

Growing, growing, gone... Microbial Growth

Microbial Nutrition

- Nutrition
 - Nutrients are acquired from the environment and used for cellular activities
- Essential nutrient
 - Any substance, whether in elemental or molecular form, that must be provided to an organism
- Macronutrients
 - Required in relatively large quantities
 - Play principal roles in cell structure and metabolism
- Micronutrients (trace elements)
 - Present in smaller amounts
 - Involved in enzyme function and maintenance of protein structure

Sources of essential nutrients

- Required for metabolism and growth
 - Carbon source
 - Energy source

Carbon source

- Heterotroph (depends on other life forms)
 - Organic molecules
 - Ex. Sugars, proteins, lipids
- Autotroph (self-feeders)
 - Inorganic molecules
 - Ex. CO₂

Growth factors

- Essential organic nutrients
- Not synthesized by the microbe, and must be supplemented
- Ex. Amino acids, vitamins

Energy source

- Chemoheterotrophs
- Photoautotrophs
- Chemoautotrophs

Chemoheterotrophs

- Derive both carbon and energy from organic compounds
 - Saprobic
 - decomposers of plant litter, animal matter, and dead microbes
 - Parasitic
 - Live in or on the body of a host

Extracellular Digestion in a Saprobe with a Cell Wall (Bacterium or Fungus)

- Enzymes are transported outside the wall.
- Walled cell is barrier.
- Enzymes hydrolyze the bonds on nutrients.
- Smaller molecules are transported across the wall into the cytoplasm.

Photoautotroph

- Derive their energy from sunlight
- Transform light rays into chemical energy
- Primary producers of organic matter for heterotrophs
- Primary producers of oxygen
- Ex. Algae, plants, some bacteria

Chemoorganic autotrophs

- Two types
 - Chemoorganic autotroph
 - Derives their energy from organic compounds and their carbon source from inorganic compounds
 - Lithoautotrophs
 - Neither sunlight nor organics used, rather it relies totally on inorganics

Transport mechanisms

- Diffusion
- Osmosis
- Active transport
- Endocytosis

Diffusion

- Net movement of molecules from a high concentrated area to a low concentrated area
- No energy is expended (passive)
- Concentration gradient and permeability affect movement

Facilitated diffusion

- Transport of polar molecules and ions across the membrane
- No energy is expended (passive)
- Carrier protein facilitates the binding and transport
 - Specificity
 - Saturation
 - Competition

Osmosis

- Diffusion of water through a permeable but selective membrane
- Water moves toward the higher solute concentrated areas
 - Isotonic
 - Hypotonic
 - Hypertonic

Active transport

- Transport of molecules against a gradient
- Requires energy (active)
- Ex. Permeases and protein pumps transport sugars, amino acids, organic acids, phosphates and metal ions.
- Ex. Group translocation transports and modifies specific sugars

Endocytosis

- Substances are taken, but are not transported through the membrane.
- Requires energy (active)
- Common for eucaryotes
- Ex. Phagocytosis, pinocytosis

Microbial Growth

- Microbial growth = increase in number of cells, not cell size.
- Growth can be affected by a number of factors.
- Temperature, pH, osmotic pressure, water activity, oxygen, other chemical requirements, etc.

Temperature

- Temperature
 - Minimum growth temperature
 - Optimum growth temperature
 - Maximum growth temperature
- The cardinal temperatures for species are not fixed, but often depend (to some extent) on other environmental factors.

Temperature Classes of Microorganisms

- Psychrophiles
 - Readily isolated from Arctic and Antarctic habitats.
 - Cell membranes have high levels of unsaturated fatty acids.
 - This remains semi-fluid when it is cold.
 - Membrane disruption can occur at temperatures of higher than 20°C.
- Psychrotrophs
 - Have optima between 20-30°C
 - Cause food spoilage
- Mesophiles
 - Most microorganisms fall into this category.
 - Almost all human pathogens are mesophiles.
 - Individual species can grow from 10°C to 50°C
 - Optimum growth temperature: 20°C to 40°C
 - Most human pathogens: 30°C to 40°C
 - Thermotolerant microbes survive short exposure to high temperatures; common contaminants of heated or pasteurized foods
- Thermophiles
 - These organisms love habitats that include hot water lines, hot springs and

