

Environmental Microbiology

Microbial Growth

prepared by Prof. Bulent Içgen

Microbial Growth

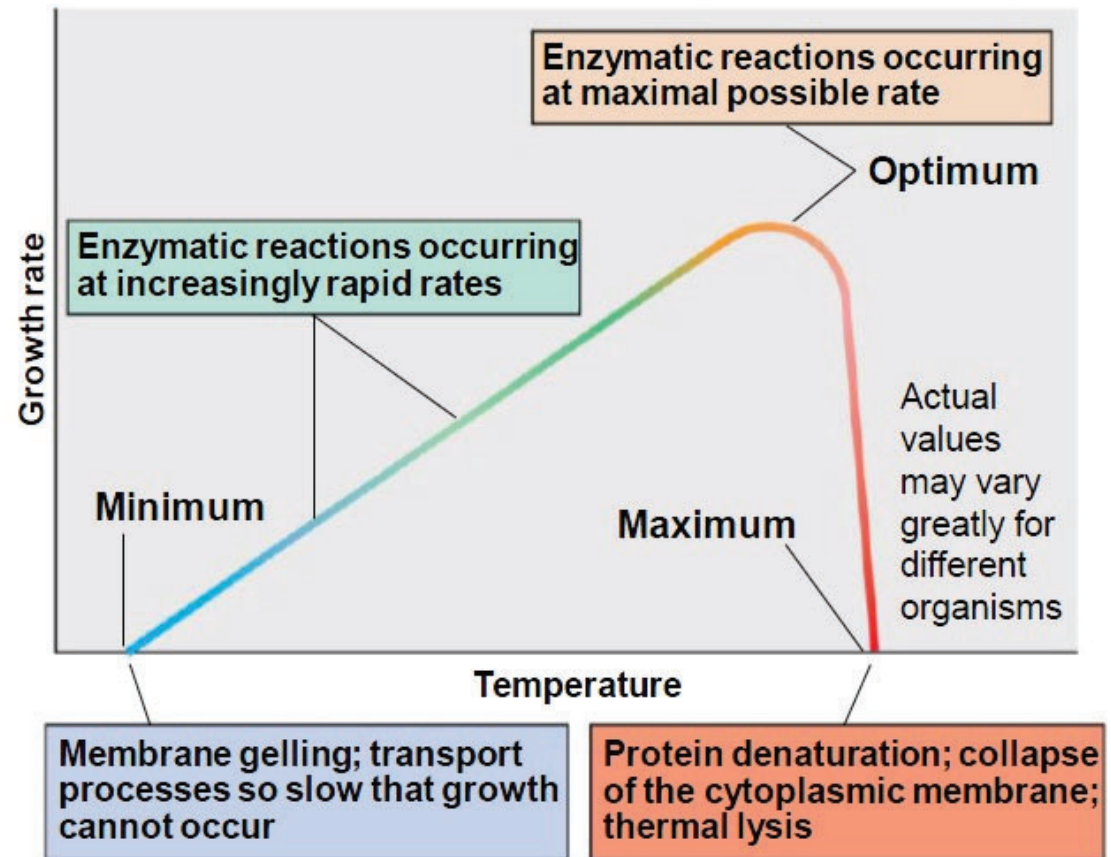
- **Microbial growth**, increase in number of cells, not cell size

The Requirements for Growth: Physical Requirements

Temperature

- Temperature is a major environmental factor controlling microbial growth
- **Cardinal temperatures** the minimum, optimum, and maximum temperatures at which an organism grows

Figure 5.18 The cardinal temperatures: minimum, optimum, and maximum



Temperature

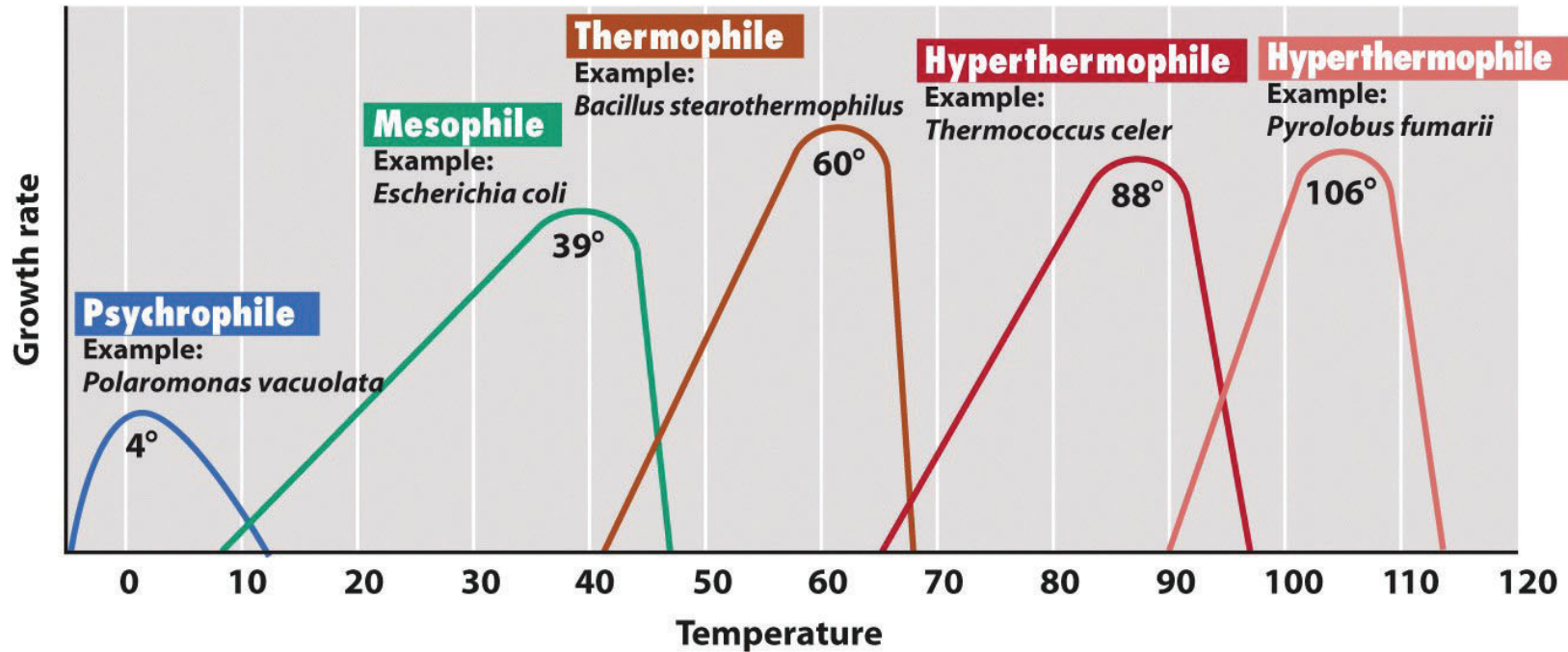
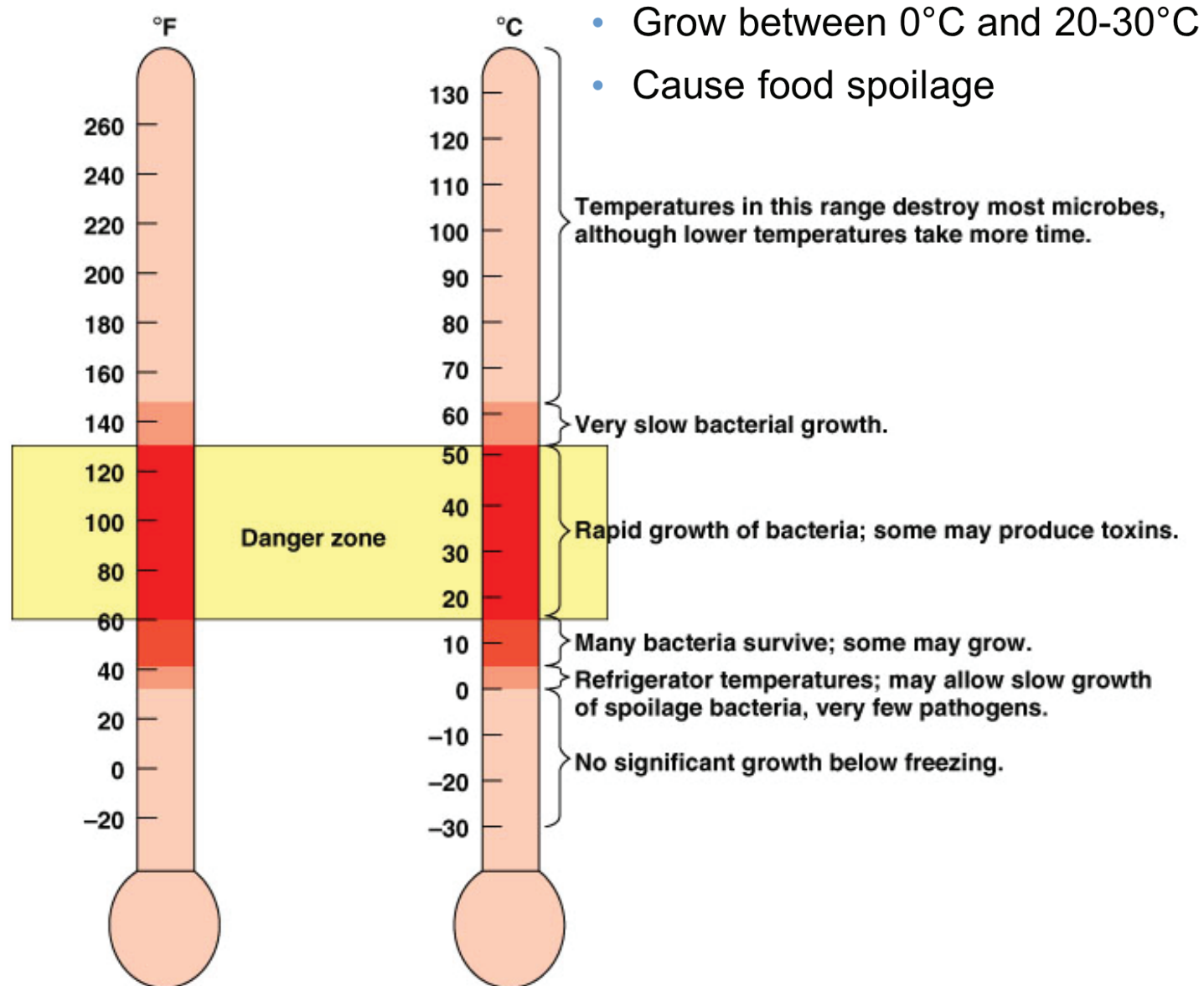


