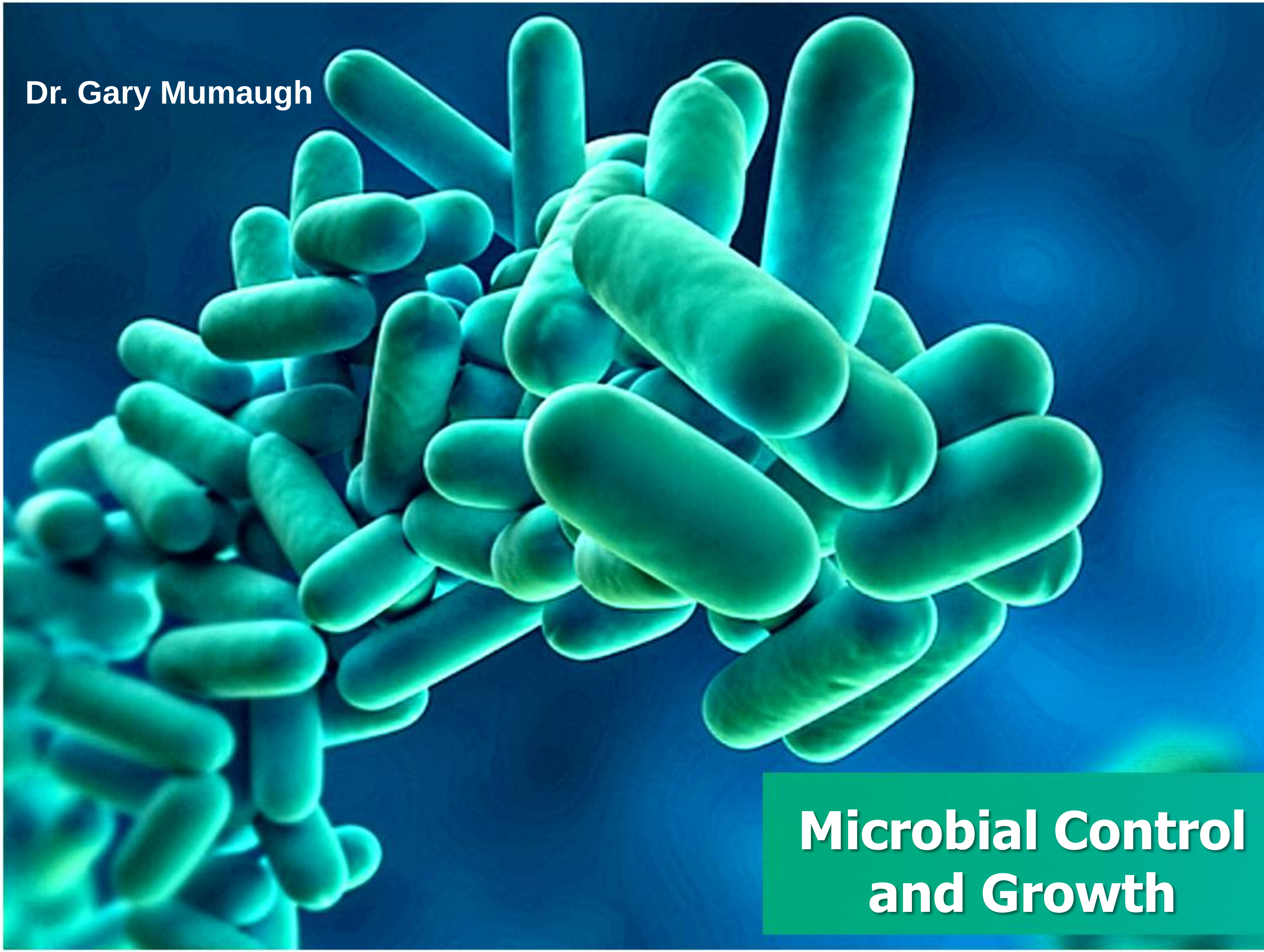

Dr. Gary Mumaugh
Microbial Control and Growth

- **Microbial Growth**
- **Microbial Control**
  - Sterilization
  - Selective Removal
  - Temperature
- **Types of Heat Treatment**
  - Incineration
  - Tyndallization
  - Autoclaving
- **Microbial Control**
  - Filtration
  - Reduction of water activity
  - Chemical treatments
  - Antiseptics and disinfectants

