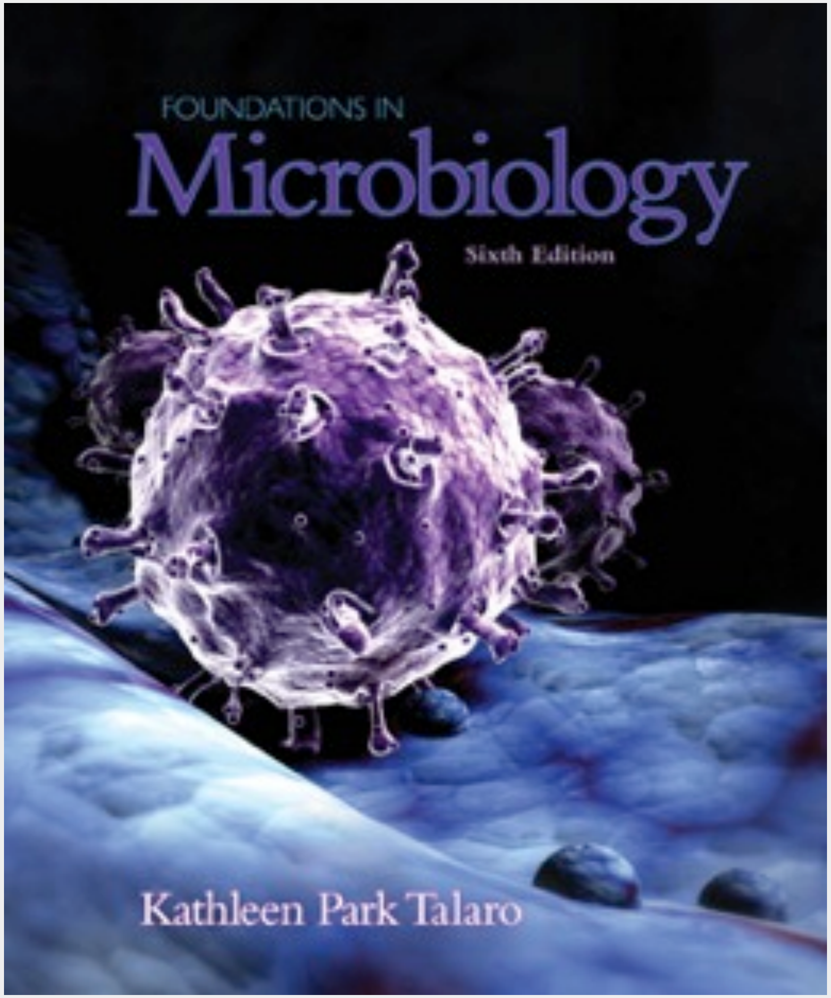
Talaro

Chapter 26

Environmental and Applied Microbiology



Home page: **http://fac.ksu.edu.sa/myalansari**

Ecology: The Interconnecting Web of Life

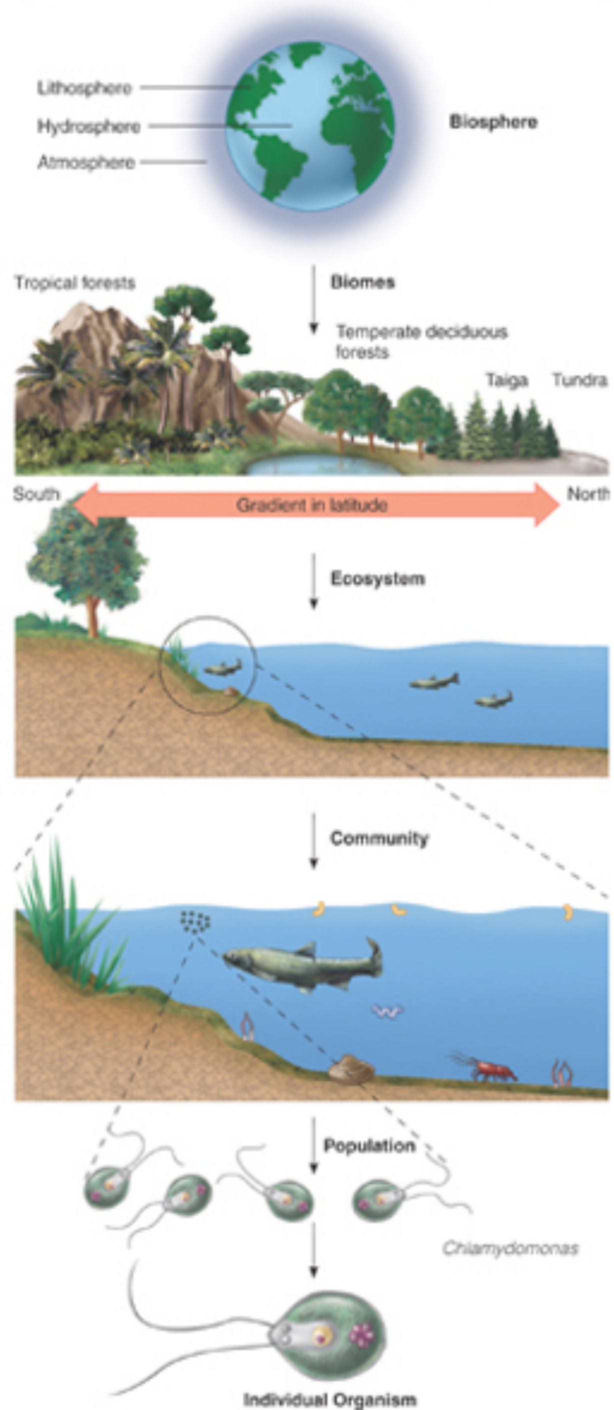
- **Environmental, or ecological, microbiology** – study of microbes in their natural habitats
- **Applied microbiology** – study of practical uses of microbes in food processing, industrial production, and biotechnology
- **Microbial ecology**- studies the interactions between microbes and their environments, involving living and nonliving components

Organization of Ecosystems

Biosphere – thick envelope of life that surrounds the earth's surface

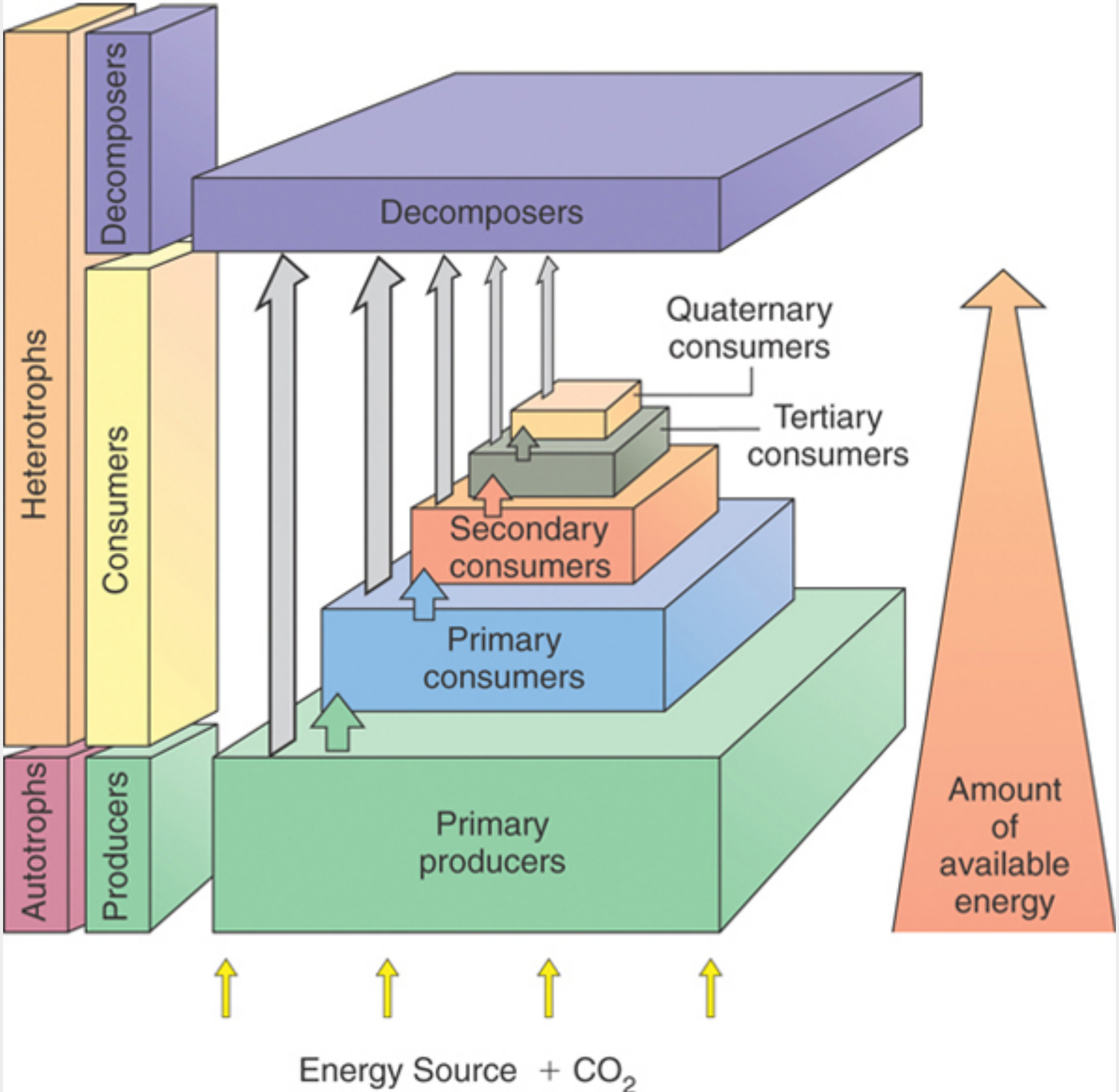
- Made up of:
  - hydrosphere (water)
  - lithosphere (soil)
  - atmosphere (air)
- Maintains and creates the conditions of temperature, light, gases, moisture, and minerals required for life processes
- **Biomes**- particular climatic regions

- **Communities** – the association of organisms that live together and that exhibit well-defined nutritional or behavioral interrelationships
- **Population** – organisms of the same species within a community
- **Habitat** – the physical location in the environment to which an organism has adapted
- **Niche** – overall role that a species, or population, serves in a community; nutritional intake, position in the community, and rate of population growth


Lithosphere
Hydrosphere
Atmosphere
Biosphere
Tropical forests
Biomes
Temperate deciduous forests
Taiga
Tundra
South
Gradient in latitude
North
Ecosystem
Community
Population
Chlamydomonas
Individual Organism

