Bacterial Growth and Nutrition

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Objectives

- Growth definition and classification
- Population growth - growth curve
- Population growth – Methods
- Physical parameters that affect growth
- Chemical parameters that affect growth
- Bacterial growth measurement

Introduction

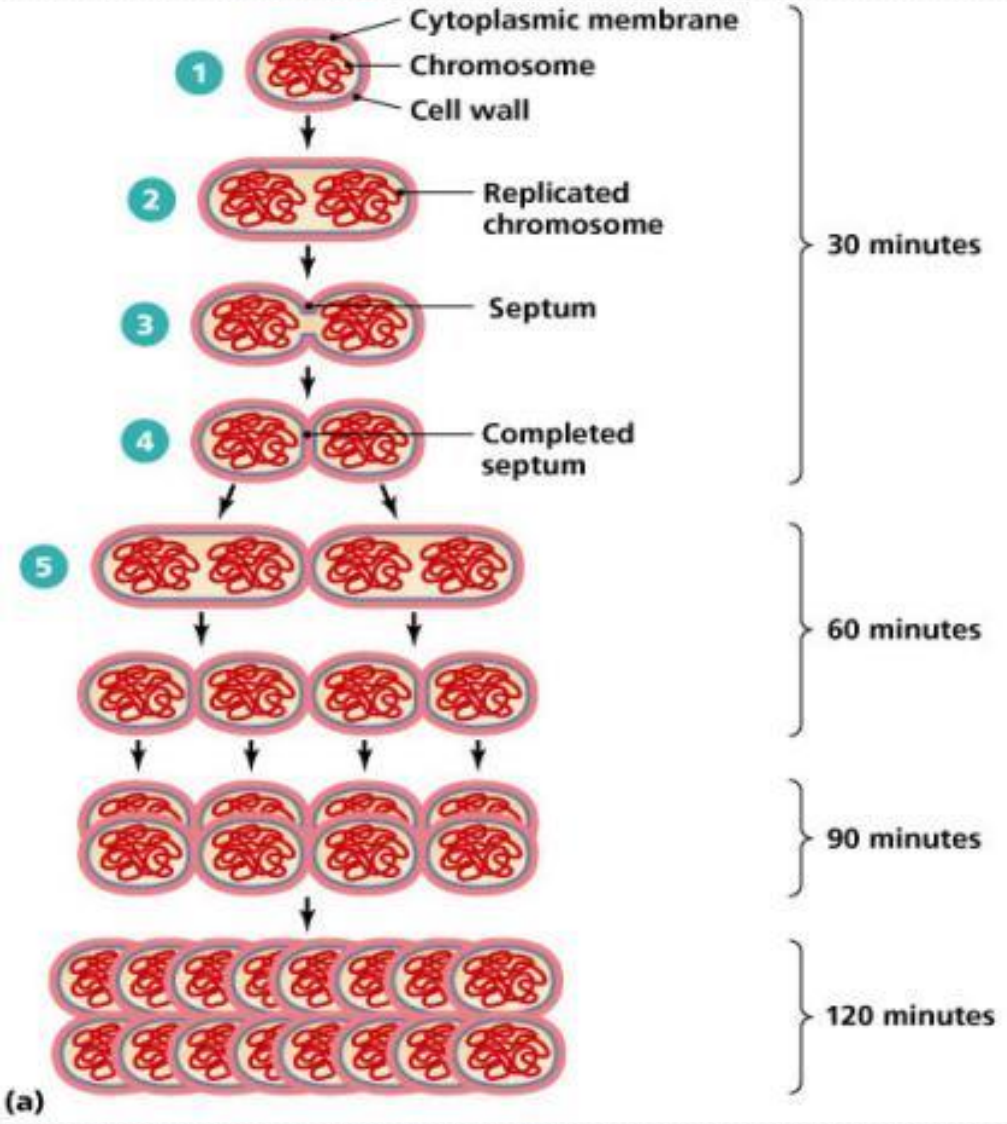
- Growth: Orderly increase in the sum of all the components of an organism, which reflects increase in number of cells
- Importance of understanding bacterial growth:

  Bacterial survival and transmission

  In vitro diagnostic (laboratory culture)

