Microbial Growth

- Growth : When we speak of bacterial growth, we are talking about an increase in population size not an increase in the size of a single bacterium
- Binary Fission: Bacterial cells replicate by a form of asexual reproduction called binary fission
- <u>Generation Time</u> time required to complete fission cycle from parent cell to 2 daughter cells. (Doubling time). In terms of a population it is the amount of time needed to double the population.
- The length of the generation time is a measure of the <u>Growth</u> <u>Rate</u> of the microbe.
- It varies depending on environmental conditions. , Different microbes have different generation times.
 - <u>Mycobacterium leprae</u> \rightarrow 10-30 days
 - <u>Staphylococcus aureus</u> \rightarrow 20-30 minutes

Growth of Microbial Populations



Microbial Growth Cycle (batch culture)

- <u>Batch culture</u>: a closed-system microbial culture of fixed volume
- Typical growth curve for population of cells grown in a closed system is characterized by four phases
 - Lag phase, Exponential phase, Stationary phase,

Death phase

The Growth Curve



Microbial Growth Cycle (batch culture)

- <u>Lag phase</u>
 - Interval of time between when a culture is inoculated and when growth begins.
- Exponential phase
 - Cells in this phase are typically in the healthiest state. Growth is at maximal rate.

• <u>Stationary phase</u>

- Growth rate of population is zero.
 - Number new divisions=number of cells dying
- Either an essential nutrient is used up or waste product of the organism accumulates in the medium
- <u>Death phase</u>
 - Lack of nutrients and increasing accumulation of wastes lead to... number of cell deaths > number of new divisions

Continuous Culture: The Chemostat

- <u>Continuous culture</u>: an open-system microbial culture of fixed volume
- <u>*Chemostat*</u>: most common type of continuous culture device



Measuring Growth (Direct Measurement)

Viable Count

- Measurement of living, reproducing population
- Two main ways to perform plate counts
 - <u>Spread-plate method</u>
 - <u>Pour-plate method</u>
- To obtain the appropriate colony number, the sample to be counted may need to be diluted (serial dilutions)

• Spread-Plate Method for the Viable Count





Sample is pipetted onto surface of agar plate (0.1 ml or less)



Sample is spread evenly over surface of agar using sterile glass spreader

mixed well with inoculum

Typical spread-plate results

Surface colonies



Pour-Plate Method for the Viable Count



sterile plate





Procedure for Viable Counting Using Serial Dilutions

Measuring Growth (Direct Measurement)

Filtration



Measuring Growth (Indirect Measurement)

- Indirect Methods
 - Metabolic activity
 - Dry weight
 - Turbidity

• Spectrophotometer-measures amount of light that passes through a sample. Absorbance is related to the number of bacteria

Turbidity





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Effects of Temperature on Microbial Growth

- Microorganisms can be classified into groups by their growth temperature optima
 - Psychrophile: low temperature
 - Mesophile: midrange temperature
 - Thermophile: high temperature
 - Hyperthermophile (extreme thermophile): very high temperature

- Organisms sensitive to changes in acidity because H⁺ and OH⁻ interfere with H bonding in proteins and nucleic acids
- Most bacteria and protozoa grow best in a narrow range around neutral pH (6.5-7.5) – these organisms are called **neutrophiles**
- Other bacteria and fungi are **acidophiles** 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

- Microbes require water to dissolve enzymes and nutrients required in metabolism
- Water is important reactant in many metabolic reactions
- Most cells die in absence of water
 - Some have cell walls that retain water
 - Endospores and cysts cease most metabolic activity in a dry environment for years
 - Two physical effects of water: Osmotic pressure
 - Hydrostatic pressure

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Osmotic Pressure

- Is the pressure exerted on a semipermeable membrane by a solution containing solutes that cannot freely cross membrane; related to concentration of dissolved molecules and ions in a solution
- Hypotonic solutions have lower solute concentrations; cells placed in these solutions will swell and burst\
- Hypertonic solutions have greater solute concentrations; cells placed in these solutions will undergo crenation (shriveling of cytoplasm)
 - This effect helps preserve some foods
- Restricts organisms to certain environments
 - Obligate halophiles grow in up to 30% salt
 - Facultative halophiles can tolerate high salt concentrations

Hydrostatic Pressure

- Water exerts pressure in proportion to its depth
- Organisms that live under extreme pressure are **barophiles**
 - Their membranes and enzymes depend on this pressure to maintain their three-dimensional, functional shape

Oxygen and Microbial Growth

- <u>Aerobes</u>: require oxygen to live
- <u>Anaerobes</u>: do not require oxygen and may even be killed by exposure
- *Facultative organisms*: can live with or without oxygen
- <u>*Microaerophiles*</u>: can use oxygen only when it is present at levels reduced from that in air

Growth : Oxygen Concentration



- Superoxide dismutase (SOD)
- Catalase
- Peroxidase