Energy and Nutritional Flow in Ecosystems

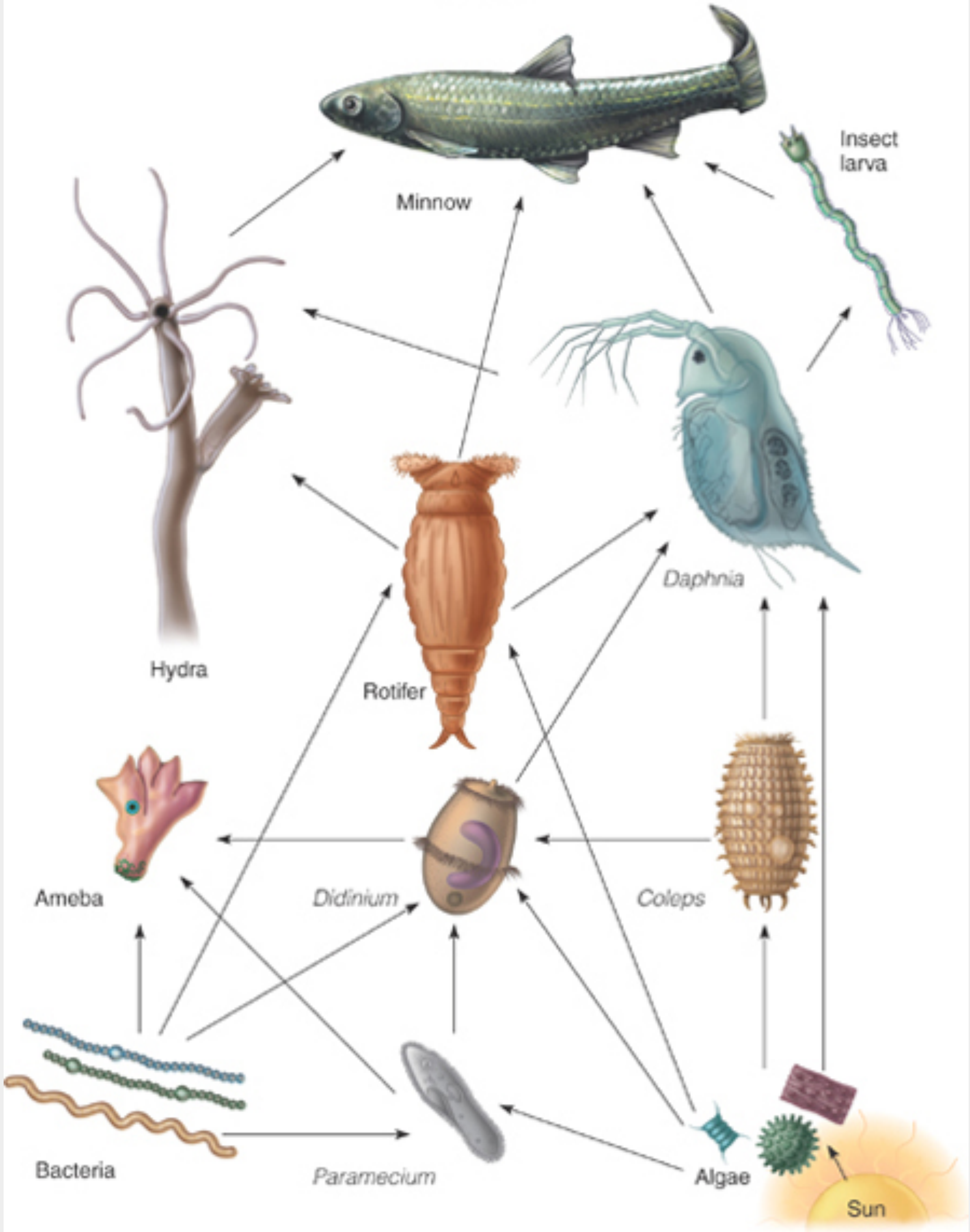
- Organisms derive nutrients and energy from their habitat.
- Food chain or energy pyramid summarizes the feeding levels:
  - **producers** – provide the fundamental energy source; only organisms that can produce organic compounds by assimilating inorganic carbon from the atmosphere; most are photosynthetic, also called autotrophs
  - **consumers** – feed on other living organisms and obtain energy from chemical bonds; primary, secondary, tertiary…
  - **decomposers** – primarily microbes, break down and absorb the organic matter of dead organisms; recycle organic matter into inorganic minerals and gases, mineralize nutrients



Heterotrophs
Decomposers
Consumers
Autotrophs
Producers
Decomposers
Quaternary consumers
Tertiary consumers
Secondary consumers
Primary consumers
Primary producers
Amount of available energy
Energy Source + CO_2

- Energy does not cycle.
- As energy is transferred to the next level, a large proportion of the energy will be lost that cannot be utilized in the system.
- Feeding relationships are represented by a food web which represents the actual nutritional structure of a community.

Food Web


Minnow
Insect larva
Hydra
Rotifer
Daphnia
Ameba
Didinium
Coleps
Bacteria
Paramecium
Algae
Sun

Ecological Interactions Between Organisms in a Community

- Dynamic interrelationships based on nutrition and shared habitat
- **Mutualism** –
- **Commensalism** –
  - **syntrophism** –
- **Synergism** –

- **Parasitism –**
- **Competition –**
- **Predator –**
- **Scavengers –**

The Natural Recycling of Bioelements

- Processes by which bioelements and essential building blocks of protoplasm are recycled between biotic and abiotic environments
- Essential elements are cycled through biological, geologic, and chemical mechanisms – **biogeochemical cycles.**
- Microorganisms remove elements from their inorganic reservoirs and convey them into the food web.

Atmospheric Cycles

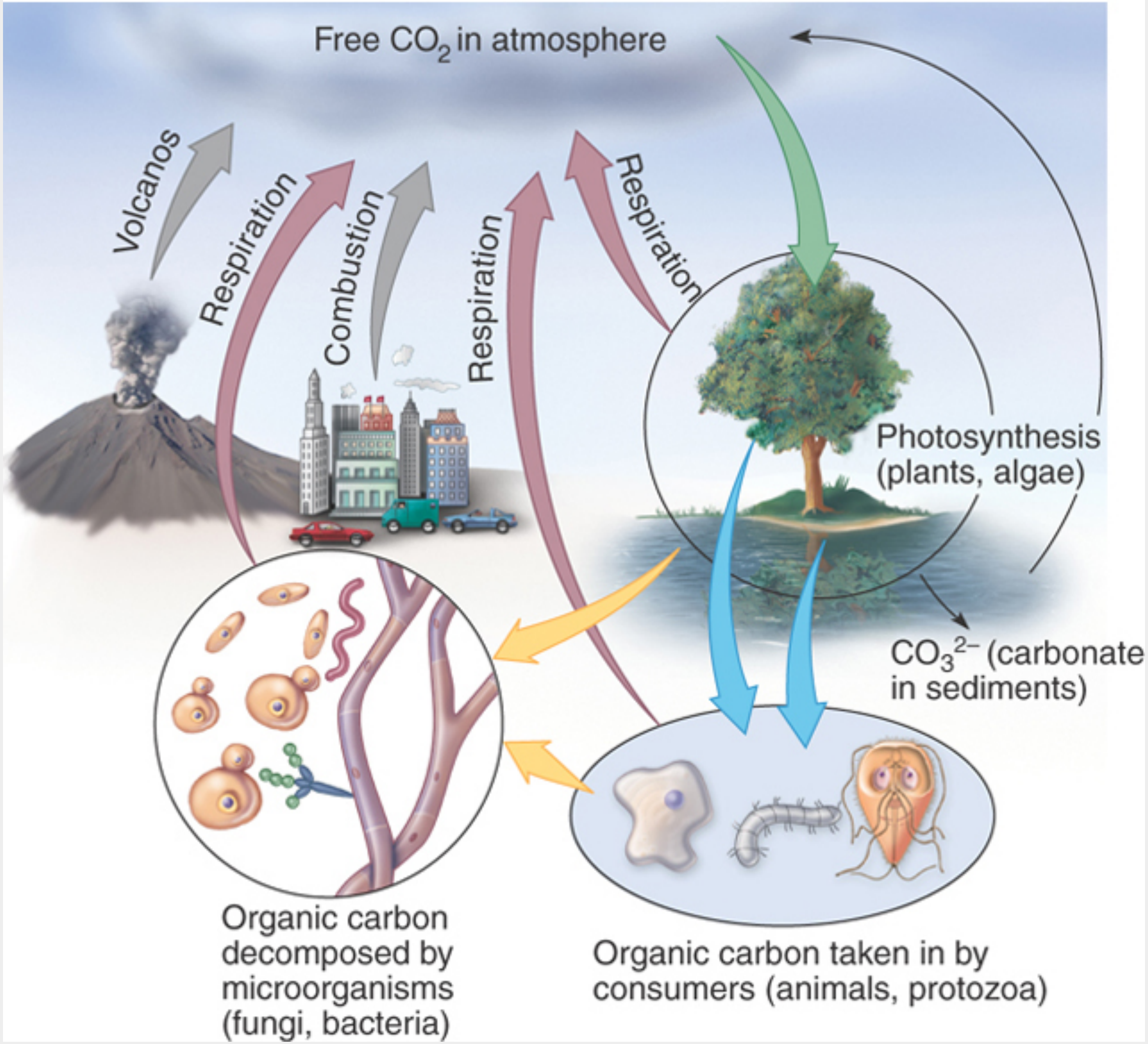
- Carbon cycle
- Photosynthesis
- Nitrogen cycle

The Carbon Cycle

- Key compounds in the carbon cycle include carbon dioxide, methane and carbonate.
- Carbon is recycled through ecosystems via photosynthesis, respiration, and fermentation of organic molecules, limestone decomposition, and methane production.

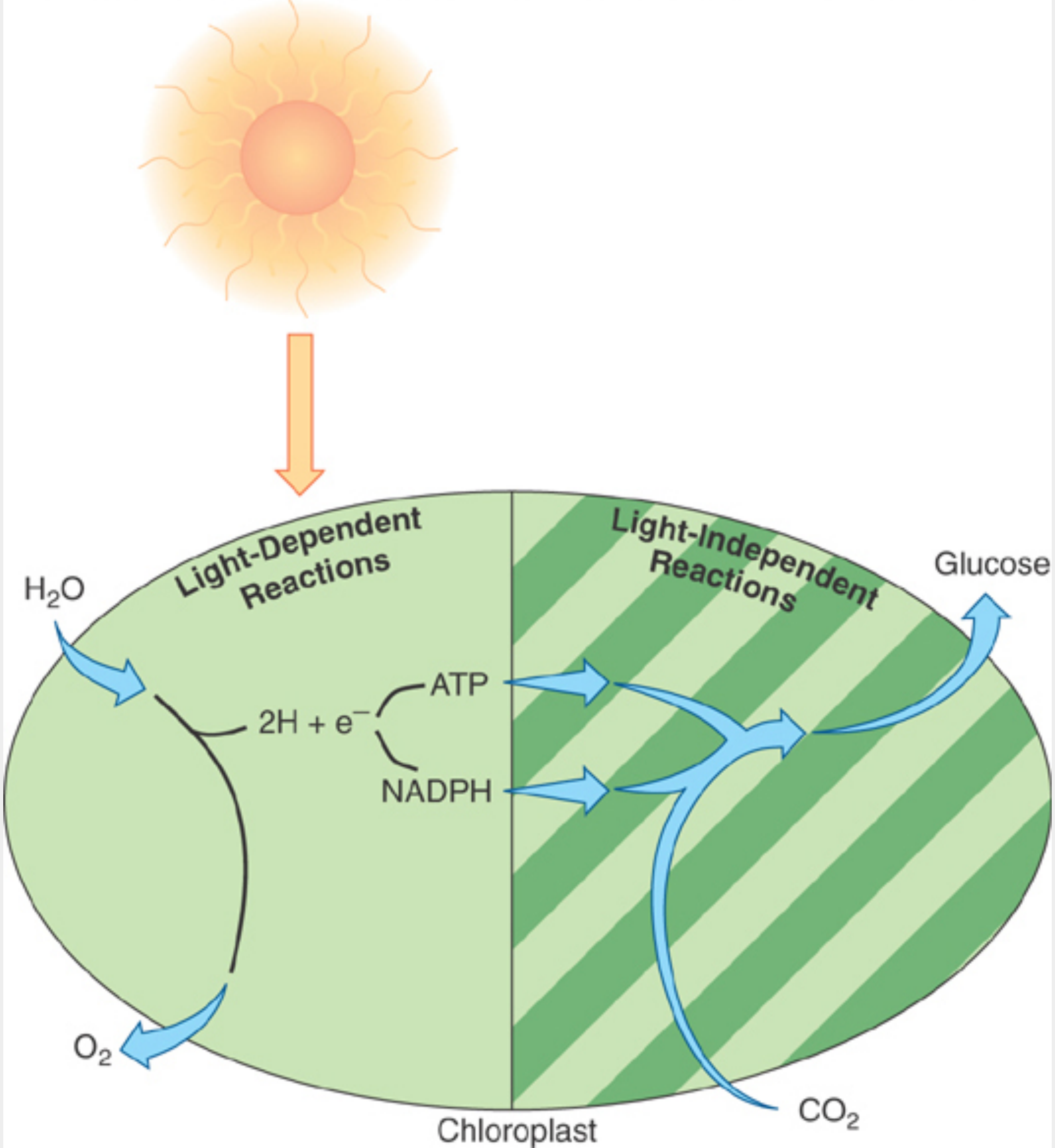
The Carbon Cycle

- Principle users of atmospheric CO_2 are photosynthetic autotrophs.
- Carbon is returned to the atmosphere as CO_2 by respiration, fermentation, decomposition of marine deposits, and burning fossil fuels.
- Methanogens reduce CO_2 and give off methane (CH_4).