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Microorganisms can be classified into groups by their growth temperature optima

- **Psychrophile:** low temperature
- **Mesophile:** midrange temperature
- **Thermophile:** high temperature
- **Hyperthermophile:** very high temperature

Psychrotrophs



- Grow between 0°C and 20-30°C
- Cause food spoilage

Molecular Adaptations to Psychrophily

➤ Production of enzymes that function optimally in the cold; features that may provide more flexibility

- More α -helices than β -sheets
- More polar and less hydrophobic amino acids
- Fewer weak bonds
- Decreased interactions between protein domains

➤ Transport processes function optimally at low temperatures

- Modified cytoplasmic membranes

➤ High unsaturated fatty acid content

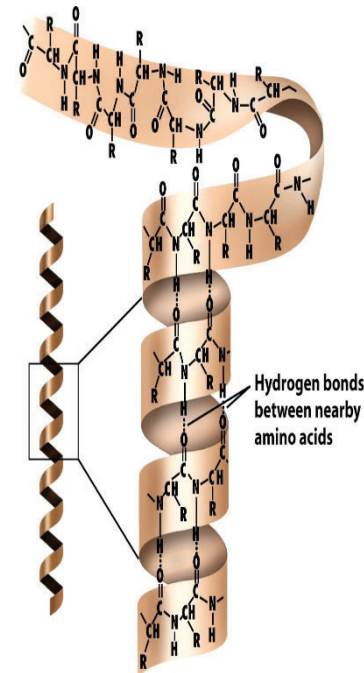


Figure 3-16a Brock Biology of Microorganisms 11/e
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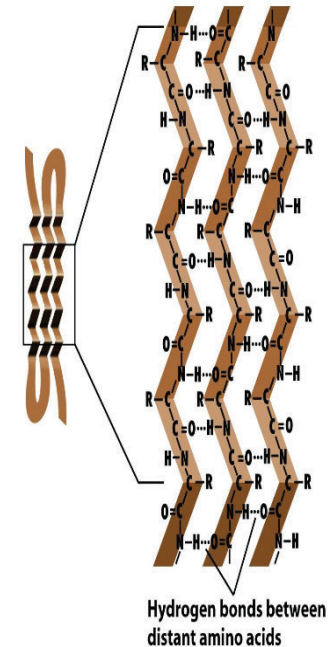


Figure 3-16b Brock Biology of Microorganisms 11/e
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5 TRY or
mobile
Gen in low temp

Adaptations of Thermophiles

↓
chaperon
↳ corrects
unfolded
mis folded } proteins

- Protein structure stabilized by a variety of means
 - more H bonds
 - more proline
 - **chaperones**
- **Histone-like proteins stabilize DNA**
→ they also protect and correct from damage repair about 40% temp.
- Membrane stabilized by variety of means
 - **more saturated**, more branched and higher molecular weight lipids, lipid **monolayers**
 - e.g., ether linkages (archaeal membranes)

The Requirements for Growth: Physical Requirements

- pH**

→ means most of the microbes are neutrophiles

Most bacteria grow between pH 6.5 and 7.5

Molds and yeasts grow between pH 5 and 6

- **acidophiles**

growth optimum between pH 0 and pH 5.5

- **neutrophiles**

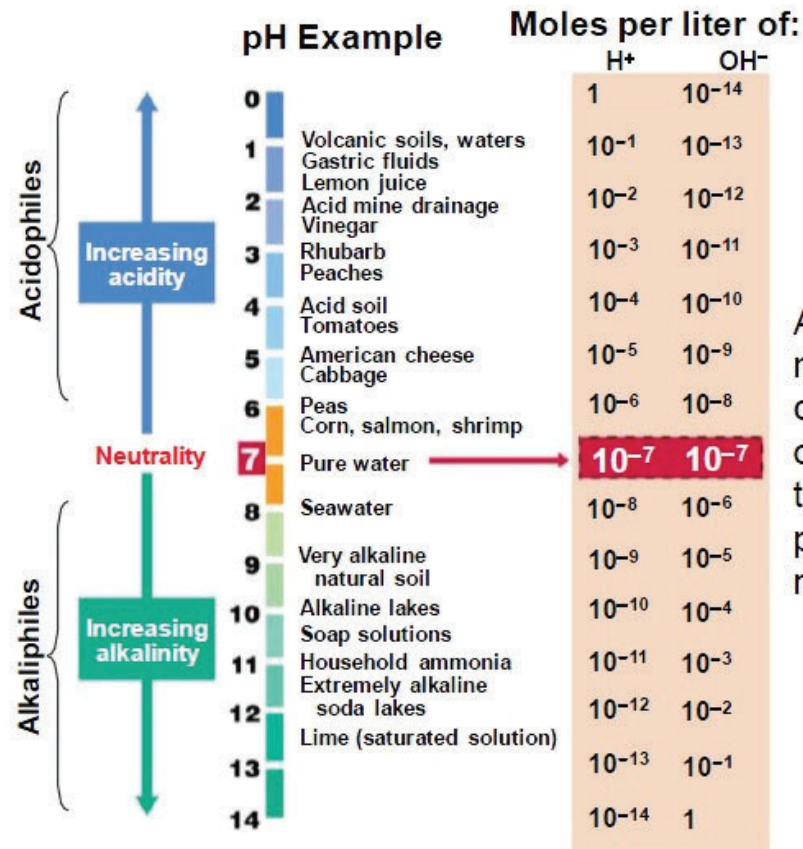
growth optimum between pH 5.5 and pH 7

- **alkalophiles**

growth optimum between pH 8.5 and pH 11.5

outside pH can change but inside of the cell pH is constant.

Figure 5.24 The pH scale



Although some microorganisms can live at very low or very high pH, the cell's internal pH remains near neutrality.

- Most acidophiles and alkalophiles maintain an **internal pH near neutrality**
 - The plasma membrane is impermeable to protons
 - Symport, antiport systems can be used to maintain pH closer to neutrality
- Synthesize proteins that provide protection
 - e.g., acid-shock proteins
- Many microorganisms change pH of their habitat by producing acidic or basic waste products
 - most media contain **buffers** to prevent growth inhibition

resist pH changes
↓
to make pH stay constant.

When bacteria grow they produce acids. → If you don't use buffers they will die from acids they produce

The Requirements for Growth: Physical Requirements

- **Osmotic Pressure**

- Hypertonic environments, increase salt or sugar, cause plasmolysis
- Extreme or obligate halophiles require high osmotic pressure
- Facultative halophiles tolerate high osmotic pressure