  Cessation of bacterial growth for treatment

Microbial growth/Binary Fission



Rapid Growth of Bacteria

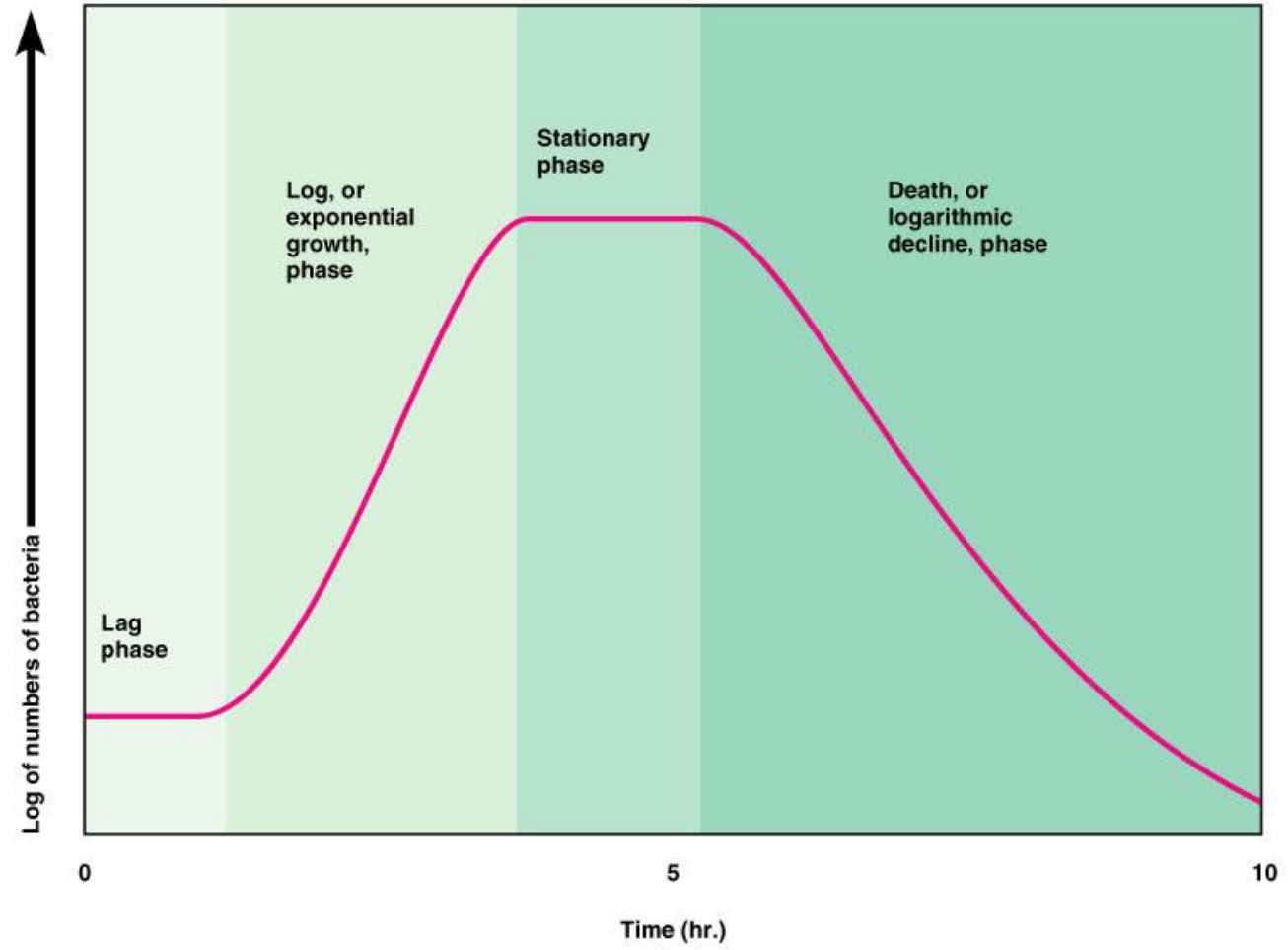
Arithmetic Numbers of Cells	Numbers Expressed as a Power of 2	Visual Representation of Numbers
1	2^0	●
2	2^1	●●
4	2^2	●●●●
8	2^3	●●●●●●●●
16	2^4	●●●●●●●●●●●●●●●●
32	2^5	●●●●●●●●●●●●●●●●●●●●●●●●●●●●●●●●

Generation time under optimal conditions

Generation time is the time it takes for a single cell to grow and divide

Organism	Generation Time
Escherichia coli	12.5 min
Staphylococcus aureus	27-30 min
Mycobacterium tuberculosis (agent of Tuberculosis)	18 – 24 hrs
Treponema pallidum (agent of Syphilis)	30 hrs