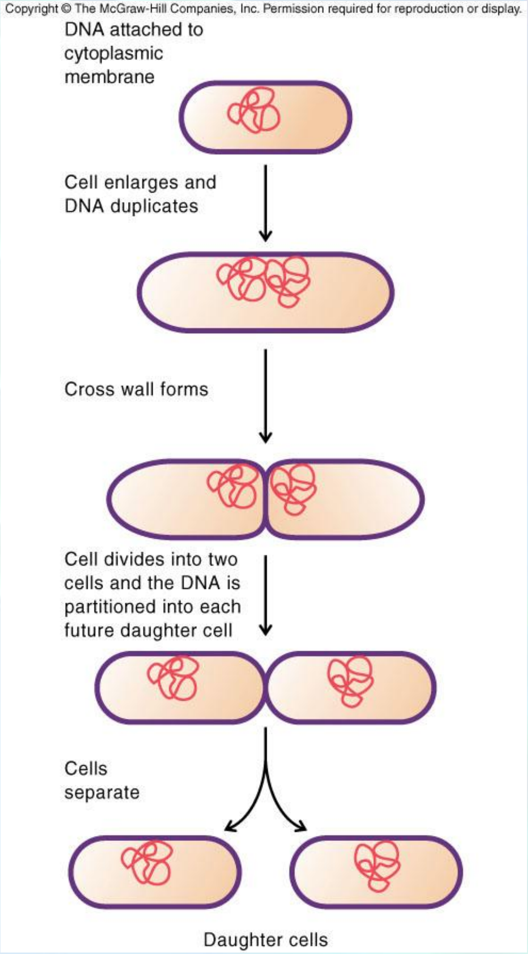
Microbial Growth: Societal Factors

- In people and animals, unwanted microbes can lead to disease
- In plants, they can damage crops and hurt the environment
- Within industrial structures, e.g., pipelines and transit systems, they can produce wear and tear, oxidation (rust), and other forms of erosion
- Knowledge about microbial growth and how to control it has dramatically helped human society

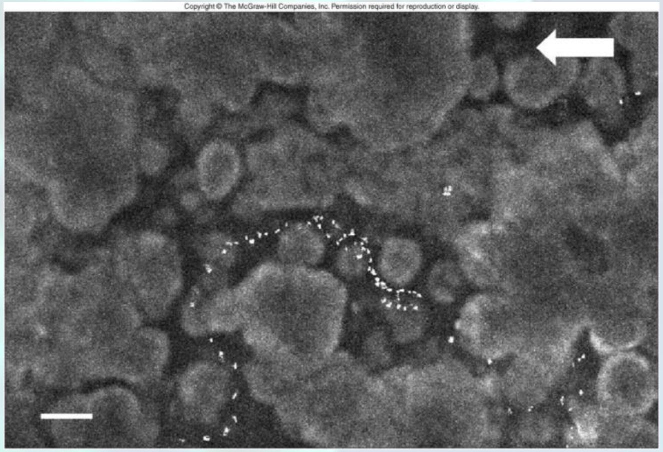
- The purpose of studying microbial control is far ranging from complete eradication to a slight inhibition of their growth

Principles of Bacterial Growth

- Prokaryotic cells divide by binary fission
  - One cell divides into two
    - Two into four etc.
  - Cell growth is exponential
    - Doubling of population with each cell division
    - Exponential growth has important health consequences
  - Generation time
    - Time it takes for population to double
    - a.k.a. doubling time
    - Varies among species



Bacterial Growth in Nature



Biofilm layer

- Conditions in nature have profound effect on microbial growth
  - Cells sense changing environment
    - Synthesize compounds useful for growth
    - Cells produce multicellular associations to increase survivability
      - Example
        - » Biofilms
        - » Slime layers

Bacterial Growth in Nature

- Biofilm
  - Formation begins when bacteria attach to surfaces
    - Other bacteria attach and grow on initial layer
  - Has characteristic architecture
    - Contains open channels for movement of nutrients and waste
  - Cells within biofilms can cause disease
    - Treatment becomes difficult
  - Factors in determining where biofilms form are: Location, Location and Location
  - Biofilms will form anywhere there is moisture and a surface with at least a minimal nutrient source

Biofilm in Healthcare

- In the 1990s doctors began to make the connection between chronic, low-grade infections and the biofilm mode of growth
- Dental professionals made the connection easily, as teeth could readily be scraped for microscopic examination
- Internal cases of chronic infection have taken longer to prove, but testing has shown that many troublesome diseases have entrenched microbial populations at their core
- Biofilms are everywhere!

