Microbial Growth
and
The Control of Microbial Growth
(Chapter 6 & 7)

Lecture Materials
for
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Primary Source for figures and content:

Chapter 6: Microbial Growth

Microbial requirements for growth:

1. Physical
   A. Temperature
      Optimal growth temperature
      Permissible range
      human pathogens optimal = 37°C
   B. pH
      most organisms optimal pH 6.5-7.5
   C. Osmotic pressure
      Most require isotonic solutions
      human blood = 0.9% NaCl, isotonic
      human skin = ~3-6% NaCl, hypertonic

2. Chemical
   A. Carbon
   B. Nitrogen
   C. Sulfur
   D. Phosphorus
      (all above: to build organic molecules)
   E. Trace elements: K⁺, Mg²⁺, Ca²⁺, Fe²⁺...
      cofactors for enzymes
F. Oxygen
   aerobe, anaerobe, facultative
G. Organic factors: vitamins, amino acids...
   (essential organic compound an organism is unable to synthesize)

Microbial Growth
- for unicellular organisms, growth = increase in cell number (population) not individual cell size
- generation time = time required for a cell to divide
- both growth and death of the population is exponential, graphed on a log scale
1. Lag Phase: little to no growth, bacteria acclimate to new environment.

2. Log Phase: period of exponential growth with constant generation time.

3. Stationary Phase: cell growth is equal to cell death.

Chapter 7: Control of Microorganisms

Sterilization = destruction or removal of all forms of microbial life, including endospores

Commercial sterilization = (canning) enough heat to kill endospores of Clostridium botulinum (thermophilic species survive)

Disinfection = destruction of vegetative pathogens on inert surfaces (disinfectant)

Antisepsis = disinfection of living tissue (antiseptic)

Degermation = mechanical removal of microbes in a limited area

Sanitization = lower microbe counts to a “safe” level to minimize disease transmission

suffix “–cide” = kill
  e.g. biocide, fungicide

suffix “-stat” or “-stasis” = stop
  inhibit growth: when agent removed, growth can resume e.g. fungistat
sepsis = bacterial contamination present
asepsis = absence of significant bacterial contamination (not sterile)
in clinical terms, absence of pathogens

Microbial Death Rates:
microbial populations that are heated or treated with chemicals will die at a constant rate (exponential decline)

(a) The curve is plotted logarithmically (solid line) and arithmetically (broken line). In this case, the cells are dying at a rate of 90% each minute.

(b) The effect of high or low initial load of microbes. If the rate of killing is the same, it will take longer to kill all members of a larger population than a smaller one. This is true for both heat and chemical treatments.
Factors that influence effectiveness of an antimicrobial treatment:

1. Number of microbes
   - more cells, more time needed to kill all

2. Environmental influences
   - organics often inhibit chemical agents
   - temperature
   - pH

3. Time of exposure
   - same agent may need longer on resistant organisms or spores
   - with heat, lower temps require longer to kill

4. Microbial characteristics
   - resistance genes, protective structures (e.g. capsules) etc. can inhibit action
   - biofilms prevent penetration
Actions of Microbial Control Agents:

1. Alteration of membrane permeability:
   - damage to proteins or lipids of membrane
     \[ \text{leak} \rightarrow \text{lysis, death} \]

2. Damage to proteins and/or nucleic acids
   - denature enzymes (no reactions)
   - prevent replication, transcription, or translation

Physical Methods of Microbial Control:

- to disinfect objects, food, and solutions
- common methods:
  - temperature: kill or inhibit growth
  - filtration: physical removal
  - desiccation: inhibit growth
  - radiation: kill
For methods that involve heat:
Thermal Death Point (TDP) = lowest temp at which all microbes in liquid suspension will be killed in 10 min
Thermal Death Time (TDT) = minimal length of time for all microbes in liquid suspension to be killed at given temp
*Both are different for different species due to microbial variation in heat tolerance
Concept of equivalent treatments:
With any heat treatment, the higher the temperature used the shorter the exposure time needed to achieve the same effect
Moist heat will always kill faster than dry heat at the same temperature

Examples of physical methods:
(on handout)
autoclave

Exhaust valve (to remove steam after sterilization)

Steam to chamber

Safety valve

Pressure gauge

Operating valve (controls steam from jacket to chamber)

Steam

Steam chamber

Air

Perforated shelf

Steam jacket

Sediment screen

Thermometer

Pressure regulator for steam supply

To waste line

Steam supply

Automatic ejector valve is thermostatically controlled and closes on contact with pure steam when air is exhausted.
low temperature

Temperatures in this range destroy most microbes, although lower temperatures take more time.

- **Very slow bacterial growth.**
- **Rapid growth of bacteria; some may produce toxins.**
- **Many bacteria survive; some may grow.**
- **Refrigerator temperatures; may allow slow growth of spoilage bacteria, very few pathogens.**
- **No significant growth below freezing.**

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Approximate temperature range at which *Bacillus cereus* multiplies in rice.
filtration

*Figure showing a filter setup with a flask of sample being poured through a membrane filter. The cotton plug in the vacuum line ensures sterility.*

osmotic pressure

**Plasma membrane**

**Cell wall**

**Cytoplasm**

**NaCl 0.85%**

**(a) Normal cell in isotonic solution.** Under these conditions, the solute concentration in the cell is equivalent to a solute concentration of 0.85% sodium chloride (NaCl). See Figure 4.18.

**H₂O**

**Plasma membrane**

**Cytoplasm**

**NaCl 10%**

**(b) Plasmolyzed cell in hypertonic solution.** If the concentration of solutes such as NaCl is higher in the surrounding medium than in the cell (the environment is hypertonic), water tends to leave the cell. Growth of the cell is inhibited.
Chemical Methods of Microbial Control
-used to “disinfect” surfaces and people
Chemical antimicrobial agents should:
1. be effective against wide range of microbes
2. be effective in presence of organic material
3. be toxic to microbe but non-toxic to people and non-corrosive to surfaces
-hard to find all in one agent: usually have to select best agent based on use application
-most chemical agents that are “safe” do not sterilize: they reduce microbe levels, but only if used at the correct concentration
Use Dilution Test:
- determine conc. of chemical agent necessary to kill 95% test organisms in 10 min
- used to determine effectiveness of disinfectant and antiseptics against various microbes

Disk-Diffusion Method
- determine efficacy of a chemical agent
- score for zone of no growth

Chemical Agents
(take notes on handout)
Microbial characteristics and microbial control
-some characteristics provide resistance:

* *Pseudomonas & Burkholderia:*
  - porins in cell wall prevent chemical entry
  - can live on any organics, even in detergents & antiseptics
* *Mycobacterium: waxy mycolic acid prevents chemical entry*
* *Endospores and cysts: thick coats prevent chemical entry*
* *Enveloped viruses more easily damaged than non-enveloped*
* *Prions are resistant to most chemical methods and even autoclaving*

Typical resistance to biocides →
Prions
Endospores of bacteria
Mycobacteria
Cysts of protozoa
Vegetative protozoa
Gram-negative bacteria
Fungi, including most fungal spore forms
Viruses without envelopes
Gram-positive bacteria
Viruses with lipid envelopes