The Growth Curve



- During lag phase, cells are recovering from a period of no growth and are making macromolecules in preparation for growth
- During log phase cultures are growing maximally
- Stationary phase occurs when nutrients are depleted and wastes accumulate (Growth rate = death rate)
- During death phase death rate is greater than growth rate

Factors Affecting Bacterial Growth

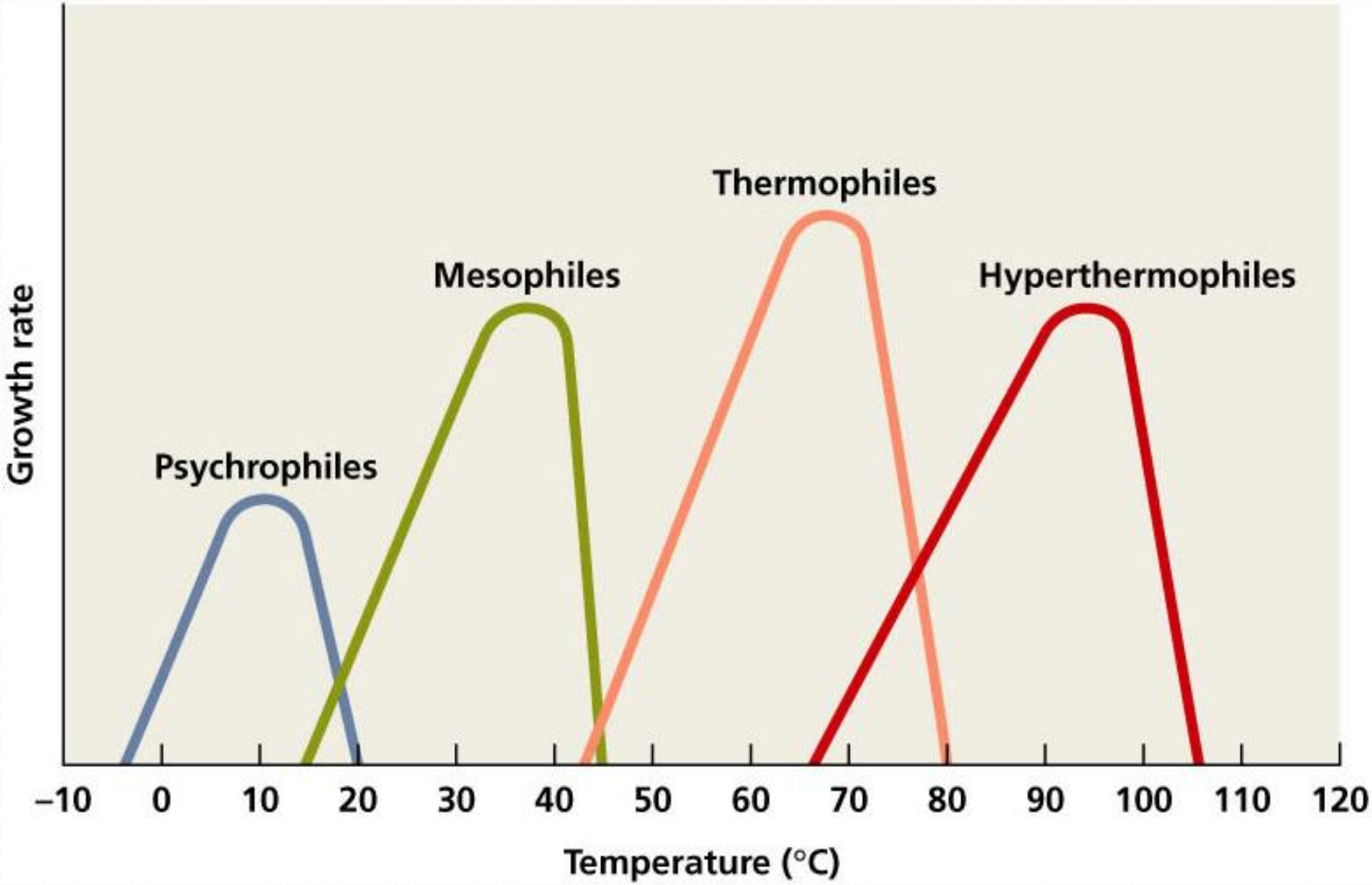
- Temperature
- pH
- Osmotic pressure
- Oxygen
- Nutrition