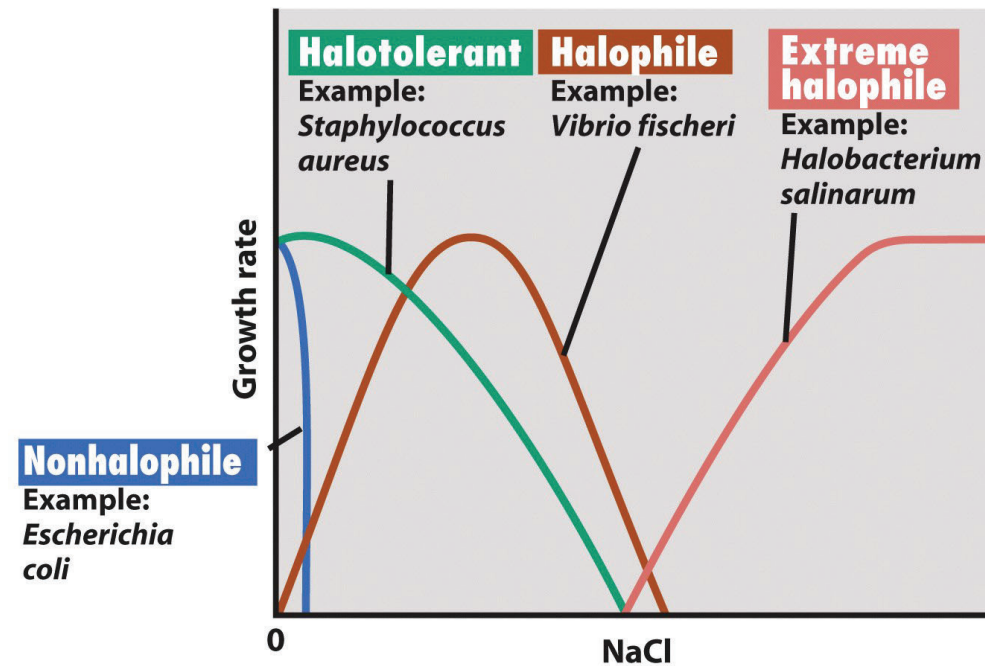


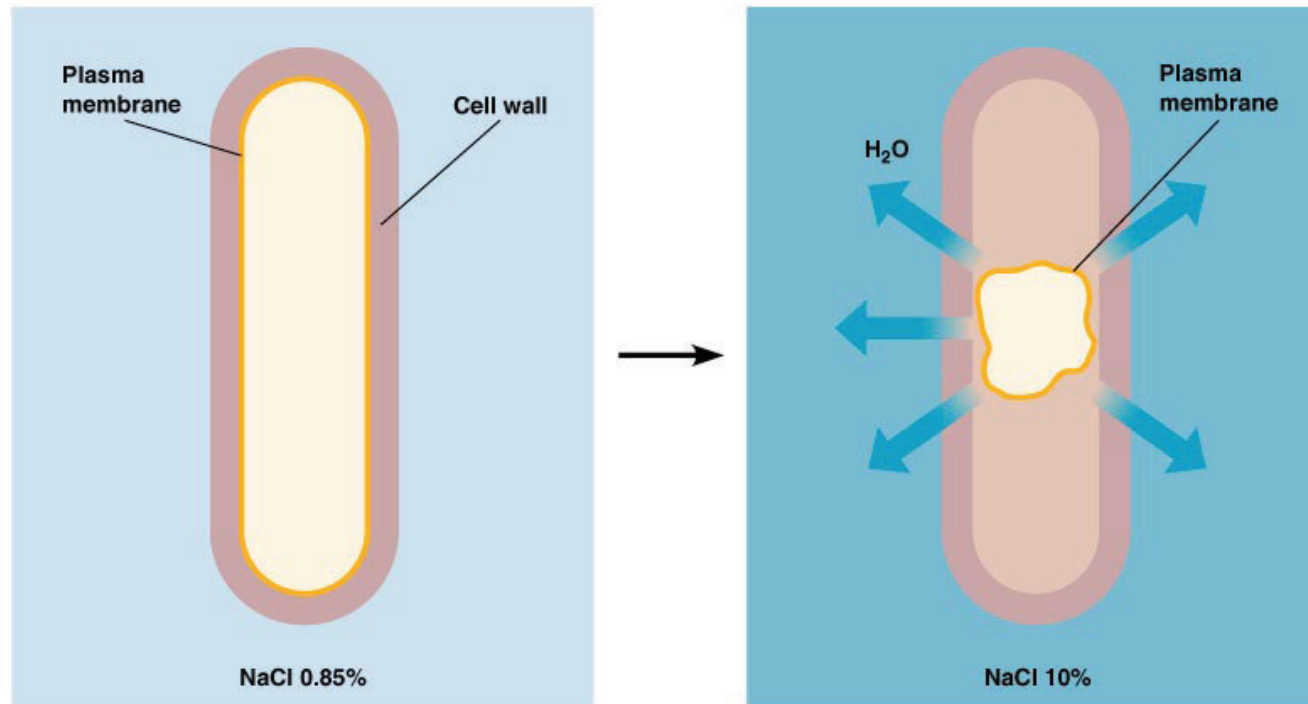
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Halophiles: organisms that grow best at reduced water potential; have a specific requirement for NaCl

Extreme halophiles: organisms that require high levels (15–30%) of NaCl for growth

Halotolerant: organisms that can tolerate some reduction in water activity of environment but generally grow best in the absence of the added solute

The Requirements for Growth: Physical Requirements



Osmophiles: organisms that live in environments high in sugar as solute

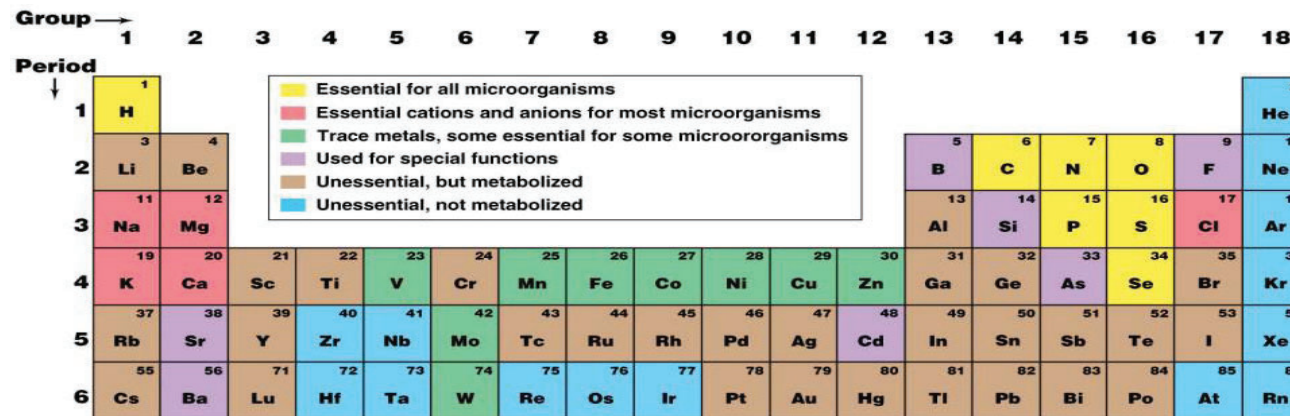
Xerophiles: organisms able to grow in very dry environments

Pressure

- **Barotolerant**
 - adversely affected by increased pressure, but not as severely as nontolerant organisms
- **Barophilic Organisms**
 - require or grow more rapidly in the presence of increased pressure

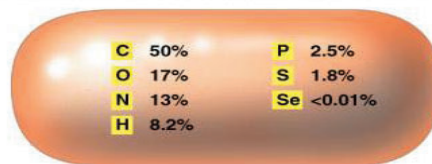
The Requirements for Growth: Chemical Requirements

Elemental and macromolecular composition of a bacterial cell



(a)

Essential elements as a percent of cell dry weight



(b)

Macromolecular composition of a cell

Macromolecule	Percent of dry weight
Protein	55
Lipid	9.1
Polysaccharide	5.0
Lipopolysaccharide	3.4
DNA	3.1
RNA	20.5

(c)

- **Nutrients**
 - Supply of monomers (or precursors of) required by cells for growth
- **Macronutrients**
 - Nutrients required in large amounts
- **Micronutrients**
 - Nutrients required in trace amount

The Requirements for Growth: Chemical Requirements

➤ 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 (S)

- In amino acids, thiamine, biotin
- Most bacteria decompose proteins
- Some bacteria use SO₄²⁻ or H₂S

➤ Phosphorus (P)

- In DNA, RNA, ATP, and membranes
- PO₄³⁻ is a source of phosphorus

The Requirements for Growth: Chemical Requirements

- Other macronutrients: K, Mg, Ca, Na
- Trace Elements
 - Inorganic elements required in small amounts
 - Usually as enzyme cofactors

Cofactors → for enzymes.

↳ If you put high conc. → can become toxic.

Table 4.1 Micronutrients (trace elements) needed by microorganisms^a

Element	Cellular function or molecule of which a part
Boron (B)	Autoinducer for quorum sensing in bacteria; also found in some polyketide antibiotics
Chromium (Cr)	Possible but not proven component for glucose metabolism (necessary in mammals)
Cobalt (Co)	Vitamin B ₁₂ ; transcobalamin (only in propionic acid bacteria)
Copper (Cu)	In respiration, cytochrome c oxidase; in photosynthesis, plastocyanin, some superoxide dismutases
Iron (Fe) ^b	Cytochromes; catalases; peroxidases; iron-sulfur proteins; oxygenases; all nitrogenases
Manganese (Mn)	Activator of many enzymes; component of certain superoxide dismutases and of the water-splitting enzyme in oxygenic phototrophs (photosystem II)
Molybdenum (Mo)	Certain flavin-containing enzymes; some nitrogenases, nitrate reductases, sulfite oxidases, DMSO-TMAO reductases; some formate dehydrogenases
Nickel (Ni)	Most hydrogenases; coenzyme F ₄₃₀ of methanogens; carbon monoxide dehydrogenase; urease
Selenium (Se)	Formate dehydrogenase; some hydrogenases; the amino acid selenocysteine
Tungsten (W)	Some formate dehydrogenases; oxotransferases of hyperthermophiles
Vanadium (V)	Vanadium nitrogenase; bromoperoxidase
Zinc (Zn)	Carbonic anhydrase; alcohol dehydrogenase; RNA and DNA polymerases; and many DNA-binding proteins

^aNot every micronutrient listed is required by all cells; some metals listed are found in enzymes or cofactors present in only specific microorganisms.

^bNeeded in greater amounts than other trace metals.

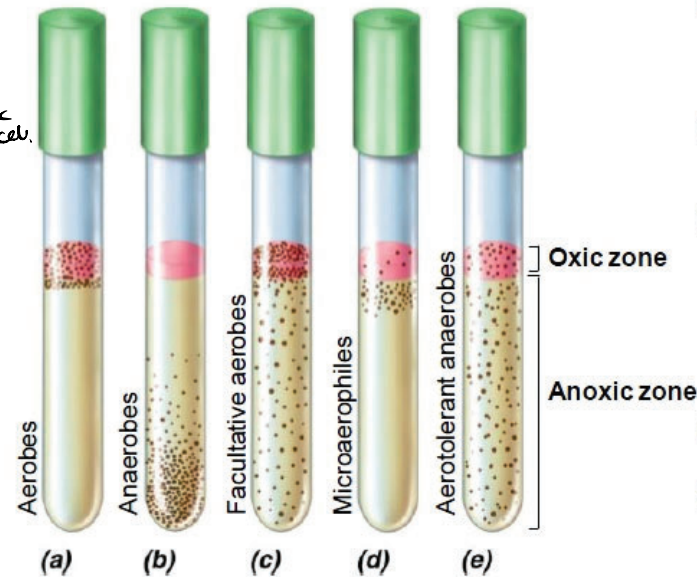
The Requirements for Growth: Chemical Requirements

Figure 5.26 Growth versus oxygen (O₂) concentration

4-6 x 10³⁰ prokaryotic cell. (estimation total)
 4-5 x 10²⁰ in soil (terrestrial env.)
 1.2 x 10²⁹ in aquatic env.
 60% of total biomass on earth is prokaryotic cell.

- Oxygen (O₂)

The redox dye, resazurin, which is pink when oxidized and colorless when reduced, has been added as a redox indicator.



- O₂ penetrates only a short distance into the tube, so obligate aerobes grow only close to the surface.
- Anaerobes, being sensitive to O₂, grow only away from the surface.
- Facultative aerobes are able to grow in either the presence or the absence of O₂ and thus grow throughout the tube. However, growth is better near the surface because these organisms can respire.
- Microaerophiles grow away from the most oxic zone.
- Aerotolerant anaerobes grow throughout the tube. Growth is not better near the surface because these organisms can only ferment.