Stream in Yellowstone National Park


Nearly 30% of food processing
drains tested positive for listeria
source: USDA

Bacterial Growth in Nature

- Interactions of mixed microbial communities
  - Prokaryotes live in mixed communities
    - Many interactions are cooperative
      - Waste of one organism nutrient for another
    - Some cells compete for nutrient
      - Synthesize toxic substance to inhibit growth of competitors

Obtaining Pure Culture

- Pure culture defined as population of cells derived from single cell
  - All cells genetically identical
- Cells grown in pure culture to study the activities of specific species
- Pure culture obtained using special techniques
  - Aseptic technique
    - Minimizes potential contamination
- Cells grown on culture media
  - Can be broth (liquid) or solid form

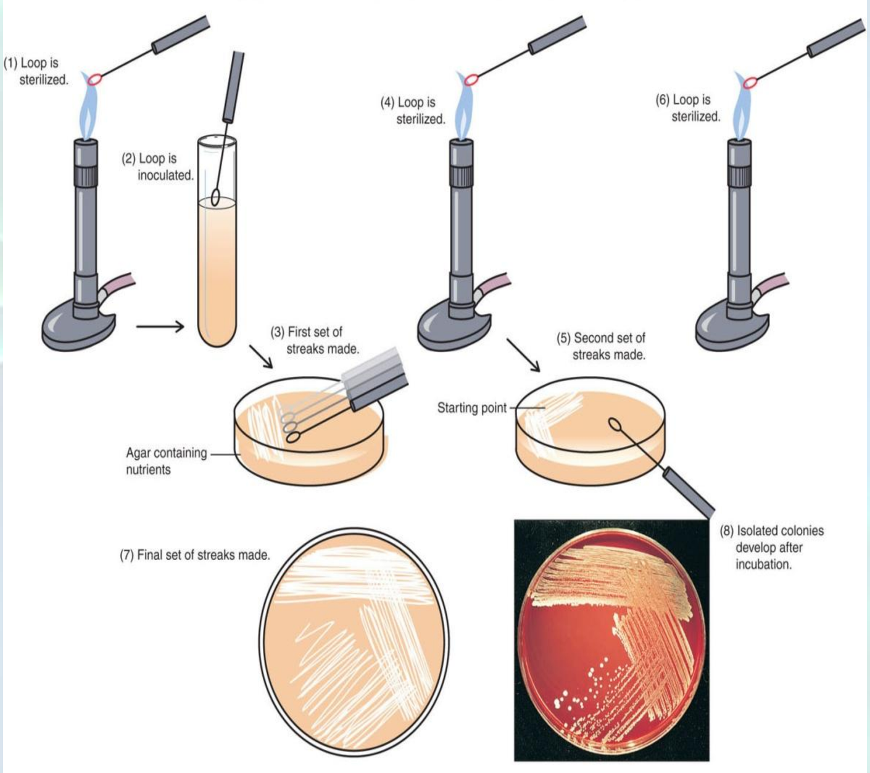
Obtaining Pure Culture

- Culture media can be liquid or solid
  - Liquid is broth media
    - Used for growing large numbers of bacteria
  - Solid media is broth media with addition of **agar**
    - Agar marine algae extract
    - Liquefies at temperatures above 95°C
    - Solidifies at 45°C

  - Bacteria grow in colonies on solid media surface
    - All cells in colony descend from single cell
    - Approximately 1 million cells produce 1 visible colony

Obtaining Pure Culture

- Streak-plate method
  - Simplest and most commonly used in bacterial isolation
  - Object is to reduce number of cells being spread
    - Solid surface dilution
    - Each successive spread decreases number of cells per streak



Environmental Factors on Growth

- As group, prokaryotes are everywhere
  - Some live in “comfortable” habitats
  - Some live in harsh environments
    - Most of these are termed extremophiles and belong to domain *Archaea*
- Major conditions that influence growth
  - Temperature
  - Oxygen
  - pH
  - Water availability