Free CO_2 in atmosphere
Volcanos
Respiration
Combustion
Respiration
Respiration
Photosynthesis (plants, algae)
CO_3^{2-} (carbonate in sediments)
Organic carbon decomposed by microorganisms (fungi, bacteria)
Organic carbon taken in by consumers (animals, protozoa)

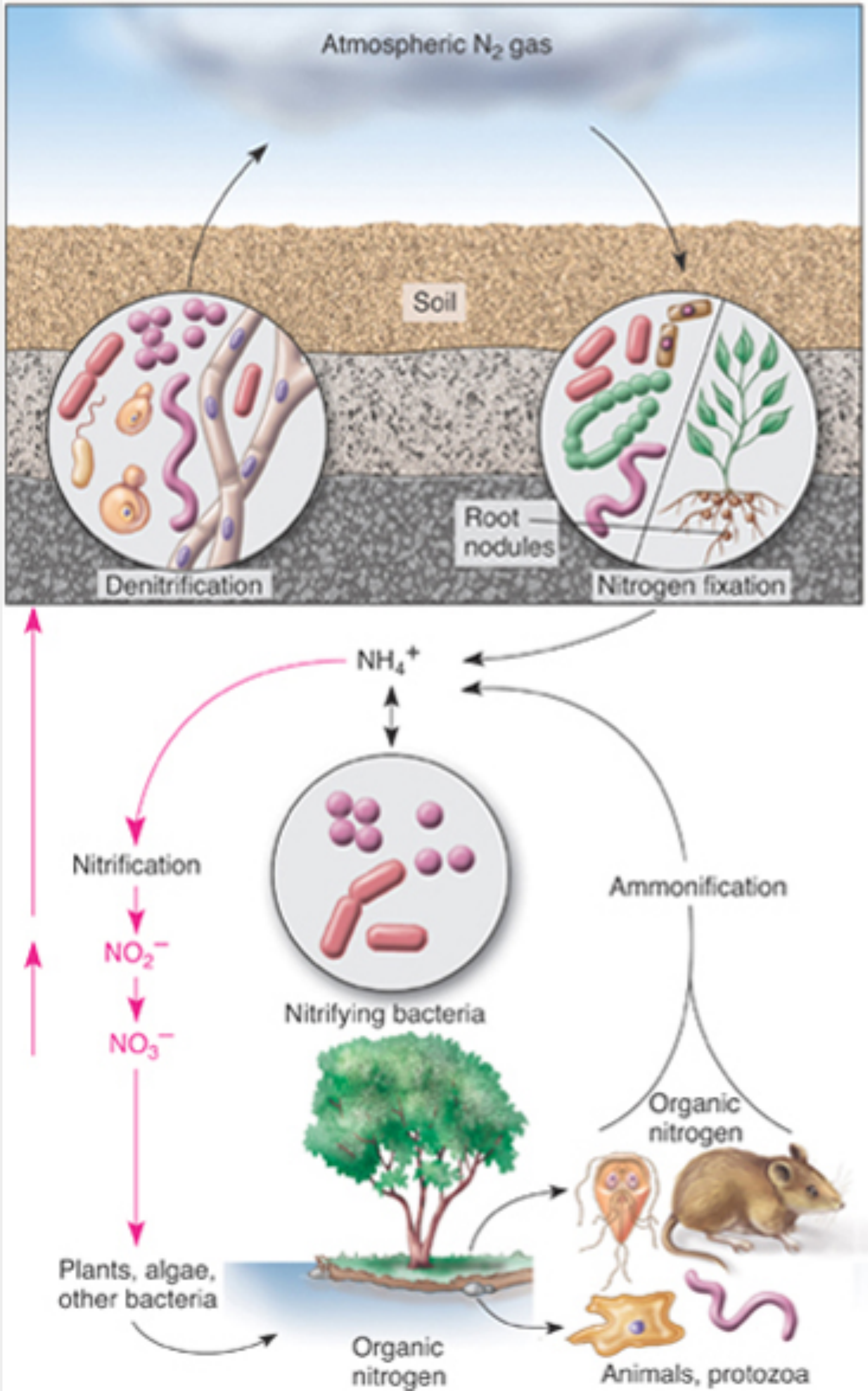
Photosynthesis

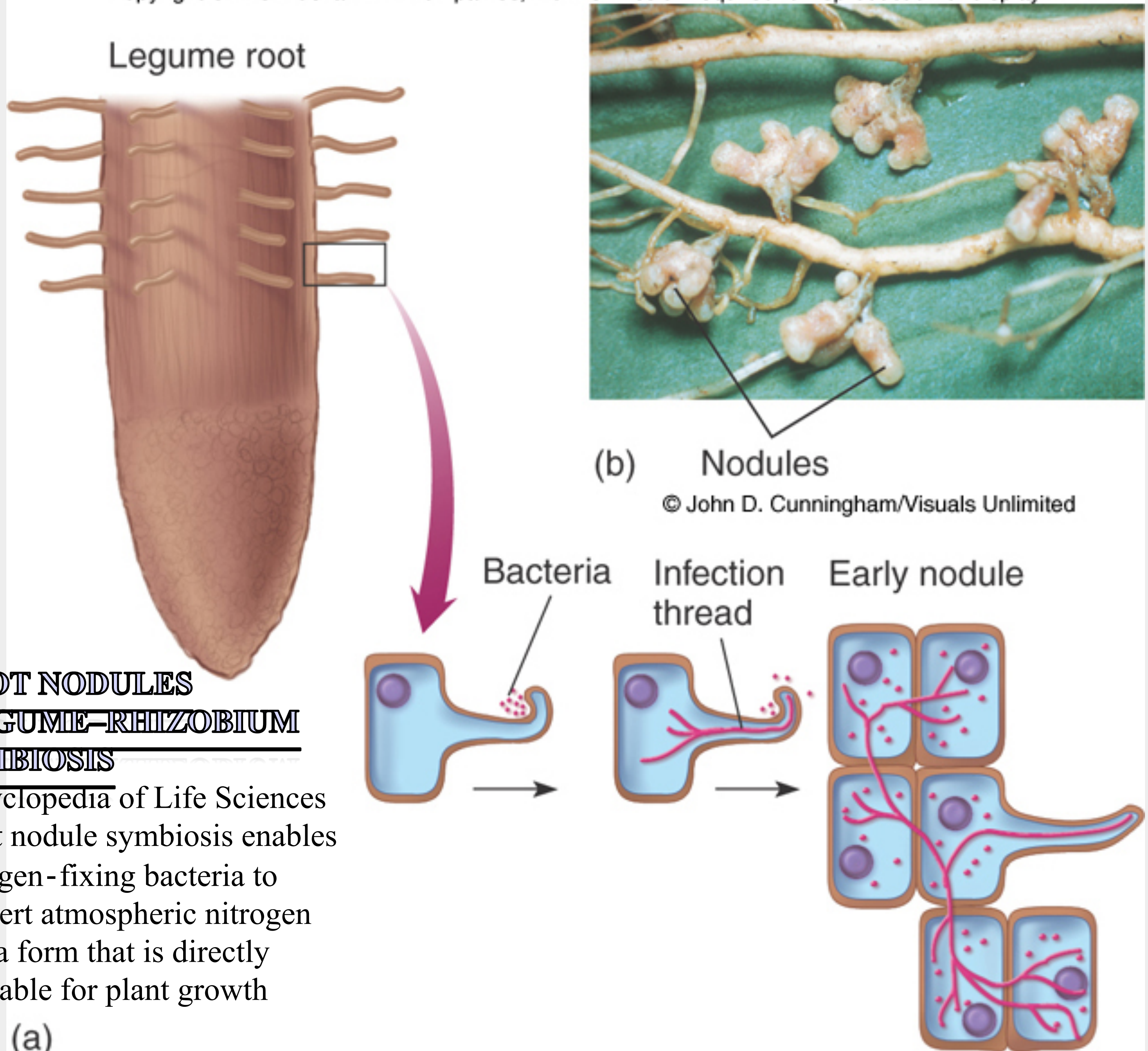
- Occurs in 2 stages
- **Light dependent** – photons are absorbed by chlorophyll, carotenoid, and phycobilin pigments
  - water split by photolysis, releasing O_2 gas and provide electrons to drive photophosphorylation
  - released light energy used to synthesize ATP and NADPH
- **Light-independent reaction** - dark reactions – Calvin cycle – uses ATP to fix CO_2 to ribulose-1,5-bisphosphate and convert it to glucose


Light-Dependent
Reactions
Light-Independent
Reactions
H_2O
2H + e⁻
ATP
NADPH
Glucose
O_2
CO_2
Chloroplast

The Nitrogen Cycle

- N_2 gas is the most abundant gas in the atmosphere, 79% of air volume.
- Involves several types of microbes
- 4 types of reactions:
  - **nitrogen fixation** –atmospheric N_2 gas is converted to NH_4 salts; nitrogen-fixing bacteria live free or in symbiotic relationships with plants
  - **ammonification** – bacteria decompose nitrogen-containing organic compounds to ammonia
  - **nitrification** – convert NH_4^+ to NO_2^- and NO_3^-
  - **denitrification** – microbial conversion of various nitrogen salts back to atmospheric N_2


Atmospheric N_2 gas
Soil
Root nodules
Denitrification
Nitrogen fixation
NH_4^+
Nitrification
NO_2^-
NO_3^-
Nitrifying bacteria
Ammonification
Organic nitrogen
Plants, algae, other bacteria
Organic nitrogen
Animals, protozoa



ROOT NODULES (LEGUME–RHIZOBIUM SYMBIOSIS

Encyclopedia of Life Sciences

Root nodule symbiosis enables nitrogen-fixing bacteria to convert atmospheric nitrogen into a form that is directly available for plant growth