- **Aerobes:** require oxygen to live
- **Anaerobes:** do not require oxygen and may even be killed by exposure
- **Facultative organisms:** can live with or without oxygen
- **Aerotolerant anaerobes:** can tolerate oxygen and grow in its presence even though they cannot use it
- **Microaerophiles:** can use oxygen only when it is present at levels reduced from that in air

Toxic Forms of Oxygen

Some enzymes are extremely sensitive to oxygen. Oxygen easily reduced to toxic products in the cell:

- Single oxygen
- Superoxide anion
- Hydrogen peroxide
- Hydroxyl radical

aerobes produce protective enzymes

- Catalase
- Peroxidase
- Superoxide dismutase
- Superoxide reductase

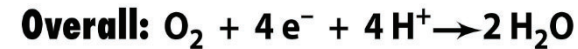
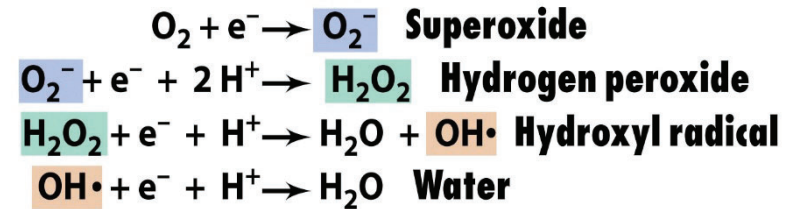
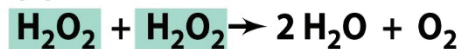
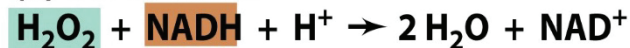


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(a) **Catalase:**



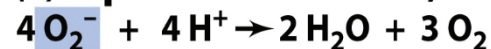
(b) **Peroxidase:**



(c) **Superoxide dismutase:**



(d) **Superoxide dismutase/catalase in combination:**



(e) **Superoxide reductase:**



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The Requirements for Growth: Chemical Requirements

o/o I can be grown in the lab. → we don't know the exact nutrient.
r/o 99% unknown.

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

Culture Media

- **Culture Medium:** Nutrients prepared for microbial growth
- **Sterile:** No living microbes *↪ get pure culture.*
- **Inoculum:** Introduction of microbes into medium *microbes you put called "inoculum"*
(inoculation) → process name.
- **Culture:** Microbes growing in/on culture medium

Agar

comes from algae

for solid medium (culture)

- 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

Culture Media

- Chemically Defined Media: Exact chemical composition is known
- Complex Media: Extracts and digests of yeasts, meat, or plants
 - Nutrient broth
 - Nutrient agar

Culture Media

TABLE 6.2

A Chemically Defined Medium for Growing a Typical Chemoheterotroph, Such as *E. coli* *exact conc. known.*

Constituent	Amount
Glucose	5.0 g
Ammonium phosphate, monobasic (NH ₄ H ₂ PO ₄)	1.0 g
Sodium chloride (NaCl)	5.0 g
Magnesium sulfate (MgSO ₄ · 7H ₂ O)	0.2 g
Potassium phosphate, dibasic (K ₂ HPO ₄)	1.0 g
Water	1 liter

TABLE 6.4

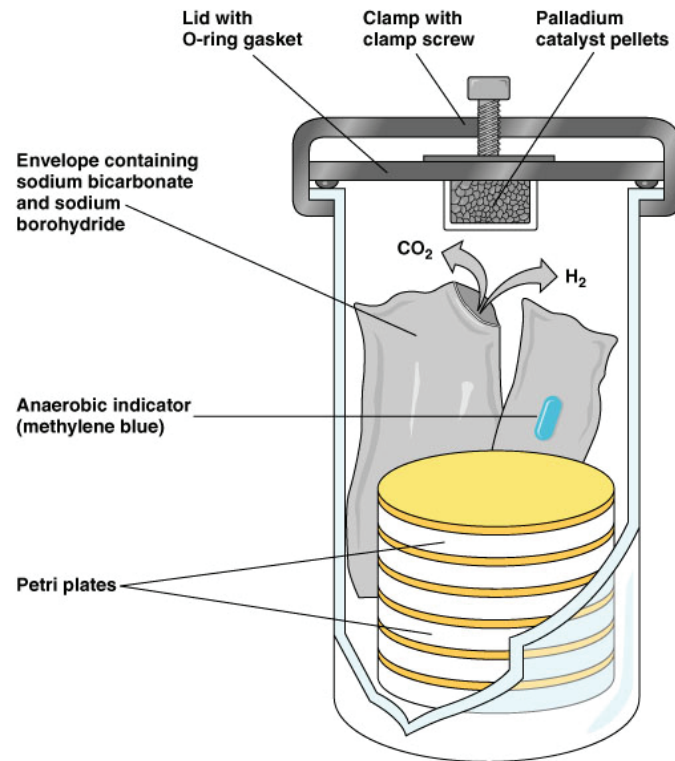
Composition of Nutrient Agar, a Complex Medium for the Growth of Heterotrophic Bacteria *cheaper*

Constituent	Amount
Peptone (partially digested protein)	5.0 g
Beef extract <i>→ meaty powder</i>	3.0 g
Sodium chloride	8.0 g
Agar	15.0 g
Water	1 liter

Anaerobic Culture Methods

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

Anaerobic Culture Methods



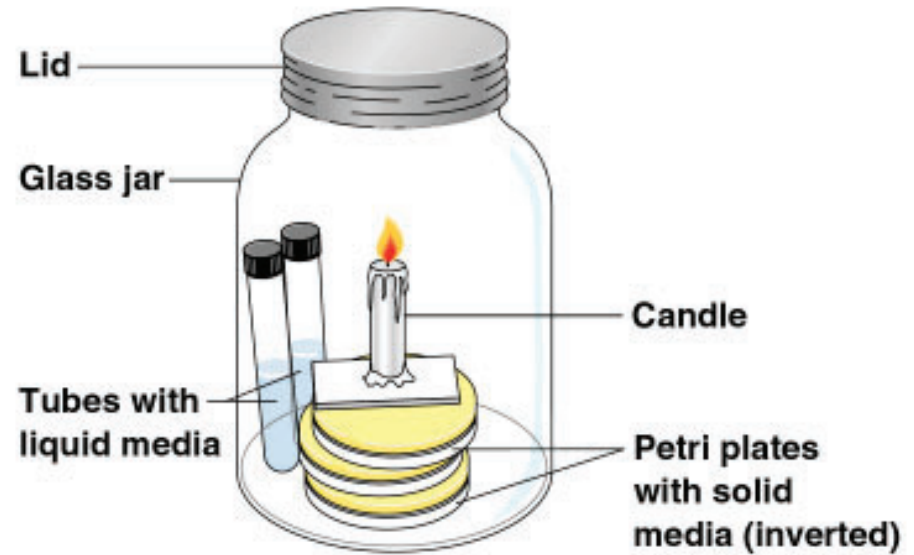
- Anaerobic jar



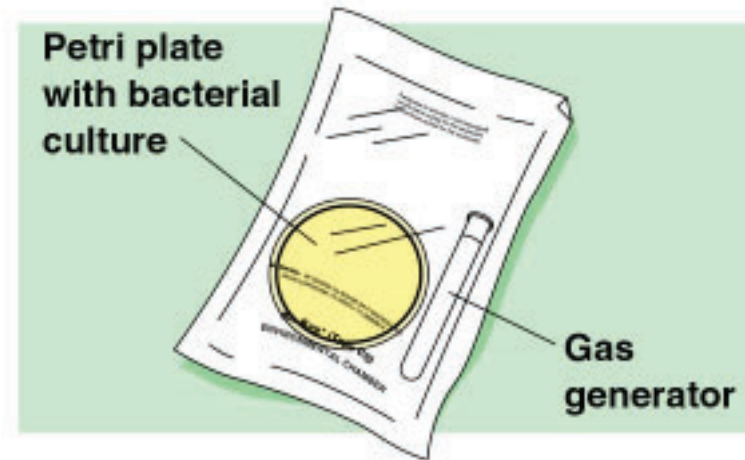
- Anaerobic chamber

Capnophiles require high CO₂

- Candle jar



- CO₂-packet



Culture Media

Nutrient solutions used to grow microbes in the laboratory

Two broad classes

- **Defined media:** precise chemical composition is known
- **Complex media:** composed of digests of chemically undefined substances (e.g., yeast and meat extracts)

Laboratory Culture

- **Pure culture:** culture containing only a single kind of microbe
 - **Contaminants:** unwanted organisms in a culture
- Cells can be grown in liquid or solid culture media
 - Solid media are prepared by addition of a gelling agent (agar or gelatin)
 - When grown on solid media, cells form isolated masses

→ colonies
pure
some ⇒ pure
direct ⇒ contaminant

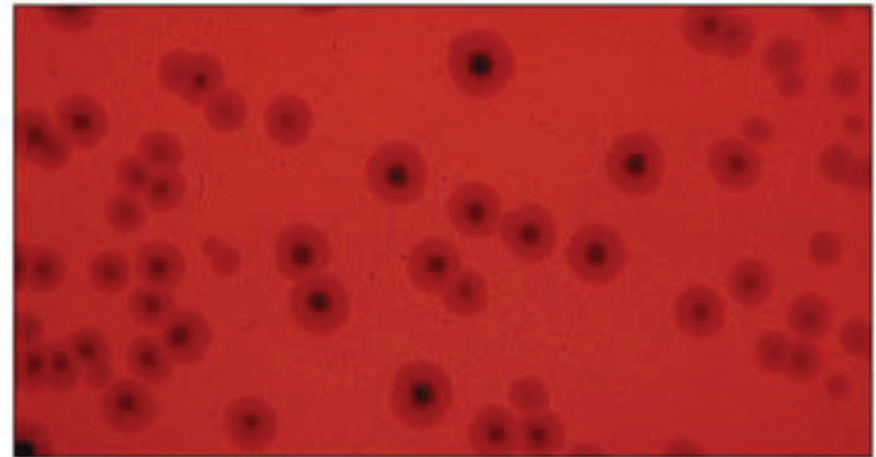
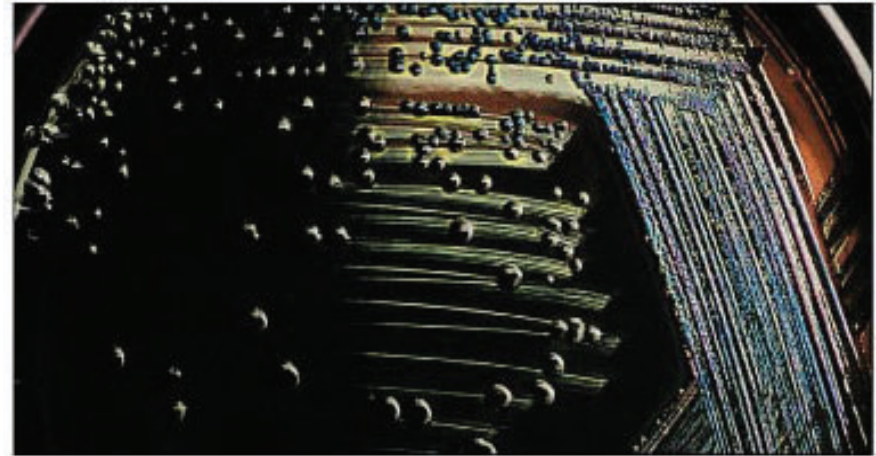
Selective Media

You grow desired ones and suppress unwanted ones

↳ sole source meaning \Rightarrow only source.

