**Chapter 6**

**Growth Requirements**

* Microbial growth – an increase in a population of microbes rather than an increase in size of an individual
* Result of microbial growth is discrete colony – an aggregation of cells arising from single parent cell
* Reproduction results in growth
* Organisms use a variety of nutrients for their energy needs and to build organic molecules and cellular structures
* Most common nutrients – those containing necessary elements such as carbon, oxygen, nitrogen, and hydrogen
* Microbes obtain nutrients from variety of sources

**Nutrients: Chemical and Energy Requirements**

* Sources of carbon, energy, and electrons
  + Two groups of organisms based on source of carbon
    - *Autotrophs* use an inorganic source of carbon (carbon dioxide)
    - *Heterotrophs* catabolize reduced organic molecules (proteins, carbohydrates, amino acids, and fatty acids)
  + Two groups of organisms based on use of chemicals or light as source of energy
    - *Chemotrophs* acquire energy from redox reactions involving inorganic and organic chemicals
    - *Phototrophs* use light as their energy source

**Growth Requirements**

* Nutrients: Chemical and Energy Requirements
  + Oxygen requirements
    - Oxygen is essential for obligate aerobes (final electron acceptor in ETC)
    - Oxygen is deadly for obligate anaerobes
    - How can this be true?
      * Neither gaseous O2 nor oxygen covalently bound in compounds is poisonous
      * Rather the forms of oxygen that are toxic are highly reactive and also excellent oxidizing agents
      * Resulting chain of oxidations causes irreparable damage to cells by oxidizing compounds such as proteins and lipids
    - Four toxic forms of oxygen

1. *Singlet oxygen* – molecular oxygen with electrons boosted to higher energy state

* + - * Occurs during photosynthesis so phototropic organisms have carotenoids that remove the excess energy of singlet oxygen

2. *Superoxide radicals* – some form during incomplete reduction of oxygen in aerobic and anaerobic respiration

* + - * So reactive that aerobes produce superoxide dismutases to detoxify them
      * Anaerobes lack superoxide dismutase and die as a result of oxidizing reactions of superoxide radicals formed in presence of oxygen

3. *Peroxide anion* – formed during reactions catalyzed by superoxide dismutase and other reactions

* + - * Aerobes contain either catalase or peroxidase to detoxify peroxide anion
      * Obligate anaerobes either lack both enzymes or have only a small amount of each

4. *Hydroxyl radical* – results from ionizing radiation and from incomplete reduction of hydrogen peroxide

* + - * The most reactive of the four toxic forms of oxygen
      * Not a threat to aerobes due to action of catalase and peroxidase
    - Aerobes – undergo aerobic respiration
    - Anaerobes – do not use aerobic metabolism
    - Facultative anaerobes – can maintain life via fermentation or anaerobic respiration or by aerobic respiration
    - Aerotolerant anaerobes – do not use aerobic metabolism but have some enzymes that detoxify oxygen’s poisonous forms
    - Microaerophiles – aerobes that require oxygen levels from 2 to 10% and have a limited ability to detoxify hydrogen peroxide and superoxide radicals
  + Nitrogen requirements
    - Anabolism often ceases due to insufficient nitrogen needed for proteins and nucleotides
    - Nitrogen acquired from organic and inorganic nutrients, plus all cells recycle nitrogen from amino acids and nucleotides
    - The reduction of nitrogen gas to ammonia (nitrogen fixation) by certain bacteria is essential to life on Earth because nitrogen is made available in a usable form
  + Other chemical requirements
    - Phosphorus is a component of phospholipid membranes, DNA, RNA, ATP, and some proteins
    - **Physical Requirements**
  + Temperature
  + Effect of temperature on proteins
  + Effect of temperature on lipid-containing membranes of cells and organelles
  + If too low, membranes become rigid and fragile
  + If too high, membranes become too fluid and cannot contain the cell or organelle
  + Sulfur is a component of sulfur-containing amino acids, disulfide bonds critical to tertiary structure of proteins, and in vitamins (thiamin and biotin)
  + Trace elements – only required in small amounts; usually found in sufficient quantities in tap water
  + Growth factors – necessary organic chemicals that cannot be synthesized by certain organisms (vitamins, certain amino acids, purines, pyrimidines, cholesterol, NADH, and heme)
* pH
  + Organisms sensitive to changes in acidity because H+ and OH- interfere with H bonding in proteins and nucleic acids
  + Neutrophiles are bacteria and protozoa that grow best in a narrow range around neutral pH (6.5-7.5)
  + Acidophiles are bacteria and fungi that grow best in acidic habitats
    - Acidic waste products can help preserve foods by preventing further microbial growth
  + Alkalinophiles live in alkaline soils and water up to pH 11.5
  + Physical effects of water
    - Hydrostatic pressure
      * Water exerts pressure in proportion to its depth

For every additional 10 m of depth, water pressure increases 1 atm

* + - * Organisms that live under extreme pressure are barophiles

Their membranes and enzymes depend on this pressure to maintain their three-dimensional, functional shape

**Associations and Biofilms**

* Organisms live in association with different species
  + Antagonistic relationships
  + Synergistic relationships
  + Symbiotic relationships
* Biofilms
  + Complex relationships among numerous microorganisms
  + Develop an extracellular matrix that adheres cells to one another, allows attachment to a substrate, sequesters nutrients, and may protect individuals in the biofilm
  + Form on surfaces often as a result of quorum sensing
  + Many microorganisms become more harmful when part of a biofilm

**Culturing Microorganisms**

* Inoculum introduced into medium (broth or solid)
  + Environmental specimens
  + Clinical specimens
  + Stored specimens
* Culture – refers to act of cultivating microorganisms or the microorganisms that are cultivated
* Obtaining Pure Cultures
  + Cultures composed of cells arising from a single progenitor
    - Progenitor is termed a CFU
  + Aseptic technique is used to prevent contamination of sterile substances or objects
  + Two common isolation techniques
    - Streak plates
    - Pour plates
* Culture Media
  + Majority of prokaryotes have never been grown in culture medium
  + Six types of general culture media
    - Defined media
    - Complex media
    - Selective media
    - Differential media
    - Anaerobic media
    - Transport media
* Special Culture Techniques
  + Techniques developed for culturing microorganisms
    - Animal and cell culture
    - Low-oxygen culture
    - Enrichment culture
* Preserving Cultures
  + Refrigeration – stores for short periods of time
  + Deep-freezing – stores for years
  + Lyophilization – stores for decades
* Generation Time
  + The time required for a bacterial cell (or population of cells) to grow and divide
  + Dependent on chemical and physical conditions
* Phases of Microbial Growth
  + Lag Phase
  + Log Phase
  + Stationary Phase
  + Death Phase
* Measuring Microbial Growth
  + Direct methods
    - Viable plate counts
    - Membrane filtration
    - Microscopic counts
    - Electronic counters
  + Indirect methods
    - Metabolic activity
    - Dry weight
    - Turbidity