Lithospheric Cycles

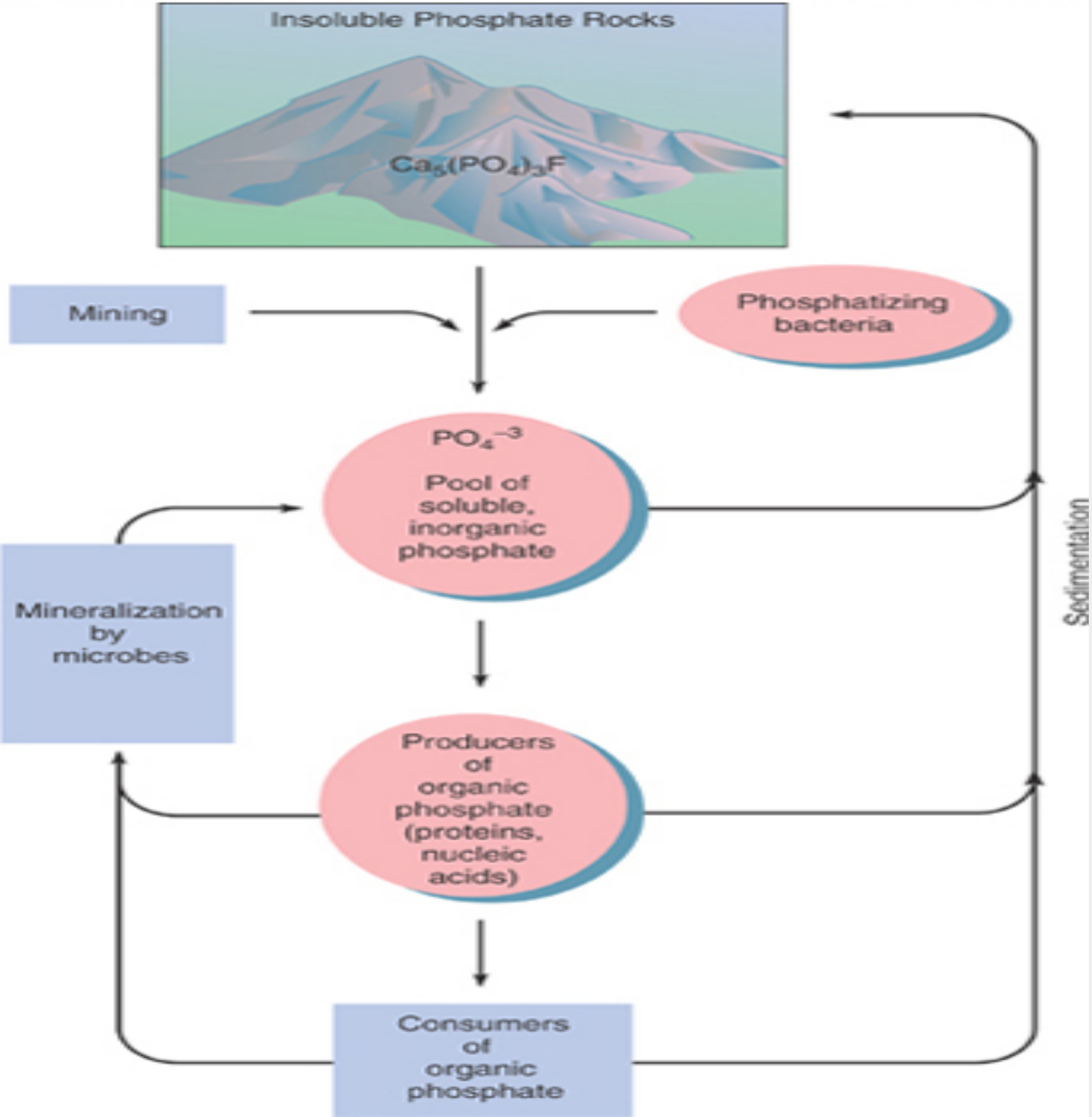
- Sulfur cycle
- Phosphorous cycle

The Sulfur Cycle

- Sulfur originates from rocks, oceans, lakes and swamps.
- Sulfur exists in the elemental form and as hydrogen sulfide gas, sulfate, and thiosulfate.
- Plants and many microbes can assimilate only SO_4 and animals require an organic source – amino acids: cystine, cysteine, and methionine.
- Bacteria convert environmental sulfurous compounds into useful substrates.

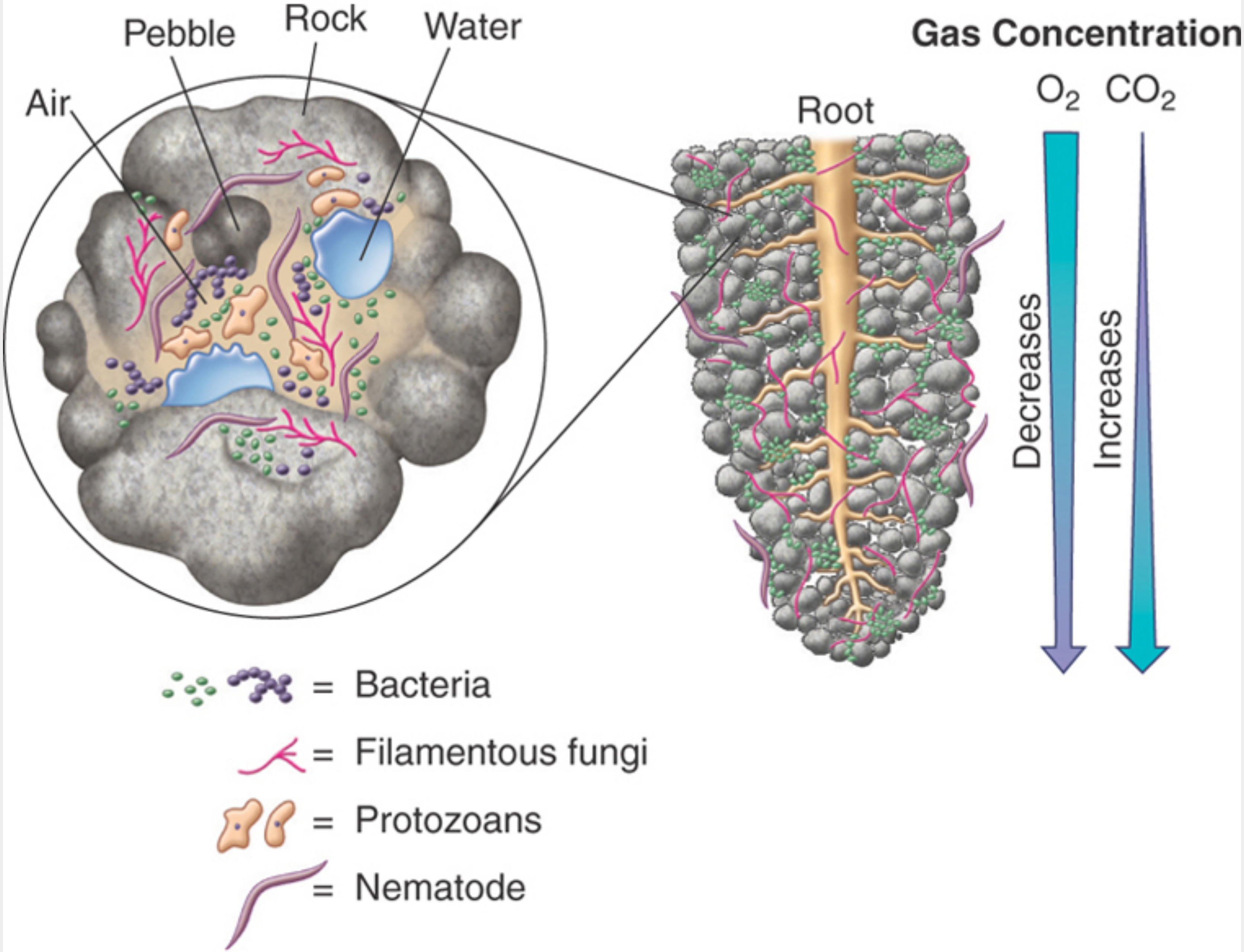
The Phosphorous Cycle

- Chief inorganic reservoir of phosphate (PO_4) is phosphate rock.
- PO_4 must be converted into a useable form (PO_4^{-3}) by the action of acid; sulfuric acid is naturally released by some bacteria.
- Organic phosphate is returned to soluble phosphate by decomposers.


Insoluble Phosphate Rocks
$Ca_5(PO_4)_3F$
Mining
Phosphatizing bacteria
PO_4^{-3}
Pool of soluble, inorganic phosphate
Mineralization by microbes
Producers of organic phosphate (proteins, nucleic acids)
Consumers of organic phosphate
Sedimentation

Soil Microbiology: The Composition of the Lithosphere

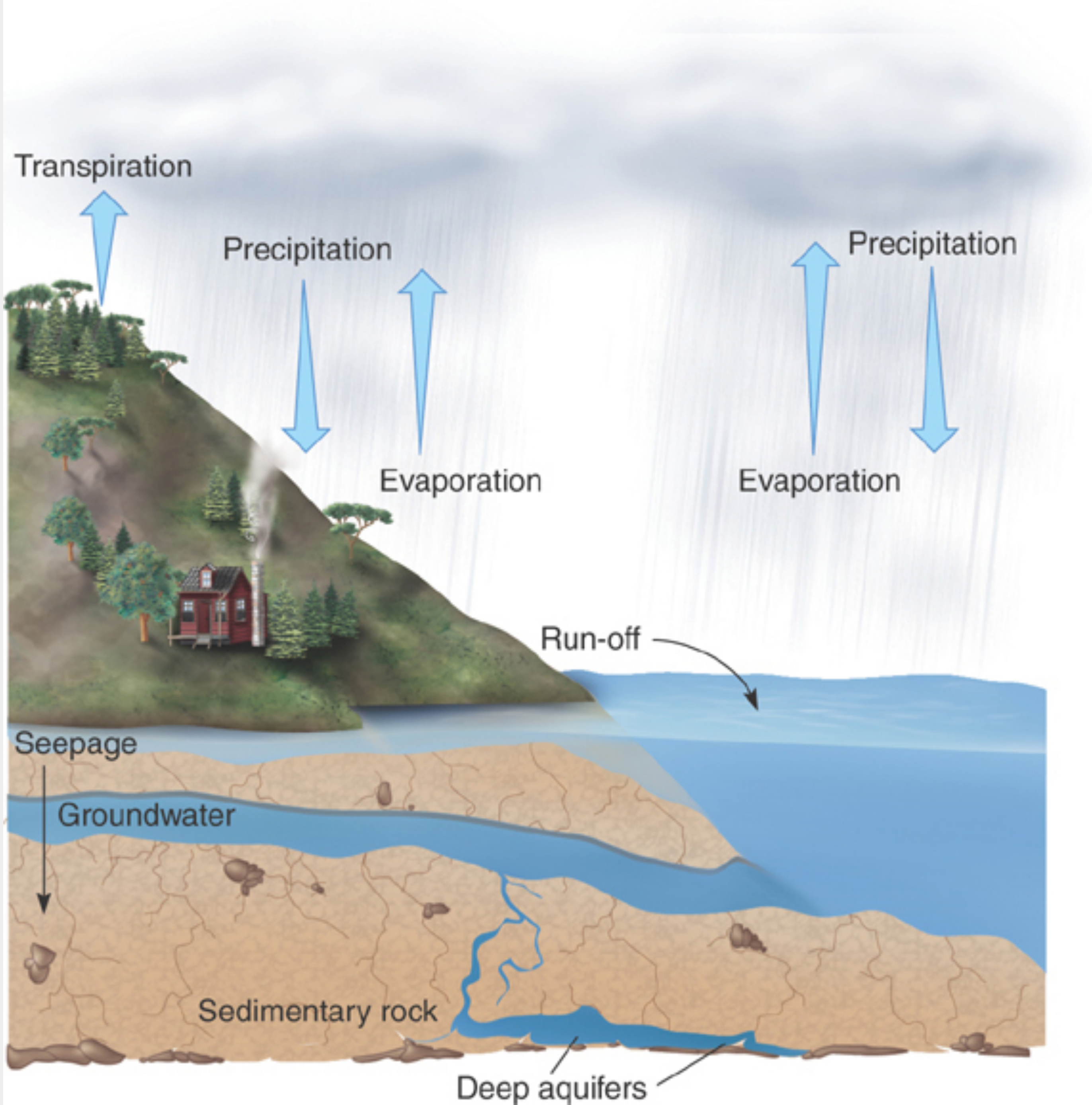
- Soil is a dynamic, complex ecosystem with a vast array of microbes, animals, and plants.
- **Lichens** – symbiotic associations between a fungus and a cyanobacterium or green algae
  - produce acid that releases minerals from rocks
- **Humus** – rich moist layer of soil containing plant and animal debris being decomposed by microbes
- **Rhizosphere** – zone of soil around plant roots contains associated bacteria, fungi and protozoa
- **Mycorrhizae** – symbiotic organs formed between fungi and certain plant roots



Pebble
Rock
Water
Air
Gas Concentration
Root
O_2
CO_2
Decreases
Increases
= Bacteria
= Filamentous fungi
= Protozoans
= Nematode