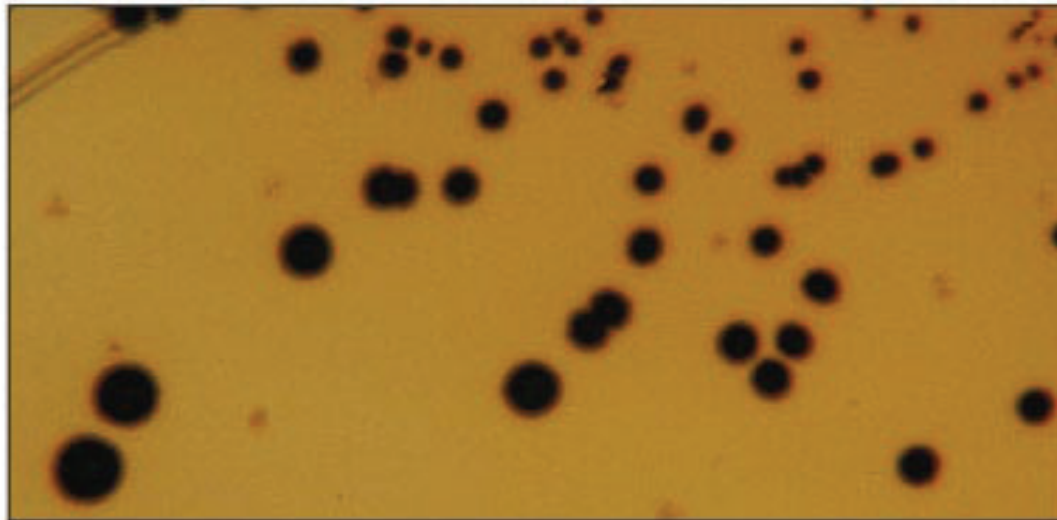
we want microbes can degrade pollutant
 \Rightarrow generally xenobiotics.

- Suppress unwanted microbes and encourage desired microbes.
- Contains compounds that selectively inhibit growth of some microbes but not others



Differential Media

- Make it easy to distinguish colonies of different microbes. Contains an indicator, usually a dye, that detects particular chemical reactions occurring during growth



Enrichment Media

Enriched Culture

phenol 10.4 μg/ml
degrader
y 1 μg/ml → you just inoculated the amount → conc. increased slowly

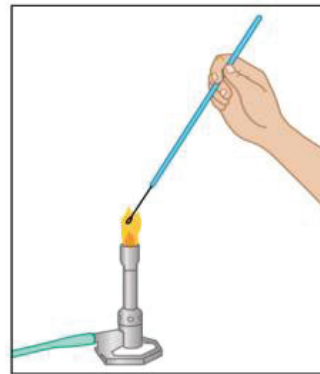
↓
different than selective

- 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

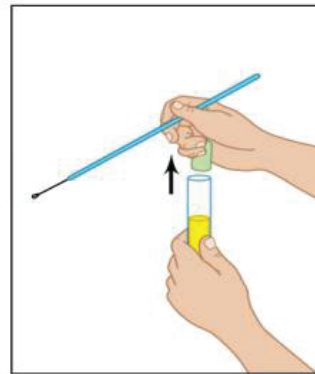
Aseptic Technique

Microbes are everywhere

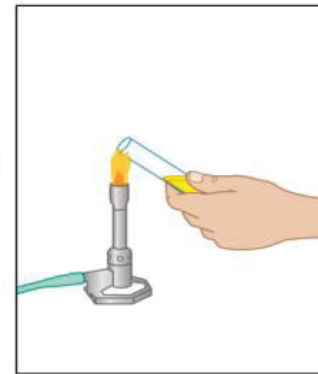
- Sterilization of media is critical
- Aseptic technique should be followed



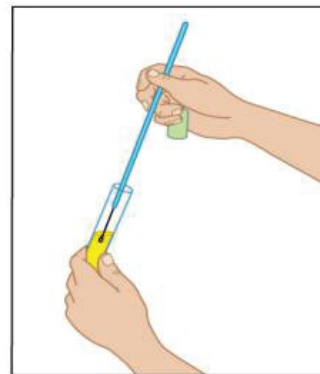
(a) Loop is heated until red hot and cooled in air briefly.



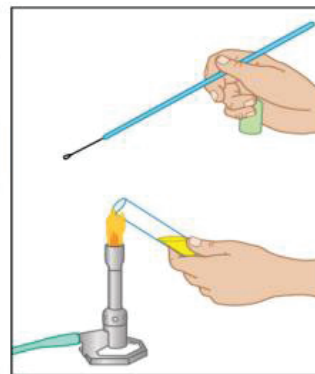
(b) Tube is uncapped.



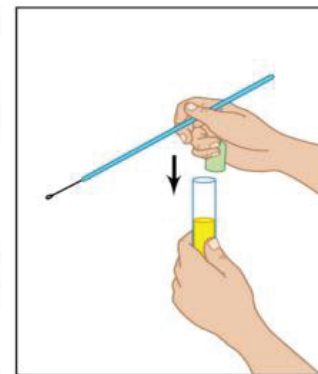
(c) Tip of tube is run through the flame..



(d) Sample is removed on sterile loop for transfer to sterile medium.



(e) The tube is reflamed



(f) The tube is recapped. Loop is reheated before being taken out of service.

Laboratory Culture

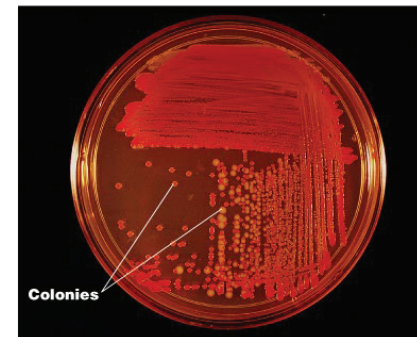
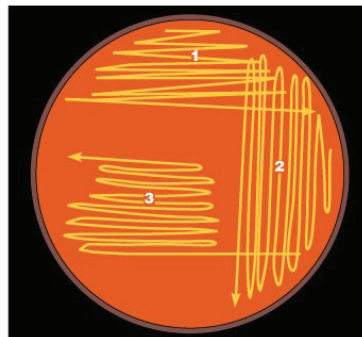
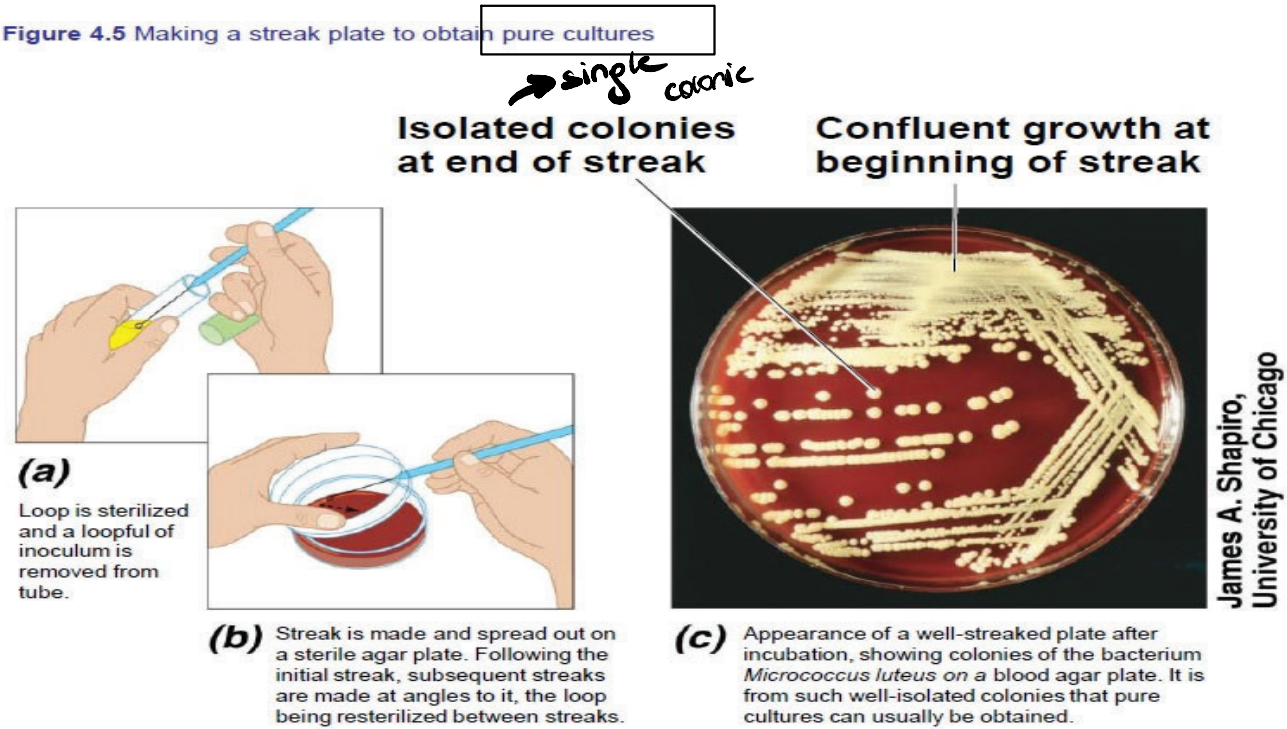
- A pure culture contains only one species or strain
- A colony is a population of cells arising from a single cell or spore or from a group of attached cells
- A colony is often called a **colony-forming unit** (CFU)