- self-heating hay stacks.
 - Their membrane lipids are more saturated than mesophiles and therefore have higher melting points.
- Hyperthermophiles
 - Do not grow well at below 55°C
 - Can be found in hot areas of the sea floor such as hyperthermal vents

pH

- pH
 - pH dramatically affects microbial growth.
 - Each species has a definite pH growth range and optimum.
 - Acidophiles have their optimum between pH 0 and 5.5
 - Neutrophiles are between pH 5.5 and 8.0
 - Alkalophiles are between 8.5 and 11.5
 - There are extreme alkalophiles which have optima of pH 10 and higher.

Osmotic Pressure

- Osmotic Pressure
 - When microorganisms are placed in hypertonic environments it will cause plasmolysis to occur.
 - When a microorganism is placed in a hypotonic environment, water will enter the cell and cause it to burst.
 - Some microorganisms have adapted to live in habitats that are hypertonic.
 - These organisms have to maintain a high internal solute concentration to retain the water.
 - These organisms are termed osmotolerant.

Water Activity

- This can be defined as the amount of water that is available to a microorganism for chemical reactions (metabolic processes).
- This can be quantified as a ratio of the vapor pressure of the solution to that of pure water.
- Water activity is inversely related to osmotic pressure.
- Most microorganisms grow well at higher water activities.

Halophiles

- Halophiles have adapted completely to saline conditions and need high levels of sodium chloride to grow.
- Extreme or obligate halophiles require high osmotic pressure.
- Facultative halophiles
 - Can survive high salt conditions but is not required
 - Ex. *Staphylococcus aureus*

Chemical Requirements: Carbon, Nitrogen, Sulfur, Phosphorus, and Trace Elements

- Carbon
 - Structural organic molecules, energy source
 - Chemoheterotrophs use organic carbon sources

- Autotrophs use CO₂
- Nitrogen
 - In amino acids, proteins
 - Most bacteria decompose proteins
 - Some bacteria use NH₄⁺ or NO₃⁻
 - A few bacteria use N₂ in nitrogen fixation
- Sulfur
 - In amino acids, thiamine, biotin
 - Most bacteria decompose proteins
 - Some bacteria use SO₄²⁻ or H₂S
- Phosphorus
 - In DNA, RNA, ATP, and membranes
 - PO₄³⁻ is a source of phosphorus
- Trace Elements
 - Inorganic elements required in small amounts
 - Usually as enzyme cofactors

Other Chemical Requirements

- Organic Growth Factors
 - Organic compounds obtained from the environment
 - Vitamins, amino acids, purines, pyrimidines

Oxygen Concentration

- Oxygen metabolites are toxic.
- These toxic metabolites must be neutralized for growth.
- Aerobes- Able to grow in the presence of atmospheric O₂.
- Anaerobes- grow in the absence of O₂.
- Obligate aerobes- completely dependent on atmospheric O₂ for growth.
- Facultative anaerobes- do not require O₂ for growth, but grow better in its presence.
- Aerotolerant anaerobes- grow equally well in the presence or absence of O₂.
- Strict anaerobes- do not tolerate O₂ at all and die in its presence.
 - Aerotolerant and strict anaerobes do not generate energy through respiration and either need to use fermentation or anaerobic respiration to fill their energy requirements.
- Microaerophiles are damaged by normal levels of atmospheric O₂ and require lower levels to survive.

Toxic Forms of Oxygen

- Singlet oxygen: O₂ boosted to a higher-energy state
- Superoxide free radicals: O₂⁻
- Hydrogen peroxide: H₂O₂
- Hydroxyl radical: OH[•]
- Many microorganisms possess enzymes that protect themselves against these forms of oxygen.
- Obligate aerobes and facultative anaerobes usually contain superoxide dismutase and

catalase.