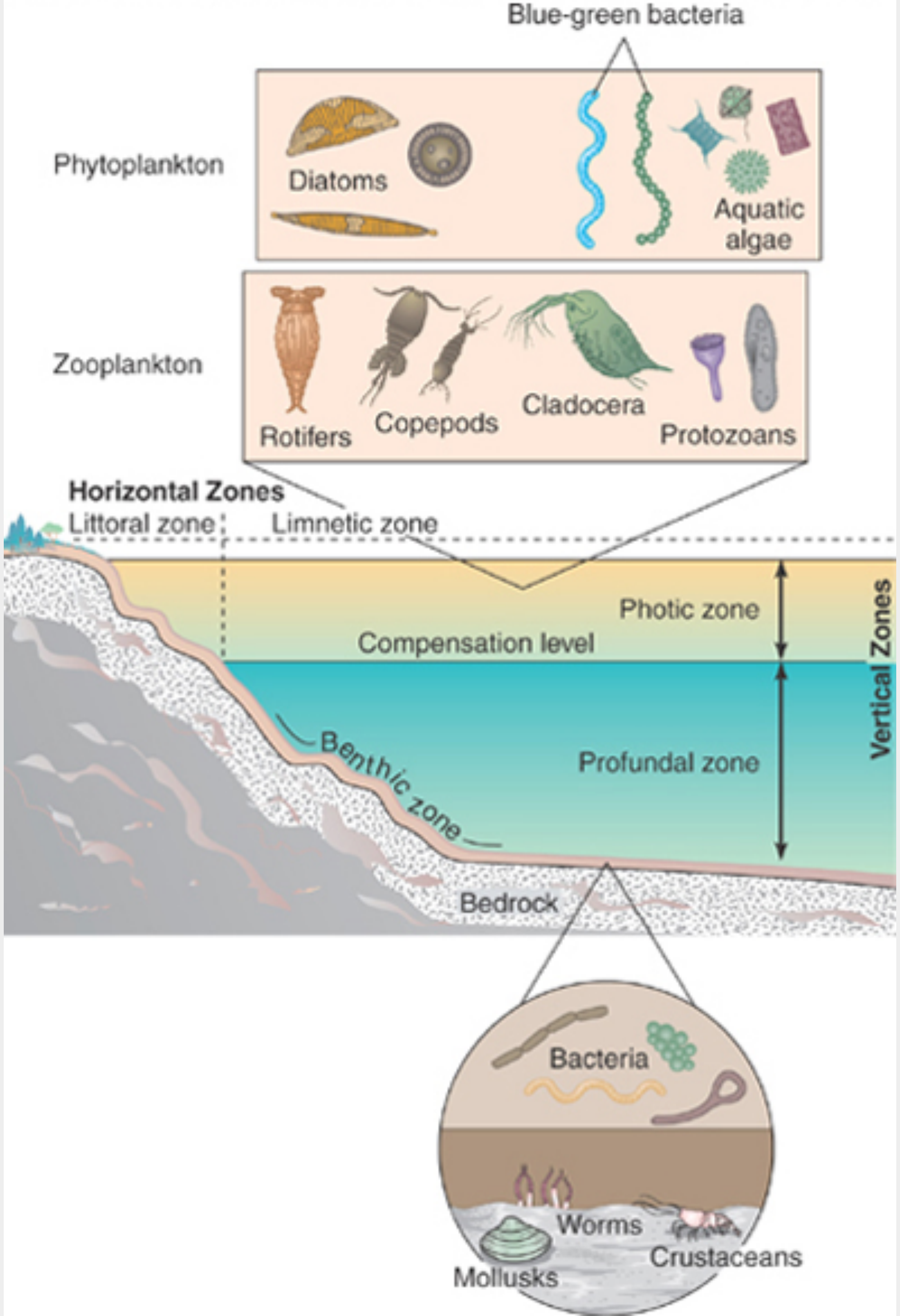
Cycles in the Hydrosphere
Aquatic Microbiology

- Water is the dominant compound on the earth; it occupies ¾ of the earth's surface.
- Continuously cycled between hydrosphere, atmosphere, and lithosphere – **hydrologic cycle**
  - Water evaporates, accumulates in the atmosphere, and returns to the earth through condensation and precipitation.
- Surface water collects in subterranean pockets forming **groundwater** source, called an **aquifer** – resurfaces through springs, geysers, and hot vents, also tapped as primary supply for 1/4th of water for human consumption


Transpiration
Precipitation
Evaporation
Precipitation
Evaporation
Run-off
Seepage
Groundwater
Sedimentary rock
Deep aquifers

The Structure of Aquatic Ecosystems

- Surface waters differ considerably in size, geographic location, and physical and chemical character.
- Sunlight, temperature, aeration, and dissolved nutrient content are factors that contribute to the development of zones.
- Lake is stratified vertically into 3 zones or strata:
  - **photic zone** – surface to lowest limit of sunlight penetration
  - **profundal zone** – edge of the photic zone to lake sediment
  - **benthic zone** – organic debris and mud forming the basin
- Stratified horizontally into 2 zones:
  - **littoral zone** – shoreline, relatively shallow water
  - **limnetic zone** – open, deeper water


Blue-green bacteria
Phytoplankton
Diatoms
Aquatic algae
Zooplankton
Rotifers
Copepods
Cladocera
Protozoans
Horizontal Zones
Littoral zone
Limnetic zone
Photic zone
Compensation level
Vertical Zones
Benthic zone
Profundal zone
Bedrock
Bacteria
Worms
Crustaceans
Mollusks

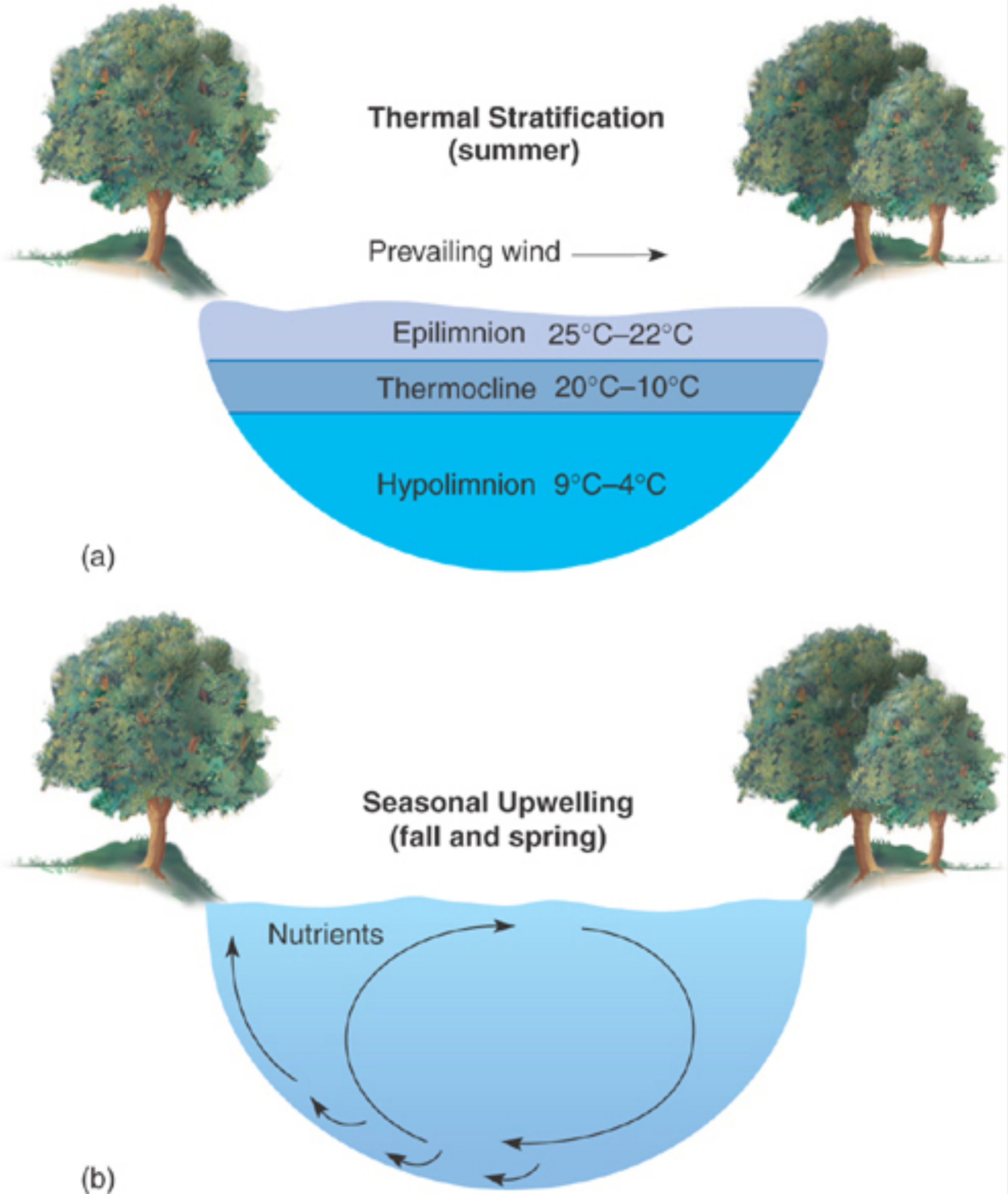
Marine Environments

- Resembles profile of lake but has variations in salinity, depth, temperature, hydrostatic pressure, and mixing
- Contains a zone, called an **estuary**, where river meets the sea; fluctuates in salinity, is very high in nutrients
- Tidal wave action subjects the coastal habitat to alternate period of submersion and exposure.
- **Abyssal zone** – extends to a depth of 10,000m; supports communities with extreme adaptations including:
  - halophilic, psychrophilic, barophilic, and in some areas, anaerobes

Aquatic Communities

- Microbial distribution is associated with sunlight, temperature, oxygen levels, and available nutrients.
- Photic zone is most productive-contains **plankton**
  - **phytoplankton** – variety of photosynthetic algae and cyanobacteria
  - **zooplankton** – microscopic consumers; filter feed, prey, or scavenge
- Benthic zone supports variety of organisms including aerobic and anaerobic bacterial decomposers.

- Large bodies of standing water develop thermal stratification.
- **Epilimnion** – upper region, warmest
- **Hypolimnion** – deeper, cooler
- **Thermocline** – buffer zone between warmest and coolest layers; ordinarily prevents the mixing of the two
- Currents, brought on by temperature change, cause upwelling of nutrient-rich benthic sediments and outbreaks of abundant microbial growth – red tides.


Thermal Stratification
(summer)
Prevailing wind
Epilimnion 25°C–22°C
Thermocline 20°C–10°C
Hypolimnion 9°C–4°C
(a)
Seasonal Upwelling
(fall and spring)
Nutrients
(b)

- Nutrient range is variable.
- **Oligotrophic** – nutrient-deficient aquatic ecosystem; supports few microorganisms; many bacteriophage
- **Eutrophication** – addition of excess quantities of nutrients; naturally or by effluents from sewage, agriculture or industry; encourages heavy surface growth of algae (bloom) which cuts off the O_2 supply; disturbs the ecological balance
- Only anaerobic and facultative anaerobes will survive.

Microbiology of Drinking Water Supplies

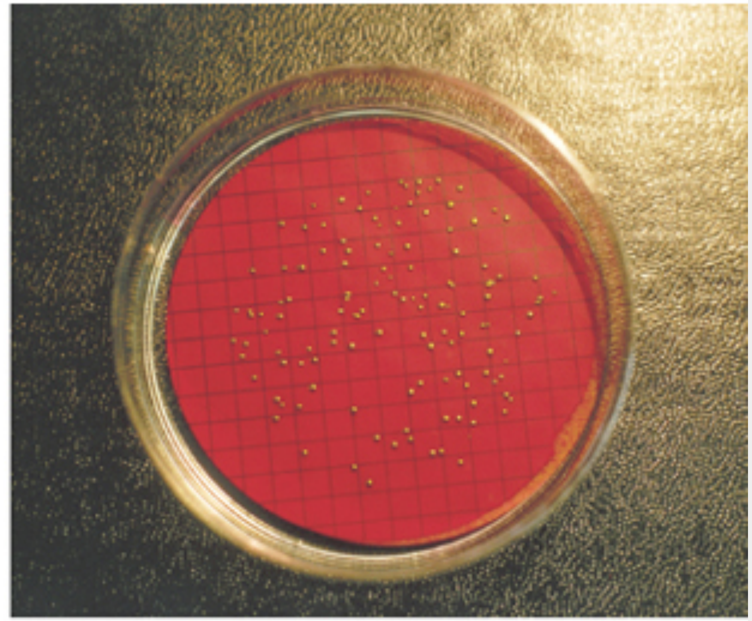
- **Potable** (drinkable) water – free of pathogens, toxins, turbidity, odor, color, and taste
- Most prominent water-borne pathogens – *Giardia, Cryptosporidium, Campylobacter, Salmonella, Shigella, Vibrio, Mycobacterium*, HAV and Norwalk viruses
- Most assays of water purity focus on detecting fecal contamination – indicator bacteria *E.coli, Enterobacter, Citrobacter*.