Pure culture techniques

- Streak plate
- Pour plate
- Spread plate

Streak Plate

Figure 4.5 Making a streak plate to obtain pure cultures



Preserving Bacteria Cultures

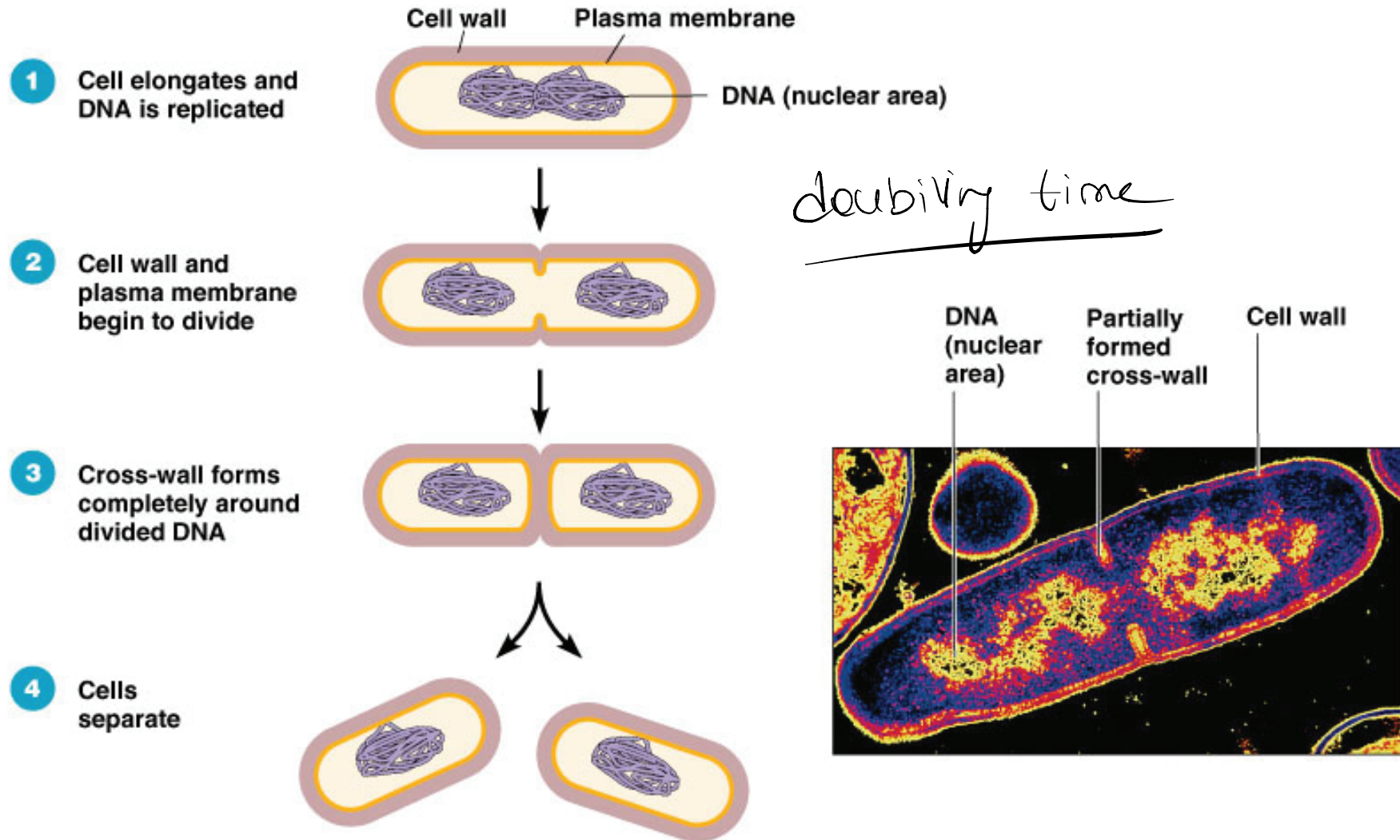
glycerol → protect
from
ice formation

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

Reproduction in Prokaryotes

- Binary fission → most common
- Budding → ameba
- Conidiospores (actinomycetes)
- Fragmentation of filaments → yeast

Binary Fission



(a) A diagram of the sequence of cell division.

(b) A thin section of a cell of *Bacillus licheniformis* starting to divide.

Measurement of Microbial Growth

Can measure changes in number of cells in a population

- **Direct cell counts**
 - counting chambers
 - on membrane filters
- **Viable cell counts** → count living ones only.
 - plating methods
 - membrane filtration methods

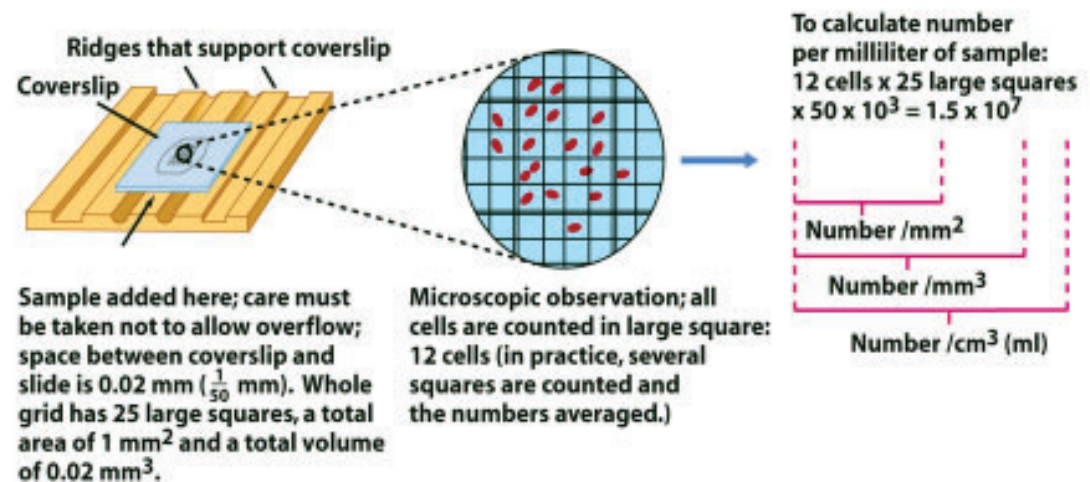
Can measure changes in mass of population

- dry weight
- quantity of a particular cell constituent
- turbidometric measures

Counting Chambers

- easy, inexpensive, and quick
- cannot distinguish living from dead cells
- examples: Petroff-Hauser or hemocytometers

Microscopic Direct Counting



Sample added here; care must be taken not to allow overflow; space between coverslip and slide is 0.02 mm ($\frac{1}{50}$ mm). Whole grid has 25 large squares, a total area of 1 mm² and a total volume of 0.02 mm³.

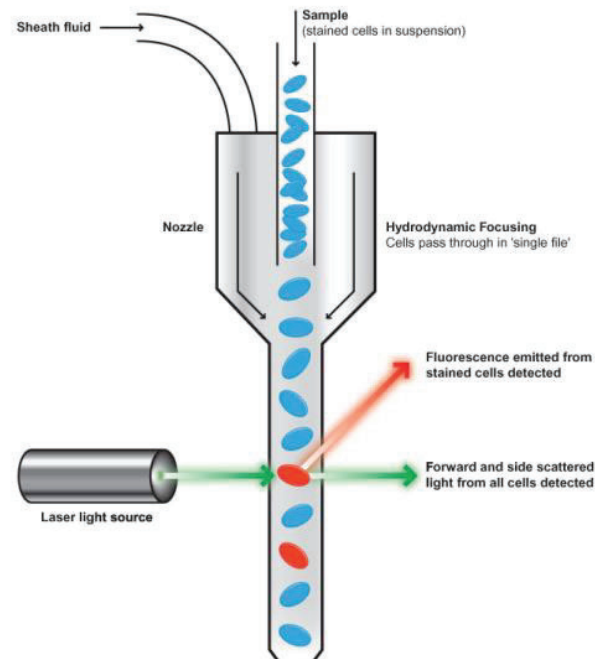
Microscopic observation; all cells are counted in large square: 12 cells (in practice, several squares are counted and the numbers averaged.)

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****Results can be unreliable**

Microscopic Counts...

- A second method for enumerating cells in liquid samples is with a **flow cytometer**
 - Uses laser beams, fluorescent dyes, and electronics



Microscopic Counts....

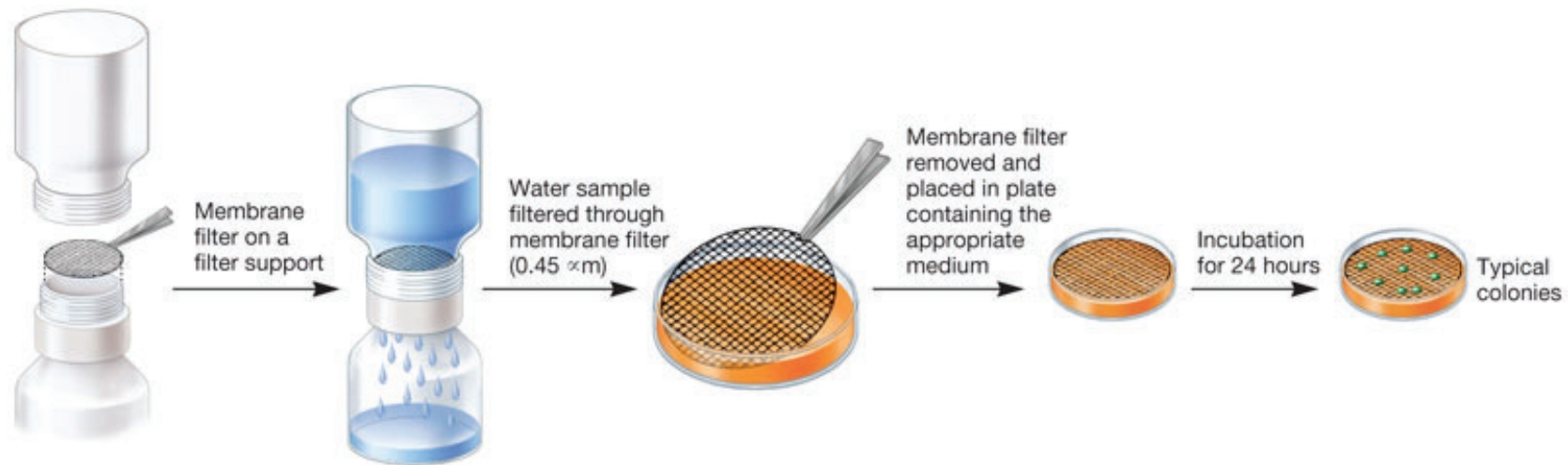
Limitations of microscopic counts

- Cannot distinguish between live and dead cells.
- Small cells can be overlooked
- Cell suspensions of low density ($<10^6$ cells/ml) hard to count
- Motile cells need to be immobilized
- Debris in sample can be mistaken for cells

Direct Counts on Membrane Filters

sieving → sievek

- Cells filtered through special membrane that provides dark background for observing cells
- Cells are stained with fluorescent dyes
- Useful for counting bacteria
- With certain dyes, can distinguish living from dead cells



