**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