Water Quality Assays

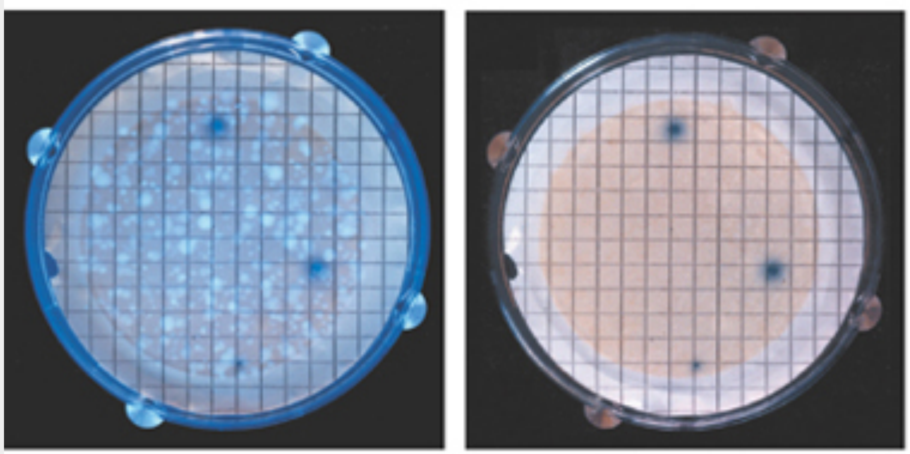
- **Standard plate count** – total number of bacteria that develop colonies represents an estimate of the viable population in the sample
- **Membrane filter method** – after filtration, filter is placed on selective and differential media, incubated, colonies are presumptively identified and counted
- **Most probable number** (MPN) – presumptive, confirmatory and completed tests
- No acceptable level for fecal coliforms, enterococci, viruses, or pathogenic protozoans in drinking water

(a) Membrane filter technique. The water sample is filtered through a sterile membrane filter assembly and collected in a flask.

(b) The filter is removed and placed in a small Petri dish containing a differential selective medium such as M-FD endo agar and incubated.



(c) On M-FD endo medium, colonies of *Escherichia coli* often yield a noticeable metallic sheen. The medium permits easy differentiation of various genera of coliforms, and the grid pattern can be used as a guide for rapidly counting the colonies.



Total coliforms fluoresce under a black light.

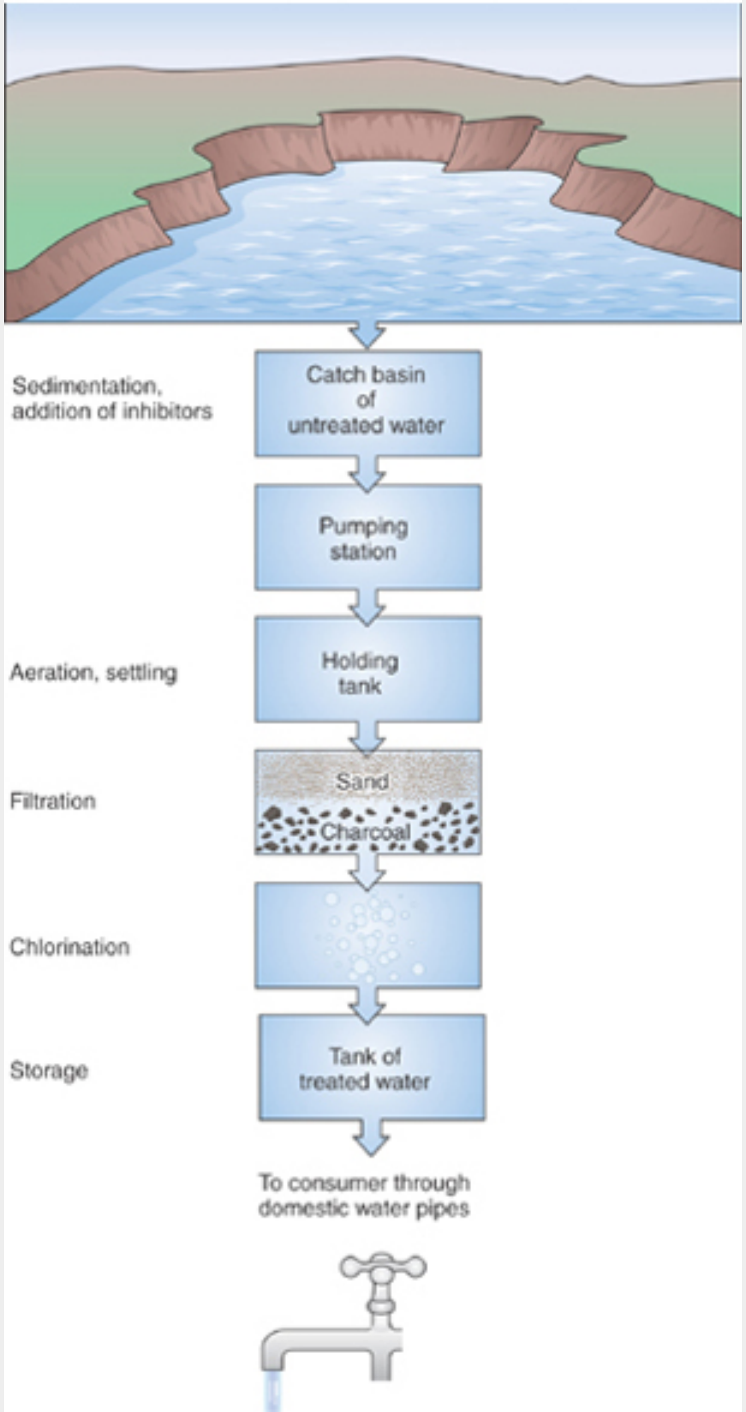
E. coli colonies are blue under natural light.

(d) Some tests for water-borne coliforms are based on formation of specialized enzymes to metabolize lactose. The MI tests shown here utilize synthetic substrates that release a colored substance when the appropriate enzymes are present. The total coliform count is indicated by the plate on the left; fecal coliforms (*E. coli*) are seen in the plate on the right. This test is especially accurate with surface or groundwater samples.

Water and Sewage Treatment

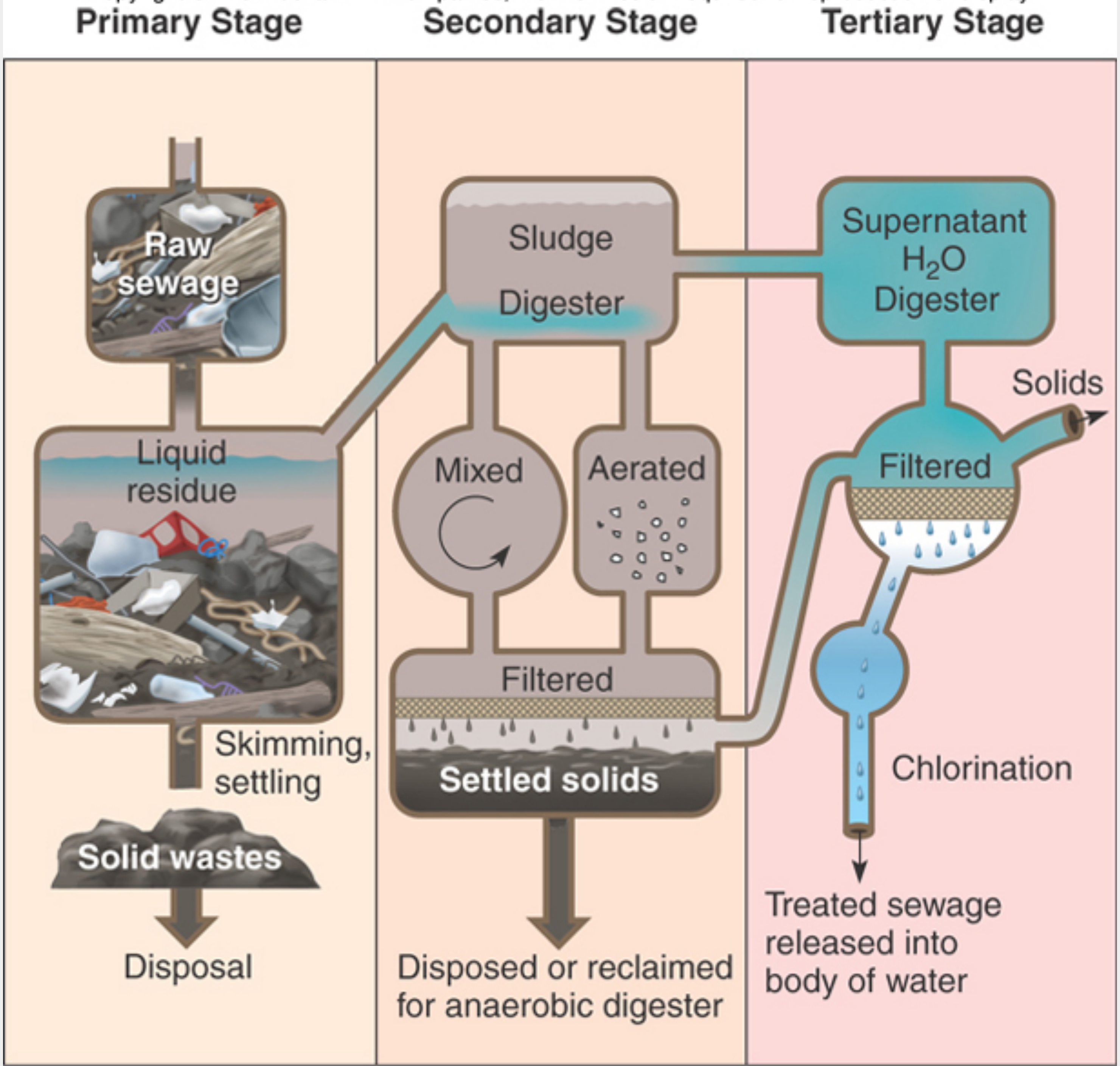
Water purification

- In most cities, water is treated in a stepwise process before it is supplied to consumers.
- Impoundment in large, protected reservoir – storage and sedimentation; treated to prevent overgrowth of cyanobacteria
- Pumped to holding tanks for further settling, aeration, and filtration; chemical treatment with a chlorine, ozone, or peroxide disinfectant


Sedimentation,
addition of inhibitors
Catch basin
of
untreated water
Pumping
station
Aeration, settling
Holding
tank
Filtration
Sand
Charcoal
Chlorination
Storage
Tank of
treated water
To consumer through
domestic water pipes

Sewage treatment

- Sewage – used wastewater containing chemicals, debris, and microorganisms
- Typically requires 3 phases:
  - primary phase – removes floating, bulky physical objects
  - secondary phase – removes the organic matter by biodegradation, natural bioremediation in a large digester forming sludge which is aerated by injection and stirred
  - tertiary phase – filtration, disinfection and removal of chemical pollutants
- Gradually released


Primary Stage
Secondary Stage
Tertiary Stage
Raw sewage
Liquid residue
Skimming, settling
Solid wastes
Disposal
Sludge Digester
Mixed
Aerated
Filtered
Settled solids
Disposed or reclaimed for anaerobic digester
Supernatant H_2O Digester
Solids
Filtered
Chlorination
Treated sewage released into body of water

Applied Microbiology and Biotechnology

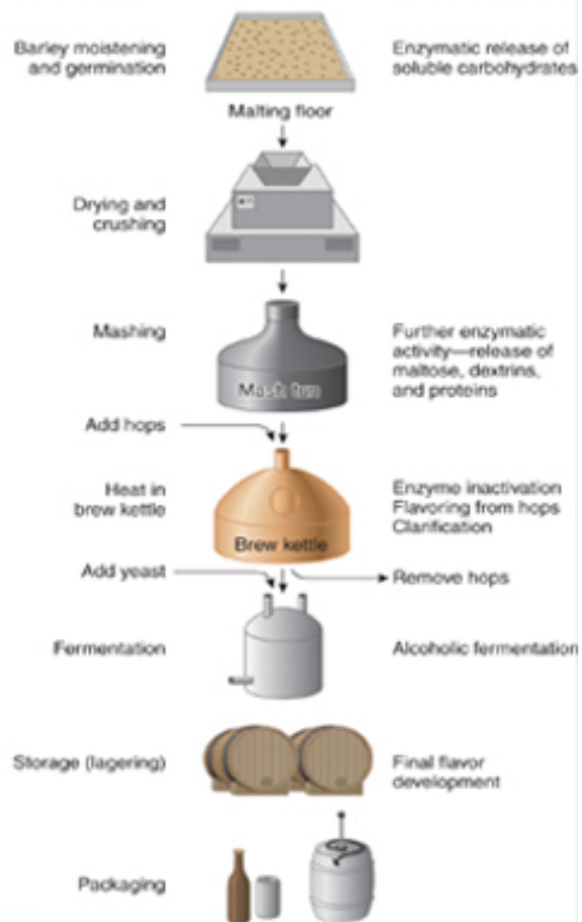
- Practical applications of microorganisms in manufacturing products or carrying out particular decomposition processes is called **biotechnology**. Many use **fermentation**.
  - food science
  - industry
  - medicine
  - agriculture
  - environmental protection

Microorganisms and Food

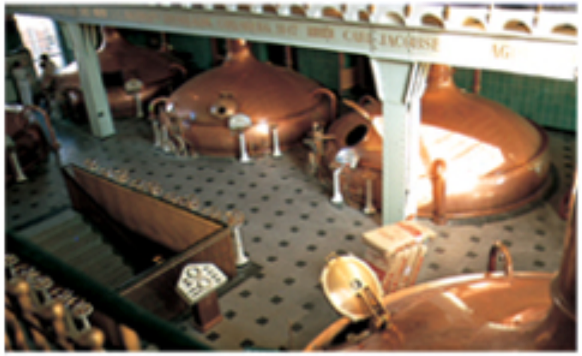
Microbes and humans compete for nutrients in food.