Temperature

- Hydrogen bonds will break at high temperatures leads to protein denaturation
- Lipids will be more liquid
- Outside membrane cannot preserve the integrity of the cell and it will disintegrate

- Minimum Temperature: Temperature below which growth ceases, or lowest temperature at which microbes will grow
- Optimum Temperature: Temperature at which its growth rate is the fastest
- Maximum Temperature: Temperature above which growth ceases, or highest temperature at which microbes will grow

Classification of Microorganisms by Temperature



Temperature Classes of Organisms

- **Psychrophiles (0-20°C)**
  - **Cold temperature optima**
  - **Most extreme representatives inhabit permanently cold environments**
- **Mesophiles (20 – 45°C)**
  - **Midrange temperature optima**
  - **Found in warm-blooded animals and in aquatic environments in temperate and tropical latitudes**
- **Thermophiles (50- 80°C)**
  - **Growth temperature optima between 50°C and 80°C**
- **Hyperthermophiles**
  - **Optima greater than 80°C**
  - **These organisms inhabit hot environments including boiling hot springs**

pH and Microbial Growth

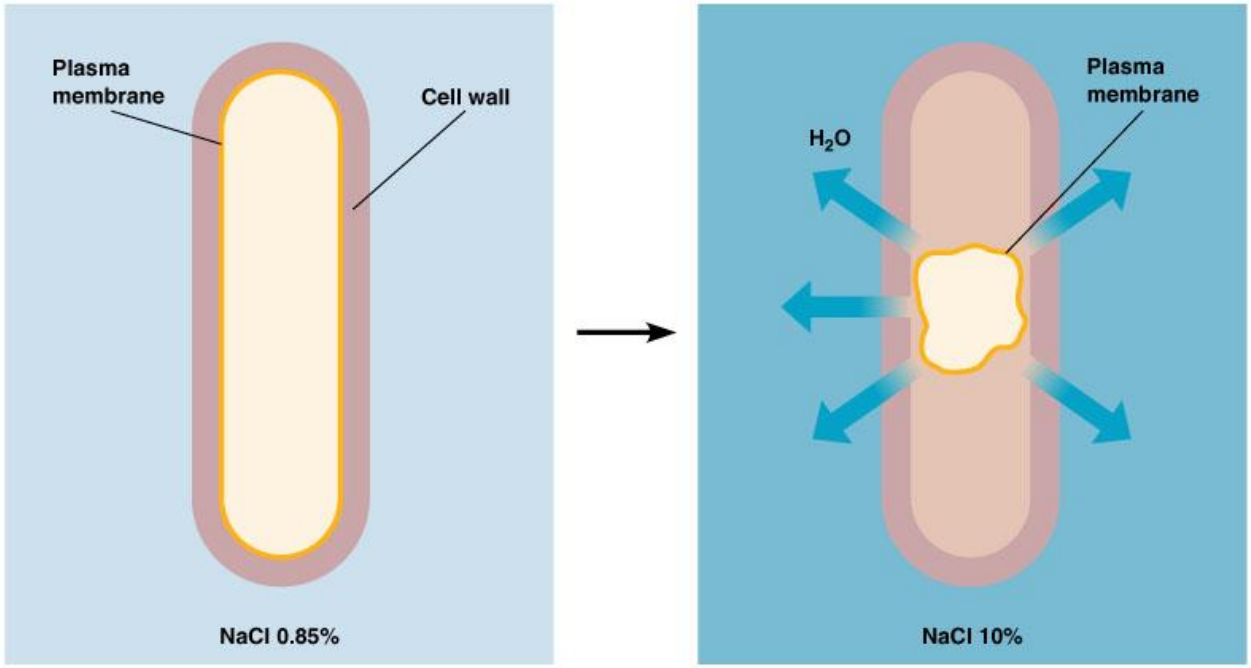
- **Each organism has a pH range and a pH optimum**
  - Acidophiles: Grow optimally between ~pH 0 and 5.5
  - Neutrophiles: Growoptimally between pH 5.5 and 8
  - Alkalophiles: Grow optimally between pH 8 – 11.5

- **Most bacteria grow between pH 6.5 and 7.5**

  Molds and yeasts grow between pH 5 and 6

- **Human blood and tissues has pH 7.2±0.2**

Osmotic Effects on Microbial Growth



- Osmotic pressure depends on the surrounding solute concentration and water availability
- Hypertonic environments, increase salt or sugar, cause plasmolysis

Classification

- Osmophiles: organisms which thrive in high solute
- Osmotolerant: organisms which tolerate high solute