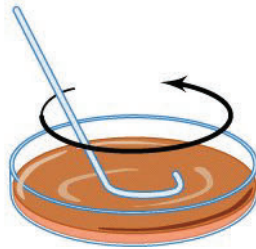
especially useful for analyzing aquatic samples

The Viable Cell Counting

Spread-plate method

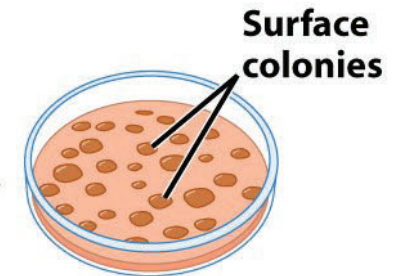
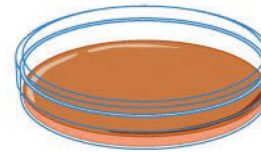


Sample is pipetted onto surface of agar plate (0.1 ml or less)



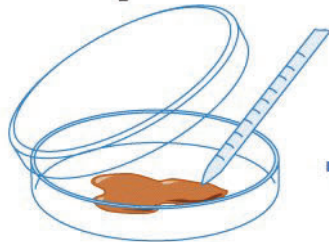
Sample is spread evenly over surface of agar using sterile glass spreader

Incubation

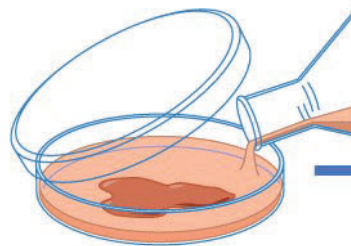


Typical spread-plate results

Pour-plate method

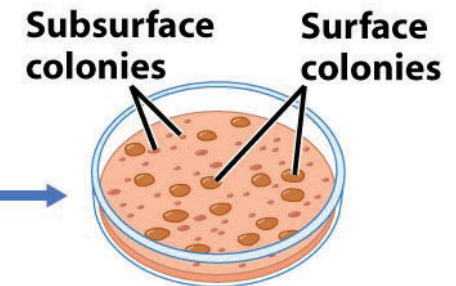
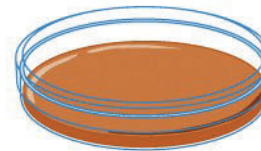


Sample is pipetted into sterile plate



Sterile medium is added and mixed well with inoculum

Incubation



Typical pour-plate results

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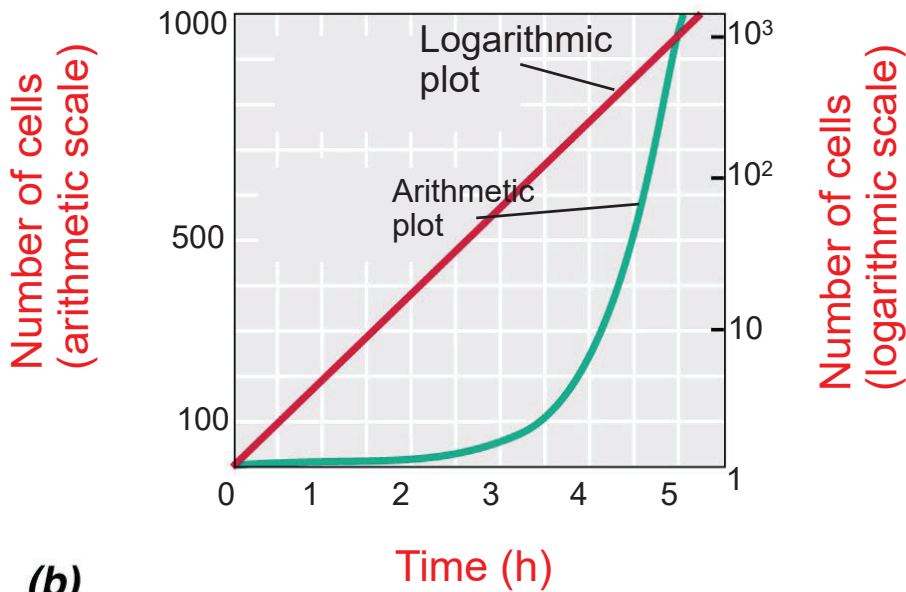
y increase the surface area

Measurement of Cell Mass

- Dry weight
 - time consuming and not very sensitive
- Quantity of a particular cell constituent
 - protein, DNA, ATP, or chlorophyll
- Turbidometric measures (light scattering)
 - quick, easy, and sensitive

Time (h)	Total number of cells	Time (h)	Total number of cells
0	1	4	256 (2^8)
0.5	2	4.5	512 (2^9)
1	4	5	1,024 (2^{10})
1.5	8	5.5	2,048 (2^{11})
2	16	6	4,096 (2^{12})
2.5	32	.	.
3	64	.	.
3.5	128	10	1,048,576 (2^{19})

(a)



(b)

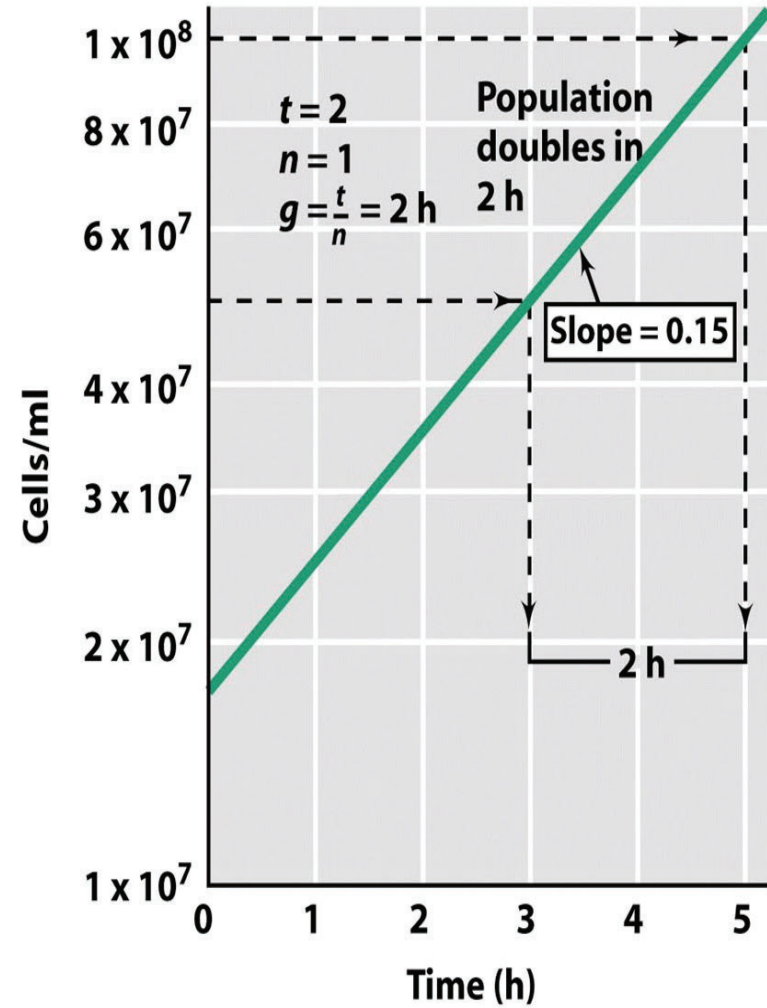


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The Mathematics of Exponential Growth

A relationship exists between the initial number of cells present in a culture and the number present after a period of exponential growth:

$$N = N_0 2^n$$

generation number

$$n = \log N - \log N_0 / \log 2$$

N_0 = initial cell numbers, N = final cell numbers

The Mathematics of Exponential Growth

- Doubling time (g) is calculated as
 $g = t/n$
- Specific growth rate (k) is calculated as

$$k = 3.32 \times 1/t \times (\text{Log } N_t - \text{Log } N_o)$$

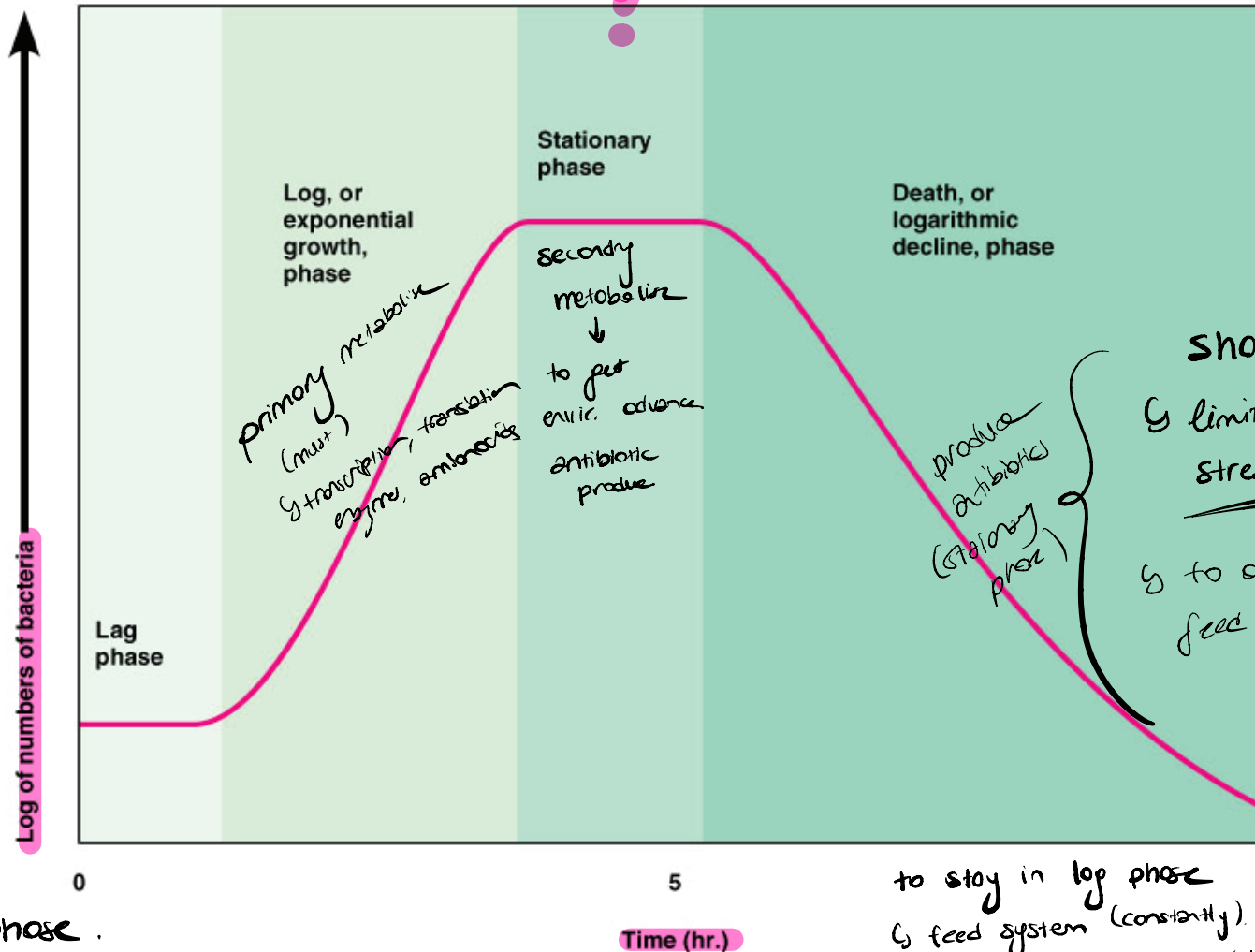
$$k = n/t \text{ (\# of generations per unit time)}$$