Culture Media

- Culture Medium: Nutrients prepared for microbial growth
- Sterile: No living microbes
- Inoculum: Introduction of microbes into medium
- Culture: Microbes growing in/on culture medium

Agar

- Complex polysaccharide
- Used as solidifying agent for culture media in Petri plates, slants, and deeps
- Generally not metabolized by microbes
- Liquefies at 100°C
- Solidifies ~40°C
- Chemically Defined Media: Exact chemical composition is known
- Complex Media: Extracts and digests of yeasts, meat, or plants
 - Nutrient broth
 - Nutrient agar

Anaerobic Culture Methods

- Reducing media
 - Contain chemicals (thioglycollate or oxyrase) that combine O₂
 - Heated to drive off O₂

Anaerobic Culture Methods

- Anaerobic jar
- Anaerobic chamber

Capnophiles require high CO₂

- Candle jar
- CO₂-packet

Selective Media

- Suppress unwanted microbes and encourage desired microbes.

Differential Media

- Make it easy to distinguish colonies of different microbes.

Enrichment Media

- Encourages growth of desired microbe.
- Assume a soil sample contains a few phenol-degrading bacteria and thousands of other bacteria.
 - Inoculate phenol-containing culture medium with the soil and incubate
 - Transfer 1 ml to another flask of the phenol medium and incubate
 - Transfer 1 ml to another flask of the phenol medium and incubate
 - Only phenol-metabolizing bacteria will be growing
- A pure culture contains only one species or strain
- A colony is a population of cells arising from a single cell or spore or from a group of

- attached cells
- A colony is often called a colony-forming unit (CFU)

Preserving Bacteria Cultures

- Deep-freezing: -50°C to -95°C
- Lyophilization (freeze-drying): Frozen (-54° to -72°C) and dehydrated in a vacuum
- Cryovials or Cryobeads

Reproduction in Prokaryotes

- Binary fission
- Budding
- Conidiospores (actinomycetes)
- Fragmentation of filaments

Binary fission

- The division of a bacterial cell
- Parental cell enlarges and duplicates its DNA
- Septum formation divides the cell into two separate chambers
- Complete division results in two identical cells

Generation time

- The time required for a complete division cycle (doubling)
- Length of the generation time is a measure of the growth rate
- Exponentials are used to define the numbers of bacteria after growth

Growth curve

- Lag phase
- Log phase
- Stationary phase
- Death phase

Lag phase

- Cells are adjusting, enlarging, and synthesizing critical proteins and metabolites
- Not doubling at their maximum growth rate

Log phase

- Maximum exponential growth rate of cell division
- Adequate nutrients
- Favorable environment

Stationary phase

- Survival mode – depletion in nutrients, released waste can inhibit growth
- When the number of cells that stop dividing equal the number of cells that continue to divide

Death phase

- A majority of cells begin to die exponentially due to lack of nutrients
- A chemostat will provide a continuous supply of nutrients, thereby the death phase is never achieved.

Chemostat

- Continuous culture system that admits a steady stream of nutrients
- Siphons off used media and old bacterial cells to stabilize growth rate and cell number
- Maintains the culture in a biochemically active state and prevents it from entering the death phase

Practical Importance of the Growth Curve

- Antimicrobial agents rapidly accelerate the death phase
 - Microbes in the exponential phase are more vulnerable to these agents than those in the stationary phase
 - Actively growing cells are more vulnerable to conditions that disrupt cell metabolism and binary fission
- Growth patterns can correspond with the stages of infection:
 - Bacterial shedding during the early and middle stages of infection is more likely to spread it to others

Direct Measurements of Microbial Growth

- Plate Counts: Perform serial dilutions of a sample

Plate Count

- Inoculate Petri plates from serial dilutions

Plate Count

- After incubation, count colonies on plates that have 25-250 colonies (CFUs)

Direct Measurements of Microbial Growth

- Filtration
- Multiple tube MPN test
- Count positive tubes and compare to statistical MPN table.
- Direct Microscopic Count
- Coulter Counter or other automated counting device

Estimating Bacterial Numbers by Indirect methods

- Turbidity
- Metabolic activity
- Dry weight

New Methods of Analyzing Population Growth

- Polymerase chain reaction (PCR)
 - Quantifies bacteria and other microorganisms in environmental and tissue samples without isolating and culturing them
- Tests that measure ATP:
 - Used in food and pharmaceutical industries
 - May be used for rapid quantification of microbes in other environmental samples