Oxygen and Microbial Growth

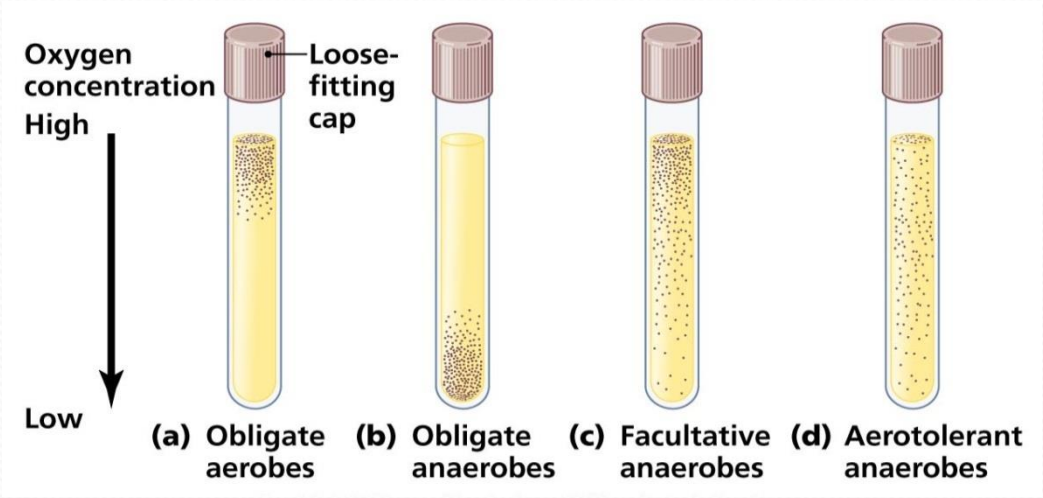
Using oxygen (O_2) in metabolism creates toxic waste

Microbes that are able to use aerobic respiration produce enzymes to detoxify oxygen:

Catalase: H_2O_2 ---→ H_2o and o_2

Superoxide dismutase (SOD): oxygen radical ---→ H_2o and O_2

Microbes that don't make these enzymes cannot exist in the presence of oxygen.



Classification of Organisms Based on O_2 Utilization

- **Aerobes :**
  - **Obligate**: require oxygen to grow
  - **Facultative**: can live with or without oxygen but grow better with oxygen
  - **Microaerphiles**: require reduced level of oxygen

- **Anaerobes :**
  - **Obligate: do not require oxygen. Obligate anaerobes are killed by oxygen**
  - **Aerotolerant anaerobes**: can tolerate oxygen but grow better without oxygen.

Microbial Nutrition

- Organisms use a variety of **nutrients** for:
  - their energy needs
  - to build organic molecules & cellular structures

- Energy Source
  - Phototroph: Uses light as an energy source
  - Chemotroph: Uses energy from the oxidation of reduced chemical compounds

Required nutrients:

- Macronutrients
- Micronutrients
- Special requirements

Macronutrients

Elements required in fairly large amounts:

- Carbon
- Nitrogen
- Sulfur
- Phosphorus

Micronutrients

Metals and organic compounds needed in very small amounts, usually as enzyme and cofactors:

