grow in the presence of oxygen but cannot use it as an electron acceptor
   d. **facultative anaerobes** can use oxygen, but can also grow without it
   e. **microaerophilic** require oxygen, but at lower levels than in air (2 - 10%)
   f. **aerobes** require oxygen for growth
   g. toxic forms of oxygen
      (1) superoxide radical, \( \text{O}_2^- \)
         (a) occurs during reduction of \( \text{O}_2 \) to \( \text{H}_2\text{O} \)
         (b) highly reactive
         (c) can cause oxidative destruction of lipids and other biochemical components
      (2) peroxide, \( \text{O}_2^{2-} \)
         (a) formed during respiratory processes
         (b) often used as a disinfectant
      (3) hydroxyl free radical, \( \text{OH} \)
         (a) formed by ionizing radiation
         (b) formed by reaction of superoxide anion with peroxide
   h. several enzymes to protect against oxygen toxicity
(1) superoxide dismutase
   (a) \( \text{O}_2^- + \text{O}_2^- \rightarrow \text{H}_2\text{O}_2 + \text{O}_2 \)
   (b) present in aerobes, facultative anaerobes, and aerotolerant anaerobes

(2) catalase
   (a) \( \text{H}_2\text{O}_2 + \text{H}_2\text{O}_2 \rightarrow 2\text{H}_2\text{O} + \text{O}_2 \)
   (b) present in aerobes and facultative anaerobes

(3) peroxidase
   (a) \( \text{H}_2\text{O}_2 + \text{NADH} + \text{H}^+ \rightarrow 2\text{H}_2\text{O} + \text{NAD}^+ \)
   (b) no oxygen produced

5. pressure
   a. most cells are **barotolerant**
   b. **barophilic** organisms grow more rapidly at high pressures
      (1) isolated from guts of deep-sea invertebrates
      (2) hard to study (cultivate)

6. radiation
   a. we are immersed in a constant background of electromagnetic radiation (emr), some of which can contain a lot of energy
      (1) often undetected by our senses
      (2) can be thought of as energy
   b. emr is absorbed by different cell components and can cause ionization (loss of electrons)
   c. emr is especially dangerous to nucleic acids
      (1) mutation, possible death
      (2) used as a sterilization method
   d. protection is through DNA repair mechanisms and or carotenoid pigments that can quench singlet oxygen (\(^1\text{O}_2\))

F. Growth limitation by environmental factors
   1. **Liebig’s law of the minimum** = total biomass of an organism will be determined by the nutrient present in the lowest concentration relative to the organism’s needs (limiting nutrient)
   2. **Shelford’s law of tolerance** = there are limits to environmental factors below and above
which a microorganism cannot survive and grow, regardless of the nutrient supply

3. growth conditions can influence cell morphology and metabolism

4. aerobes tend to be limited for carbon/energy; anaerobes tend to be limited for electron acceptors