Temperature Affecting Growth

- Each species has well- defined temperature range
  - Within range lies optimum

Psychrophile -5°C to 15°C (23-50 F)
  - Found in Arctic and Antarctic regions
- Psychrotroph 20°C to 30°C (68-88 F)
  - Important in food spoilage
- Mesophile 25°C to 45°C (77-113 F)
  - More common
  - Disease causing
- Thermophiles 45°C to 70°C (113-158 F)
  - Common in hot springs
- Hyperthermophiles 70°C to 110°C (158-230 F)
  - Usually members of *Archaea*
  - Found in hydrothermal vents

Oxygen Affecting Growth

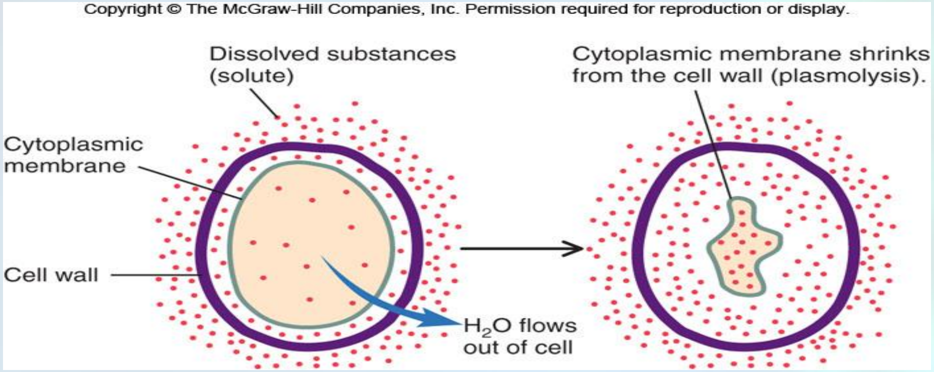
- Obligate aerobes
  - Absolute requirement for oxygen
    - Use for energy production
- Obligate anaerobes
  - No multiplication in presence of oxygen
    - May cause death
- Facultative anaerobes
  - Grow better with oxygen
    - Use fermentation in absence of oxygen
- Microaerophiles
  - Require oxygen in lower concentrations
    - Higher concentration inhibitory
- Aerotolerant anaerobes
  - Indifferent to oxygen, grow with or without
    - Do not use oxygen to produce energy

pH Affecting Growth

- Bacteria survive within various pH range
  - Neutrophiles
    - Multiply between pH of 5 to 8
- Acidophiles
    - Thrive at pH below 5.5
- Alkalophiles
    - Grow at pH above 8.5
    - Maintain neutral internal pH through sodium ion exchange

Water Affecting Growth

- All microorganisms require water for growth
- Water not available in all environments
  - In high salt environments
    - Bacteria increase internal solute concentration
    - Osmotolerant bacteria tolerate high salt environments
    - Bacteria that require high salt for cell growth termed halophiles



Nutritional Factors Affecting Growth

- Growth of prokaryotes depends on nutritional factors as well as physical environment
- Main factors to be considered are:
  - Required elements
  - Growth factors
  - Energy sources
  - Nutritional diversity

Nutritional Factors Affecting Growth

- Required elements

  Major elements

    - Carbon, oxygen, hydrogen, nitrogen, sulfur, phosphorus, potassium, magnesium, calcium and iron
      - Essential components for macromolecules

  - Organisms classified based on carbon usage
    - Heterotrophs
      - Use organism carbon as nutrient source
    - Autotrophs
      - Use inorganic carbon (CO_2) as carbon source
  - Trace elements
    - Cobalt, zinc, copper, molybdenum and manganese
      - Required in minute amounts

Nutritional Factors on Growth

- Energy Sources
  - Organisms derive energy from sunlight or chemical compounds
    - Phototrophs
      - Derive energy from sunlight
    - Chemotrophs
      - Derive energy from chemical compounds
  - Organisms often grouped according to energy source

Approaches to Control

Microbial Control: Sterilization

- Complete removal of all life forms from a given area (Includes viruses)
- Sterilization treatments are among the most dramatic of all 'growth control' methods
- Important for instruments used in medical procedures, food-related items and laboratory cultures
- Preventing cross-contamination is extremely important