Calcium, Copper, Iron, Magnesium, Manganese, and Iron

Special requirements

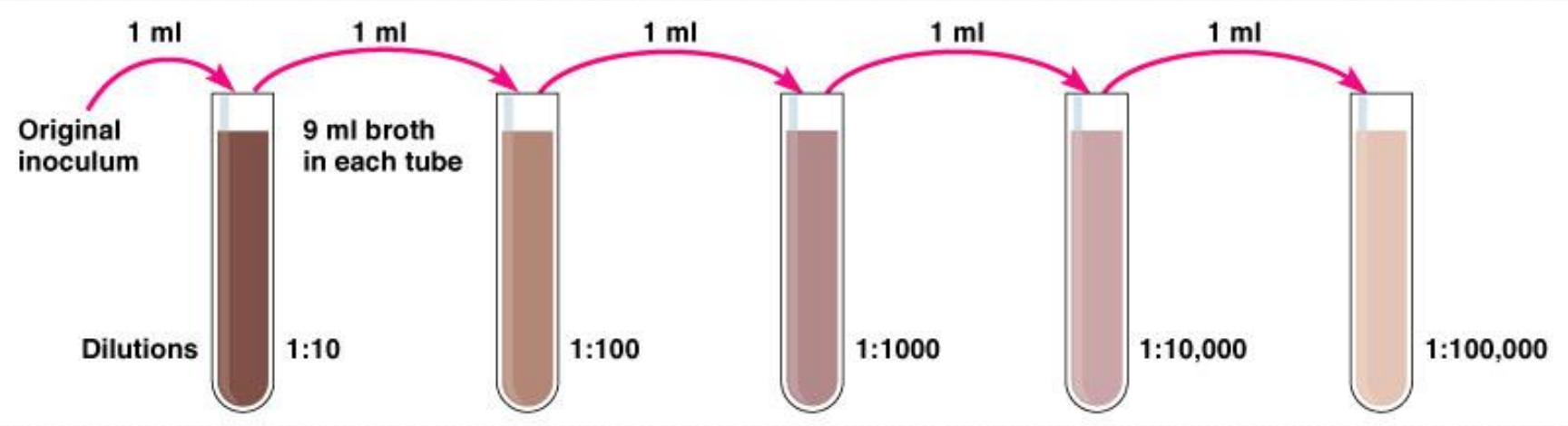
- Amino acids
- Nucleotide bases
- Enzymatic cofactors or “vitamins”

Methods Used to Measure Microbial Growth

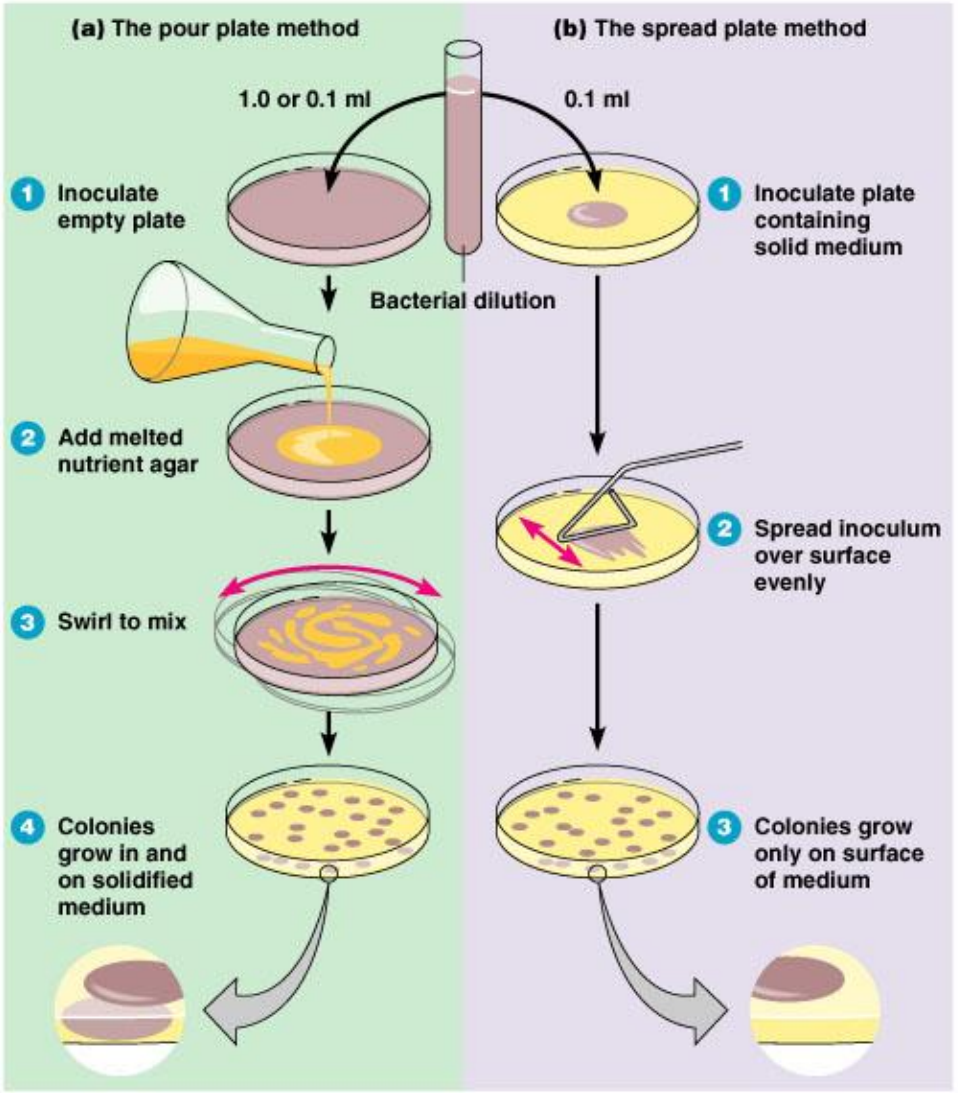
- Count colonies on plate or filter (counts live cells)
- Microscopic counts
- Mass determination
- Turbitity
- Measurement of enzymatic activity or other cell components