Division rate (v) is calculated as

$$v = 1/g$$

memorize

Growth Curve



Shorten the lag phase
 ↳ limit the nutrients.
 stress conditions.
 ↳ to avoid dead phase.
 feed them slowly.

(shorten)
 remove lag phase.
 use medium similar the one in the lab.
 because they get the lag phase in the lab.

to stay in log phase 10
 ↳ feed system (constantly).
 and also remove the waste (toxic).
 => not a closed system.

The Serial Dilutions Technique

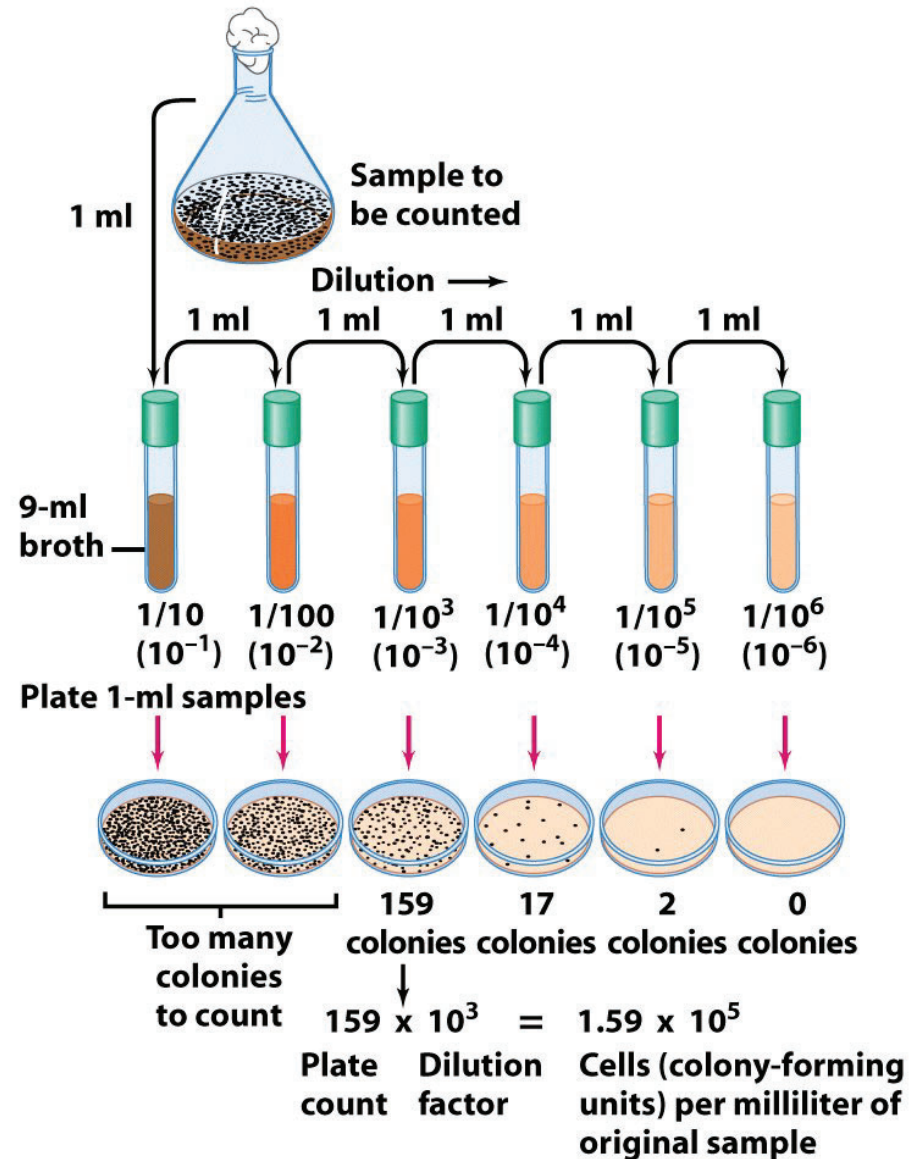


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Direct Measurements of Microbial Growth

- Multiple tube MPN test
- Count positive tubes and compare to statistical MPN table.

Combination of Positives	MPN Index/ 100 ml	95% Confidence Limits	
		Lower	Upper
4-2-0	22	9	56
4-2-1	26	12	65
4-3-0	27	12	67
4-3-1	33	15	77
4-4-0	34	16	80
5-0-0	23	9	86
5-0-1	30	10	110
5-0-2	40	20	140
5-1-0	30	10	120
5-1-1	50	20	150
5-1-2	60	30	180
5-2-0	50	20	170
5-2-1	70	30	210
5-2-2	90	40	250
5-3-0	80	30	250
5-3-1	110	40	300
5-3-2	140	60	360

Figure 6.18b

Direct Measurements of Microbial Growth

- Direct Microscopic Count

$$\text{Number of bacteria/ml} = \frac{\text{number of cells counted}}{\text{volume of area counted}}$$

$$\frac{14}{8 \times 10^{-7}} = 17,500,000$$

Indirect Measurements of Microbial Growth: Turbidity

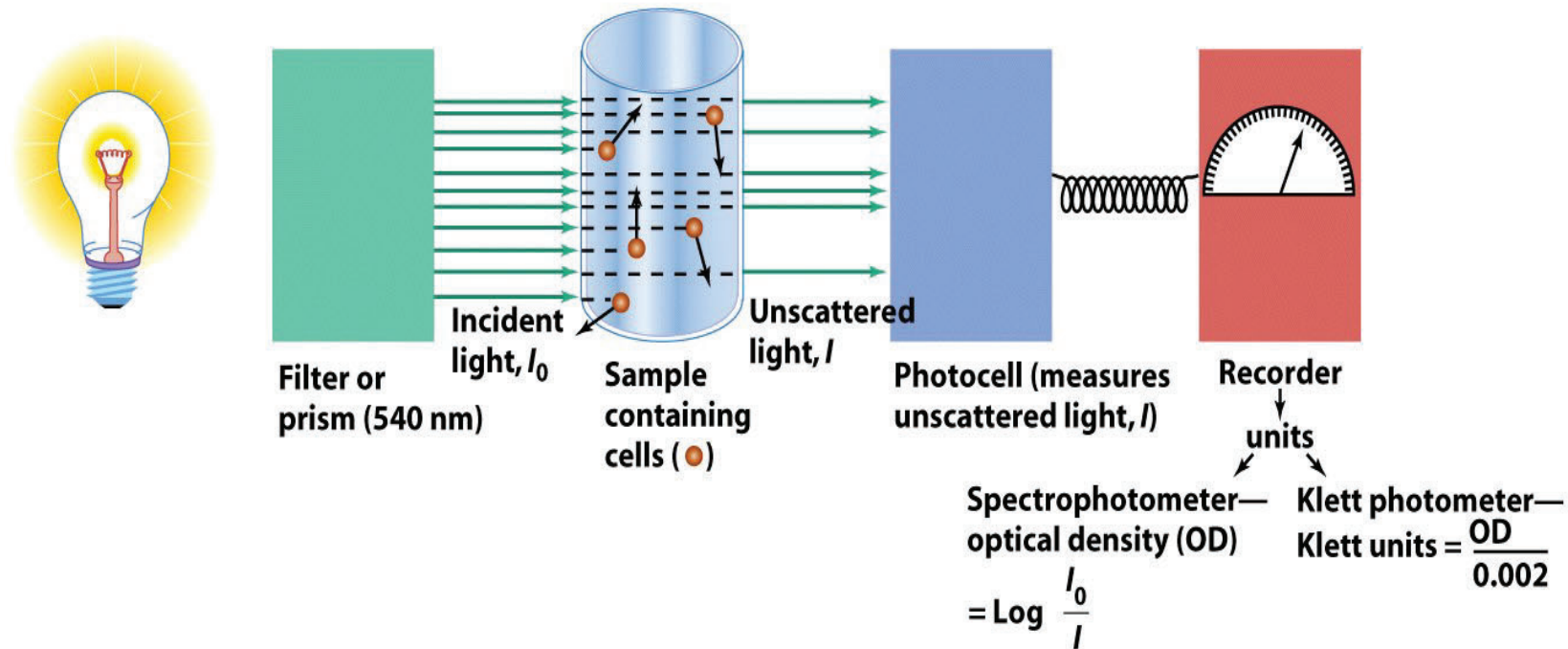


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Turbidity measurements are an indirect but very rapid and useful method of measuring microbial growth

Turbidimetric Methods

- Turbidity measurements are an indirect, rapid, and useful method of measuring microbial growth
 - Most often measured with a spectrophotometer and measurement referred to as optical density (O.D.)
- Turbidity measurements
 - Quick and easy to perform
 - Typically do not require destruction or significant disturbance of sample
 - Sometimes problematic (e.g., microbes that form clumps or biofilms in liquid medium)

Estimating Bacterial Numbers by Indirect methods

- Metabolic activity
- Dry weight

VBNC → viable But Not Countable

↓

To learn them
molecular methods
(QPCR, universal primers)
PCR