Microbes, through fermentation, can impart desirable aroma, flavor, or texture to foods.

- Bread – yeast leaven dough by giving off CO_2
- Beer – fermentation of wort
- Wine –fermentation of fruit juices
- Vegetable products – sauerkraut, pickles, and soybean derivatives
- Vinegar –fermentation of plant juices
- Milk and diary products – cheeses, yogurt

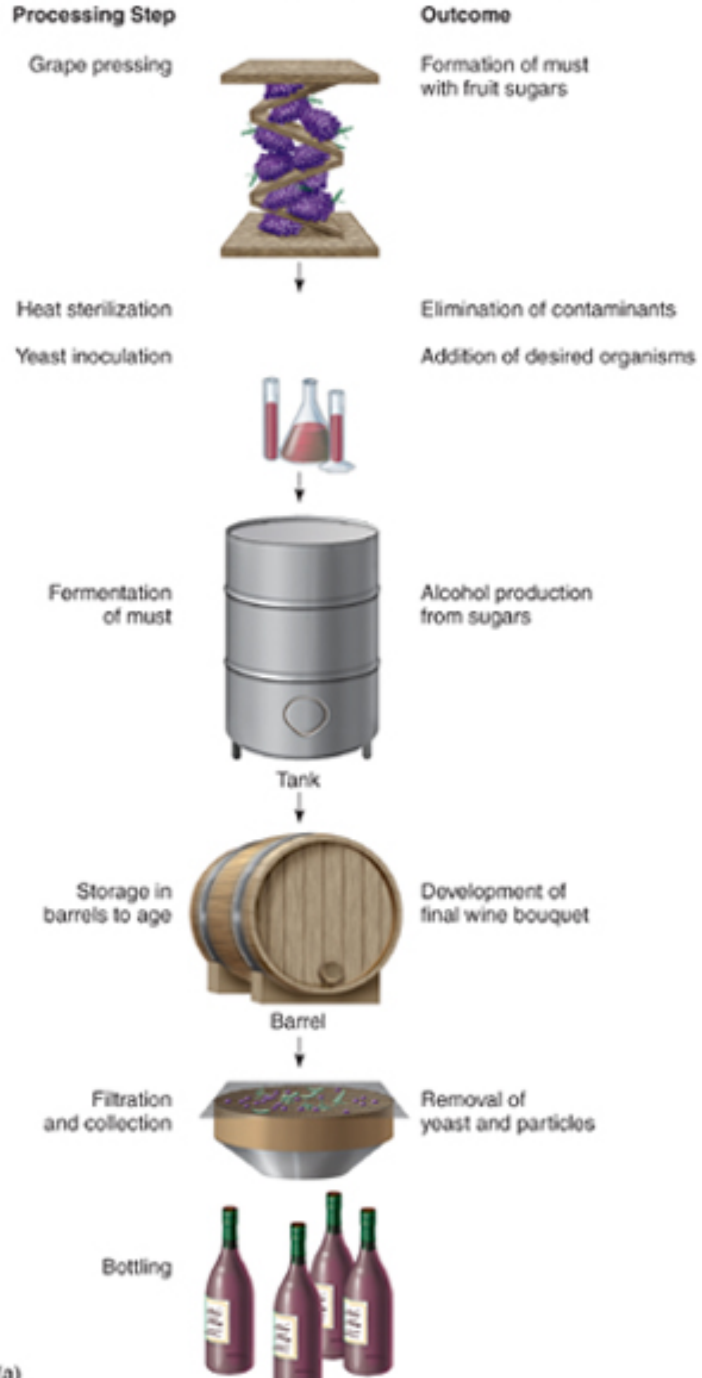


(a)



(b)

© Sanitation Districts of Los Angeles County



(a)


(b)

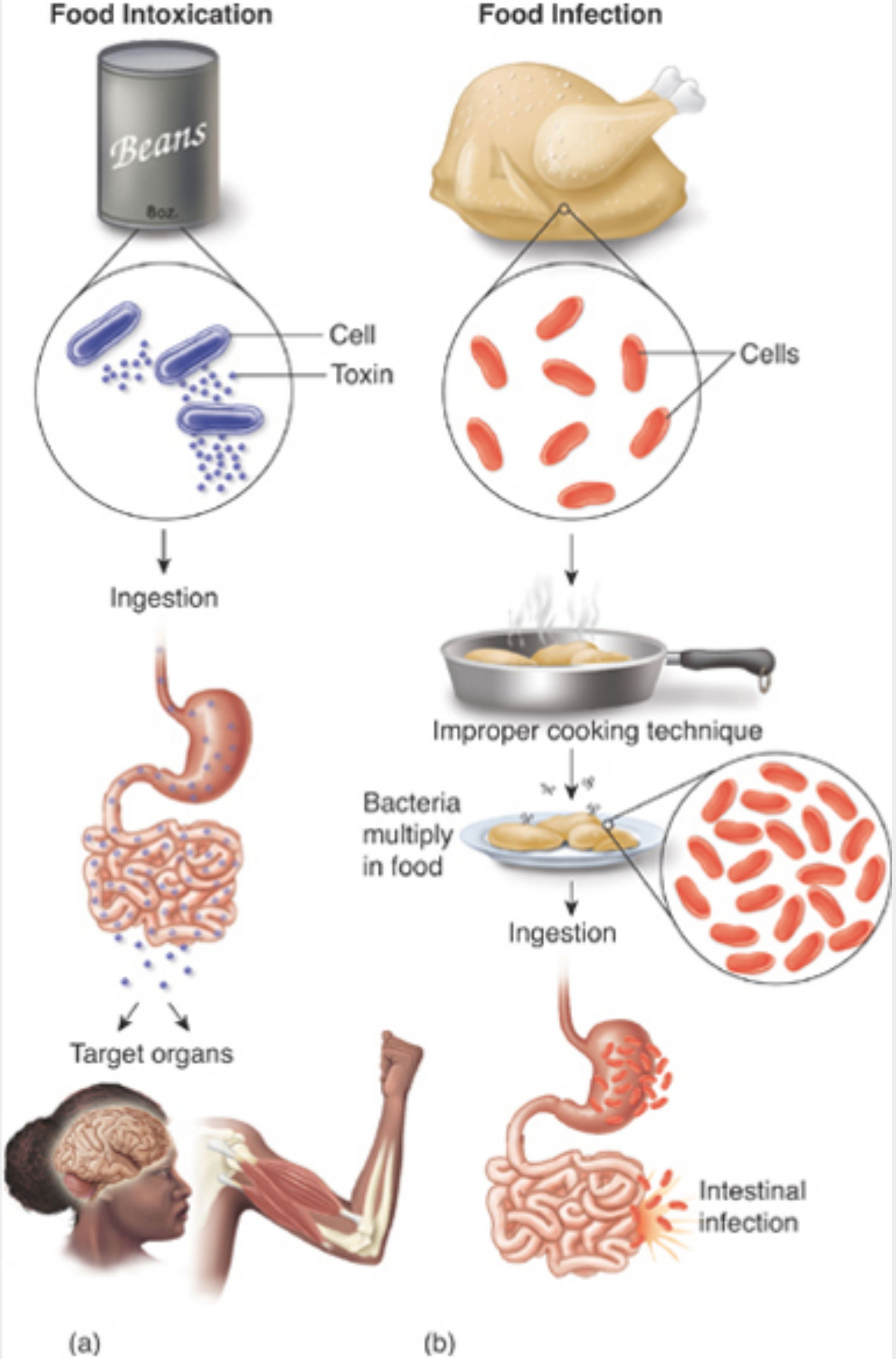
©KevinSchafer/Peter Arnold

Microorganisms as food

- Mass-produced yeasts, molds, algae, and bacteria
- Single-celled protein and filamentous mycoprotein added to animal feeds

Microbial Involvement in Food-Borne Diseases

- Food poisoning- diseases caused by ingesting food
- 2 types:
  - **food intoxication** – results from ingesting exotoxins secreted from bacterial cells growing in food
  - **food infection** – ingestion of whole microbes that target the intestine – salmonellosis, shigellosis
- Staphylococcal food intoxication - most common in U.S.
- Other common agents – *Campylobacter, Salmonella, Clostridium perfringens, and Shigella*


Food Intoxication
Food Infection
Beans
8oz.
Cell
Toxin
Cells
Ingestion
Improper cooking technique
Bacteria multiply in food
Ingestion
Target organs
Intestinal infection
(a)
(b)

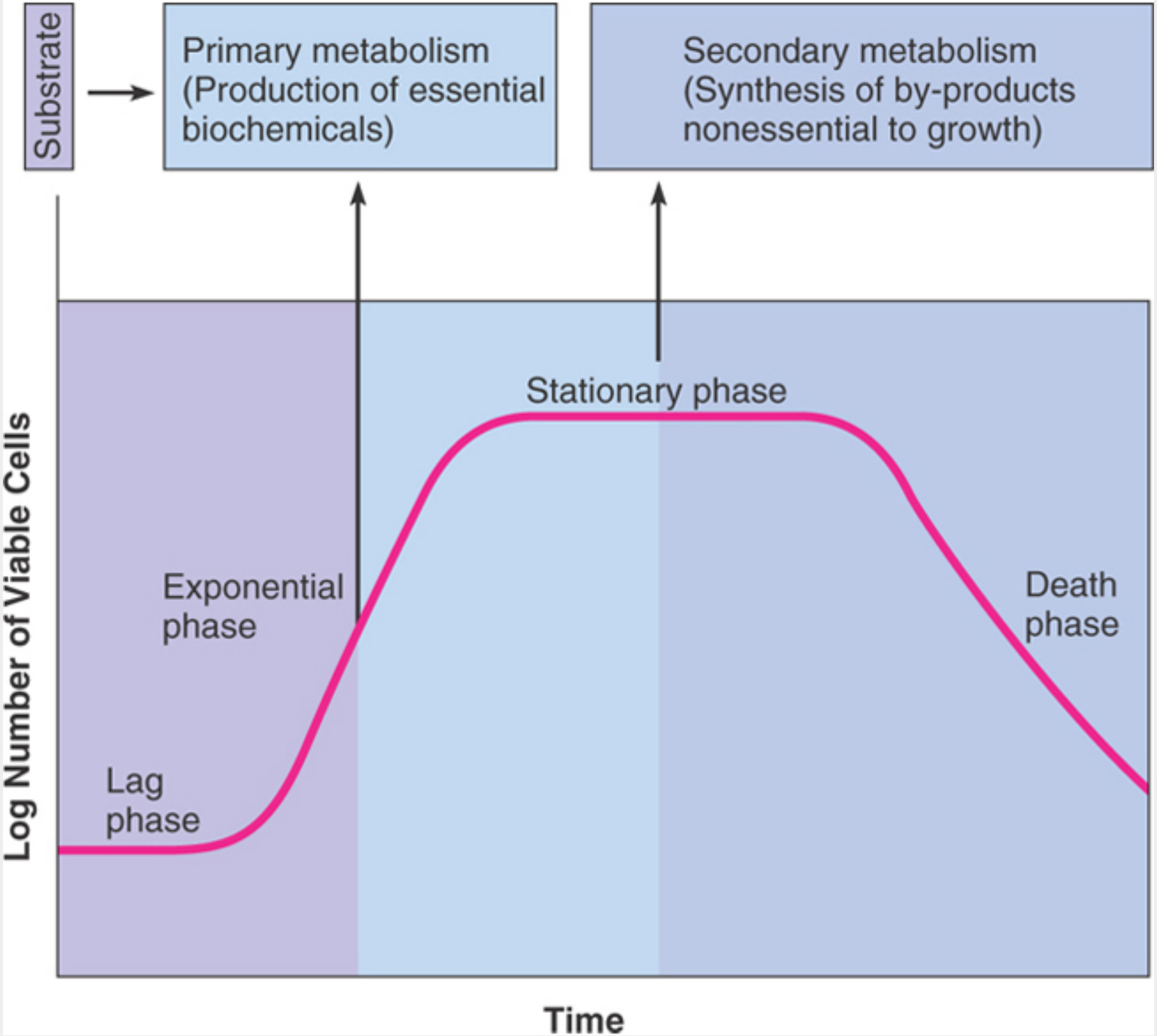
Prevention Measures for Food Poisoning and Spoilage