Viable Count

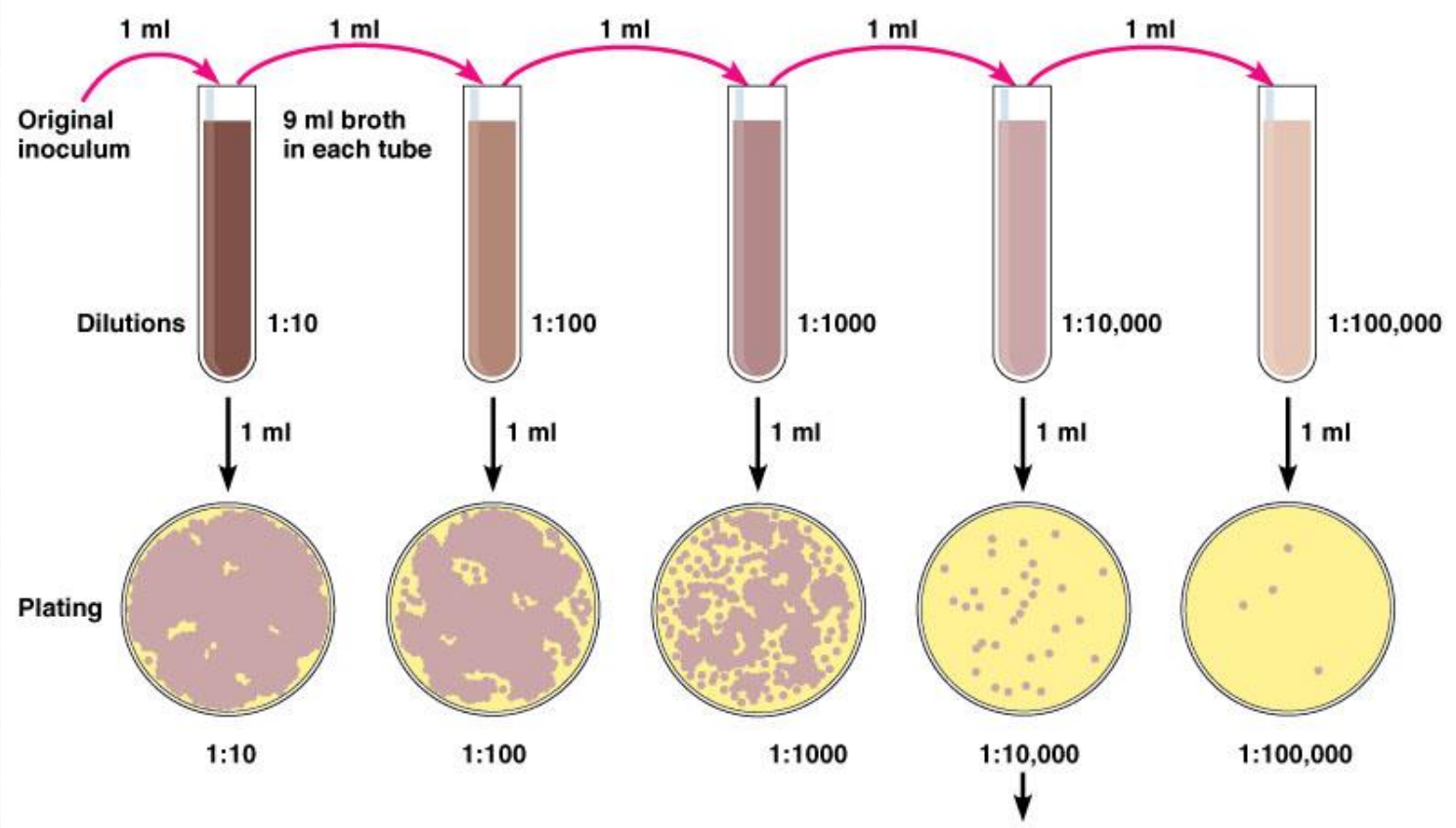
1. Perform serial dilution of original sample (1:10 dilution)