Microbial Control: Selective Control

- This method is applicable in cases when only a select group or division of microbes are known to be 'harmful' and that the removal of this group will not damage the product
- Example of 'selective removal' is pasteurization
  - Is a process by which milk is heated in an effort to kill off lethal pathogens
    - e.g., Strains of salmonella and E. coli
    - Typically, the pasteurization process is used to kill-off harmful bacteria

- Sometimes pasteurization is used to isolate harmless bacteria as opposed to kill it
- Example of the separation method is bread
  - Contains a preservative known as sodium benzoate--this inhibits the growth of molds and prolongs the shelf life of the product

Microbial Control: Temperature

- Temperature (both extreme hot and cold variances) are a part of the previously mentioned pasteurization method
- Critical enzymes are either killed outright or are dematured
- Two methods of heat to curtail the growth of microbes
  - Dry heat--a process involving incubation in an oven-like environment
  - Moist heat--a process utilizing steam within a pressure-oriented encasement

Types of Heat Treatments Incineration

- Ancient heat-killing methods
- Typically destroys all living things, in addition, to the sample in which they are contained
- Incineration was primarily used to ward off the spread of infectious disease
  - In the 14th century--during the time of the black plague, individuals were known to burn the corpses (along with the material possessions) of those who died of the epidemic
  - Incineration is still required by law for the disposal of body parts and the removal of animals suspected of being infected with anthrax

Types of Heat Treatments Tyndallization

- Archaic method of sterilization was repeated boiling
  - While boiling a solution for 30 minutes at room temperature is apt to kill-off the majority of vegetative cells, it will not have the same effect upon bacterial endospores.
- Boiled then cooled; incubated for several hours; and then re-boiled. The entire cycle is then repeated three times.
- Used to sterilize media before the invention of the autoclave
  - Major drawback was that it was time-consuming