- Prevent incorporation of microbes into food
  - aspetic technique
  - handwashing and proper hygiene
- Prevent survival or multiplication of microbes in food.
  - heat- autoclaving, pasteurization, cooking
  - cold- refrigeration, freezing
  - radiation- UV, ionizing
  - desiccation
  - chemical preservatives – NaCl, organic acids

General Concepts in Industrial Microbiology

- Bulk production of organic compounds such as antibiotics, hormones, vitamins, acids, solvents, and enzymes
- Any processes involving fermentation

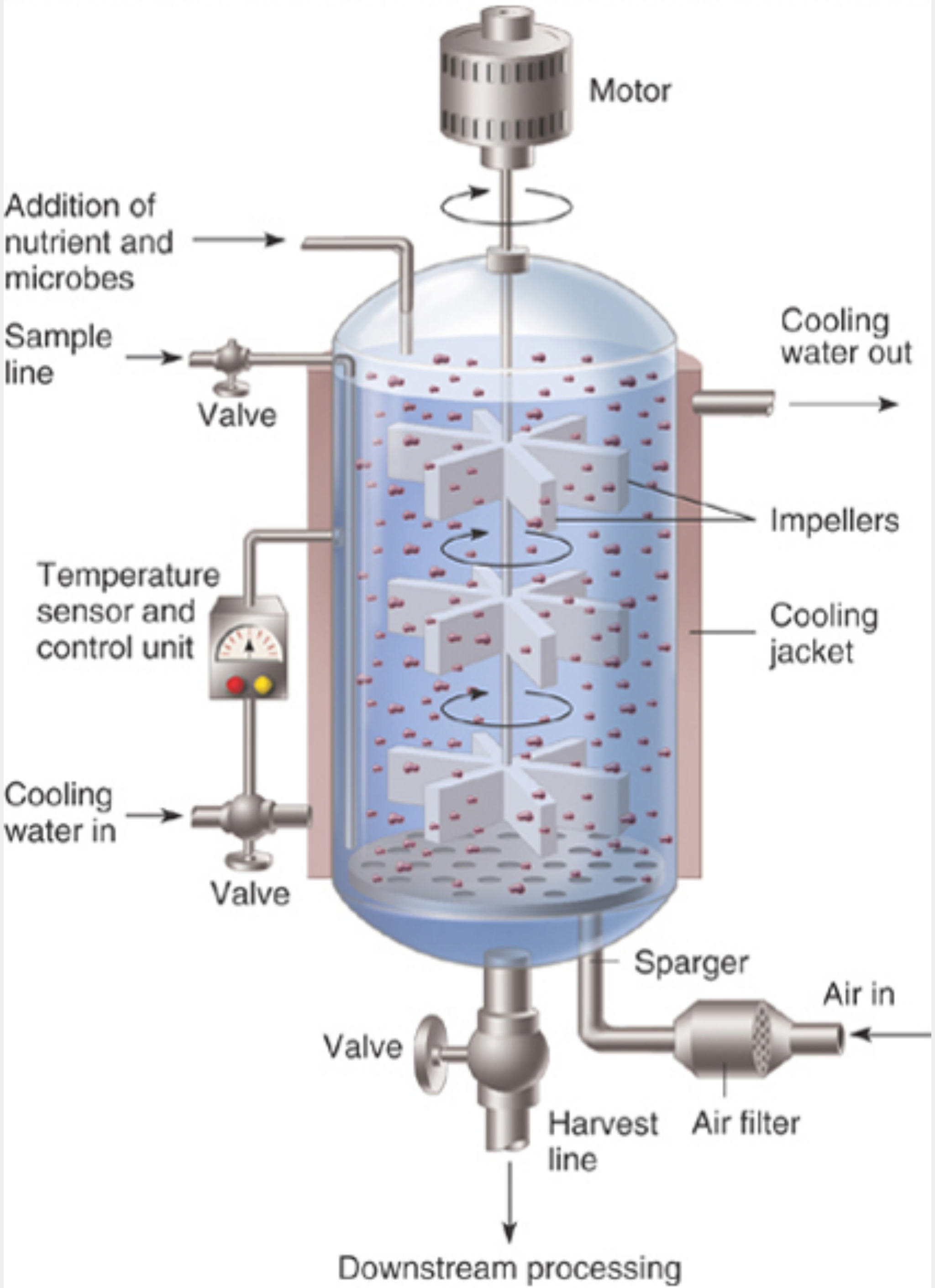
- Mutant strains of bacteria and fungi that synthesize large amounts of metabolites
- **Primary metabolites** - produced during major metabolic pathways and are essential to microbe's function – amino acids, organic acids synthesized during logarithmic growth
- **Secondary metabolites** – by-products of metabolism that may not be critical to microbe's function – vitamins, antibiotics, and steroids synthesized during stationary phase


Substrate
Primary metabolism (Production of essential biochemicals)
Secondary metabolism (Synthesis of by-products nonessential to growth)
Log Number of Viable Cells
Lag phase
Exponential phase
Stationary phase
Death phase
Time

- Many syntheses occur in sequential fashion involving more than one organism.
- **Biotransformation** – waste product of one organism becomes the building block of the next

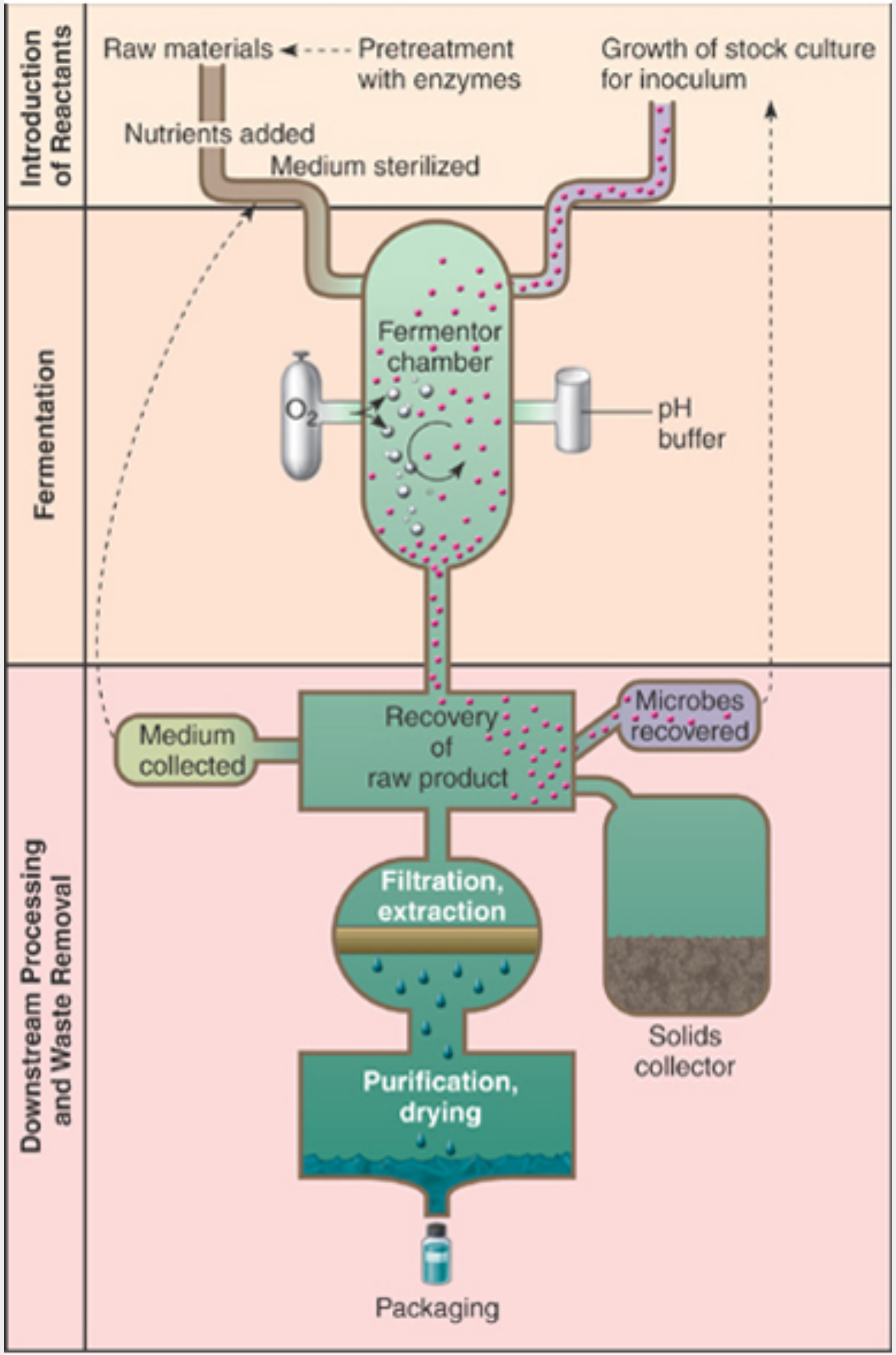
From Microbial Factories to Industrial Factories

- Produce appropriate levels of growth and fermentation in a carefully controlled environment
- Commercial fermentation carried out in **fermentors** – large culture devices with mechanisms for controlling environment


Motor
Addition of nutrient and microbes
Sample line
Valve
Cooling water out
Impellers
Temperature sensor and control unit
Cooling jacket
Cooling water in
Valve
Sparger
Air in
Valve
Harvest line
Air filter
Downstream processing

Substance Production

- Steps in mass production:
- Introduction of microbes and sterile media into reaction chamber
- Fermentation
- Downstream processing (recovery, purification, packaging)
- Removal of waste
- Carried out aseptically and monitored for rate of flow and quality of product



Introduction of Reactants
Fermentation
Downstream Processing and Waste Removal
Raw materials
Pretreatment with enzymes
Growth of stock culture for inoculum
Nutrients added
Medium sterilized
Fermentor chamber
O_2
pH buffer
Recovery of raw product
Medium collected
Microbes recovered
Filtration, extraction
Solids collector
Purification, drying
Packaging

- **Batch fermentations** – substrate added to system all at once and taken through a limited run until product is harvested
- **Continuous feed** systems – nutrients are continuously fed into the reactor and product is siphoned off throughout run

- Pharmaceutical products
  - antibiotics
  - vitamins
  - vaccines
- Miscellaneous products
  - biopesticides
  - enzymes
  - amino acids
  - organic acids
  - solvents
  - natural flavor compounds