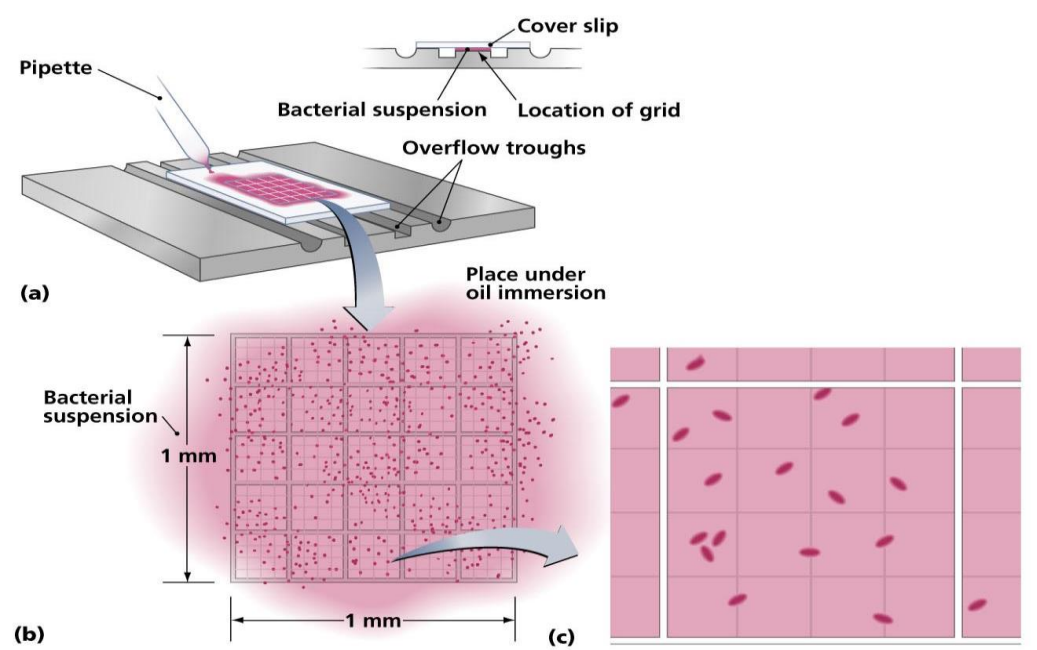
2. Incubate plates with samples from each serial dilution



3. Count plates that have 25-250 colonies and correct The dilution factor



Microscopic counts

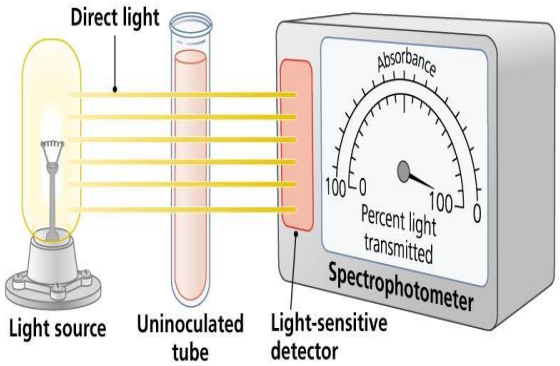


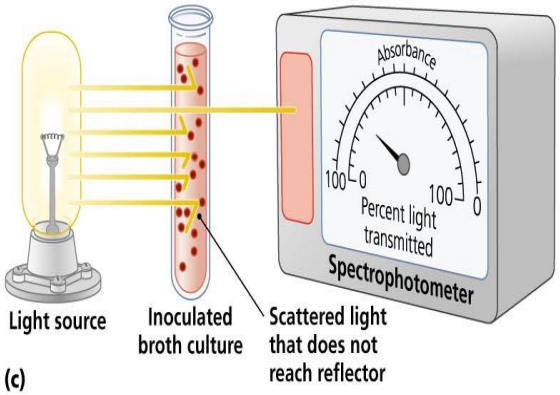
- Need a microscope, special slides, high power objective lens
- Typically only counting total microbe numbers, but differential counts can also be done

Turbidity

(a)

(b)





(c)

- Cells act like large particles that scatter visible light
- A spectrophotometer sends a beam of visible light through a culture and measures how much light is scattered
- Scales read in either absorbance or % transmission
- Measures both live and dead cells

Mass Determination

- Cells are removed from a broth culture by centrifugation and weighed to determine the “wet mass.”
- The cells can be dried out and weighed to determine the “dry mass”

Thank you…