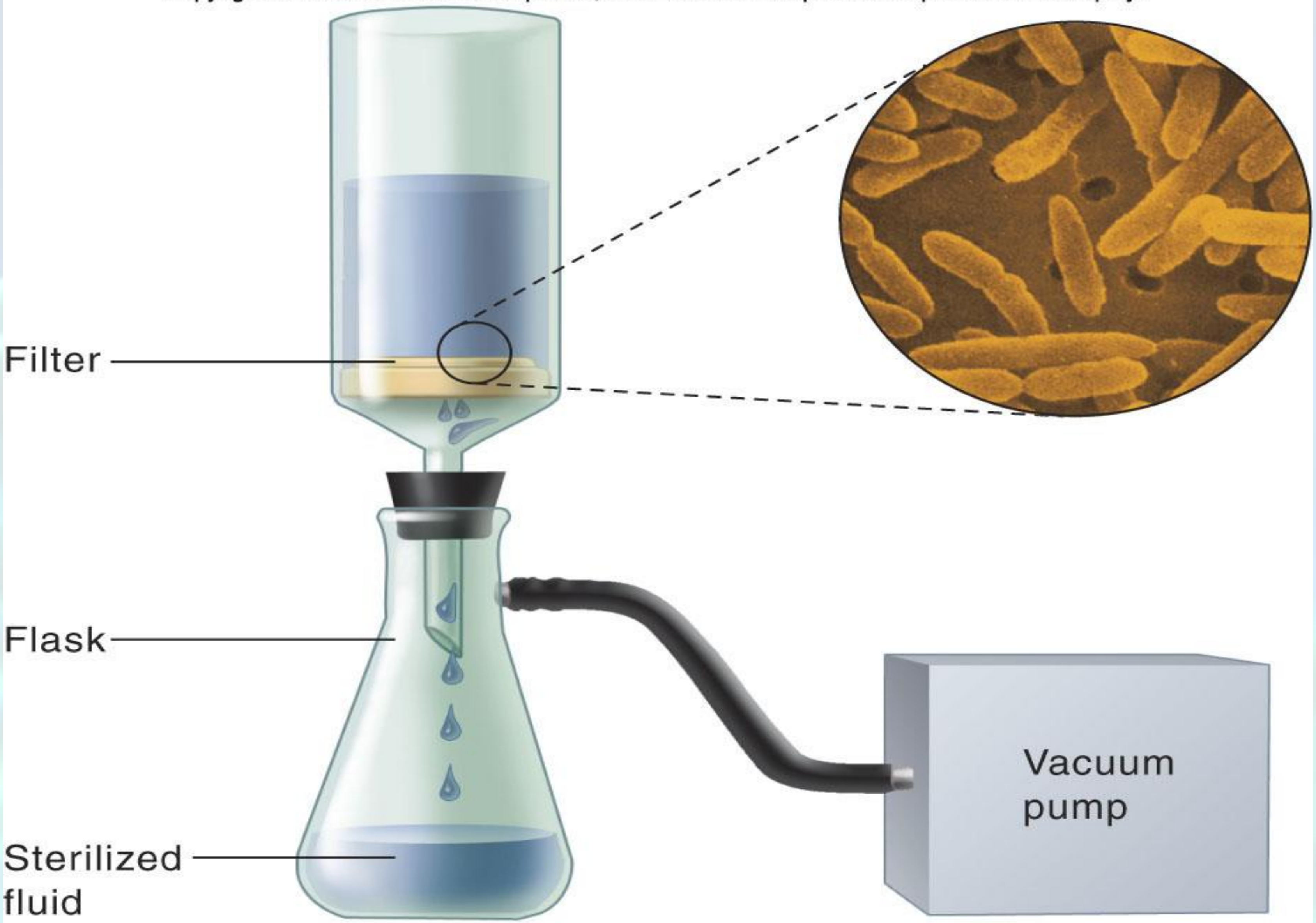
Microbial Control: Autoclaving

- Most common method of sterilization currently used in laboratories and hospital settings
- Complex pressure cooker that employs stream under pressure to raise the temperature to 121 C for at least 15 minutes
- At this elevated temperature all living cells, including endospores and viruses, are killed

Microbial Control: Filtration

- Used to remove microbes from gases & liquids
- Examples include the brewing process whereby yeast is removed before final bottling
- Because it is ineffective in removing viruses from a solution, filtration cannot technically be considered a form of sterilization
- The process is often referred to as 'filter sterilization'

- Three major types of filters
  - Depth Filters
    - The oldest form, consisting of overlapping layers of fibrous sheets of paper, asbestos or glass fibers
    - Are able to remove the bulk of unwanted materials
  - Membrane Filter
    - The most common form in microbiology labs
  - Nucleopore Membranes
    - Exposing a very thin polycarbonate film (10 m) to nuclear radiation


Filter
Flask
Sterilized
fluid
Vacuum
pump

Microbial Control: Removal of Water Activity

Water may be removed from foods by any of the following four methods:

1. * Heating
2. * Evaporation
3. ◆Freeze-drying
4. ◆Addition of salt or sugar

* Directly remove water from the sample

◆ Relies on salt and sugar to bind up the water

Microbial Control: Chemical Treatments

- The advantage is that they offer continuous protection
  - In low dosages, the majority of chemicals are unlikely to change the physical environment
  - Adding a chemical to a sample can alter the finished product and leave behind unwanted residual effects
  - In medical situations where significant amounts of antimicrobial chemicals are used for the purpose of killing-off lethal types of microbes (e.g. cancerous agents)

Antimicrobial Agents

- Potency to kill-off or inhibit microorganisms
- Many forms of synthetic and natural compounds have antimicrobial qualities
- - static Chemicals that stop microbes from growing
- - cidal Kills cells
- - lytic in addition to killing microbes also cause them to lyse (dissolve or destruct cells, e.g. blood cells or bacteria)

Antiseptics and Disinfectants

- Antiseptics are used to prevent infection or sepsis
- Disinfectants differ in that they are not safe to apply to living tissues
- A distinction between antiseptics and disinfectants depends upon concentration
  - Common household disinfectants include chlorine compounds, as well as lye, copper sulfate and quaternary ammonium compounds.
  - Some disinfectants are powerful enough to eliminate all life forms from an area and are given the name sterilants.

- **A number of factors affect the potency of disinfectants:**
- Biofilms
  - have the ability to retard or even prevent the diffusion of disinfectants to the microbes, eliminating the effectiveness of the compound
- High concentrations of organic compounds
  - decrease the potency of disinfectants
- Endospores
  - tend to be more resistant to disinfectants than vegetative cells