Microbial Growth

Growth of Microbes

- Increase in *number* of cells, not cell size
- One cell becomes colony of millions of cells

Growth of Microbes

- Control of growth is important for
  - infection control
  - growth of industrial and biotech organisms

Factors Regulating Growth

- Nutrients
- Environmental conditions: temperature, pH, osmotic pressure
- Generation time

Chemical Requirements

- #1 = water!
- Elements
  - C (50% of cell's dry weight) HONPS
  - Trace elements
- Organic
  - Source of energy (glucose)
  - Vitamins (coenzymes)
  - Some amino acids, purines and pyrimidines

Nitrogen: Found in all the amino acids, nitrogenous bases of nucleic acids, etc.

Hydrogen: found in all biological molecules, Carbs, fats, proteins, nucleic acids, etc

Phosphorous: found in nucleic acids, ATP, and phospholipdids of membranes

Sulfur: found in 2 or 3 amino acids of microbes

Trace elements: inorganic elements needed in very tiny concentrations (manganese, cobalt, Zn, Cr)

Organic cofactors:

Vitamins	
	Required by certain bacteria, “fastidious” hard to grow
Coenzymes	Many microbes produce their own from scratch, source of our supplements (one a day, GNC)

Fastidious organisms may require enriched media to get them to grow (blood, eggs, etc)

Some organisms are almost impossible to culture because of their strict parasitic-fastidious nature (syphilis, leprosy)

Nutritional Categories

- Carbon sources
  - CO_2 = autotroph
  - organic = heterotroph
- Energy sources
  - sunlight = phototroph
  - organic = chemotroph

A “Chemoheterotroph” would…..

- Derive both carbon and energy from organic compounds

A “Chemoorganic autotroph would be….

Derives energy from organic compounds and carbon source from inorganic compounds

A related ancient group…..

Lithoautotroph

Neither sunlight nor organics used, rather it relies totally on inorganics

Nutritional Categories

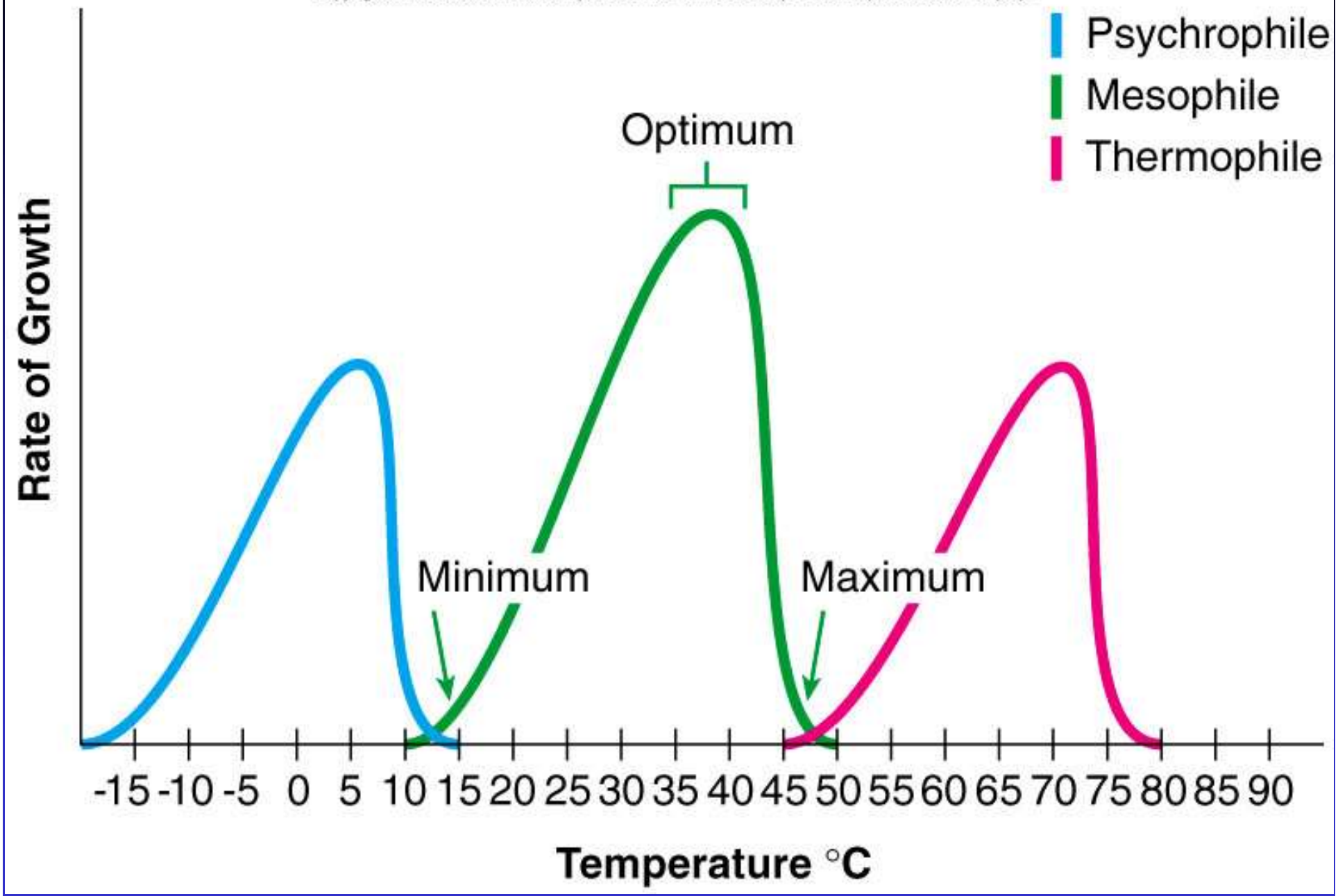
- Saprobe – lives on organic matter of dead organisms
- Parasite – lives on organic matter of living host = pathogens

Environmental Factors Influencing Growth

- Temperature
- O_2
- pH
- Osmotic Pressure
- Others: radiation, atmospheric pressure

Temperature Optima

- Psychrophiles: cold-loving
- Mesophiles: moderate temperature-loving
- Thermophiles: heat-loving
- Each has a minimum, optimum, and maximum growth temperature



Physical factors that affect bacterial growth;

Mesophiles : grow best moderate temp. 25 – 40 degrees

most of our lab microbes

Psychrophiles: adapted to survive and grow at cooler temp., even in the frig (below 25 degrees)

Listeria (in cheeses and meat)

Thermophiles: adapted to and grow at much higher temp.

Thermus aquaticus, from oceanic vents, survives at 60 degrees C

Leprosy bacilli prefer 30 degrees, most pathogens prefer 37 degrees.

Temperature Optima

- *Optimum* growth temperature is usually near the top of the growth range
- Death above the maximum temp. comes from enzyme inactivation
- Mesophiles most common group of organisms
- 40ºF (5°C) slows or stops growth of most microbes

Oxygen Requirements

- Obligate aerobes – require O_2
- Facultative anaerobes – can use O_2 but also grow without it
- Obligate anaerobes – die in the presence of O_2

Oxygen:

Obligate aerobes: require molecular oxygen (as final electron acceptor in catabolism)

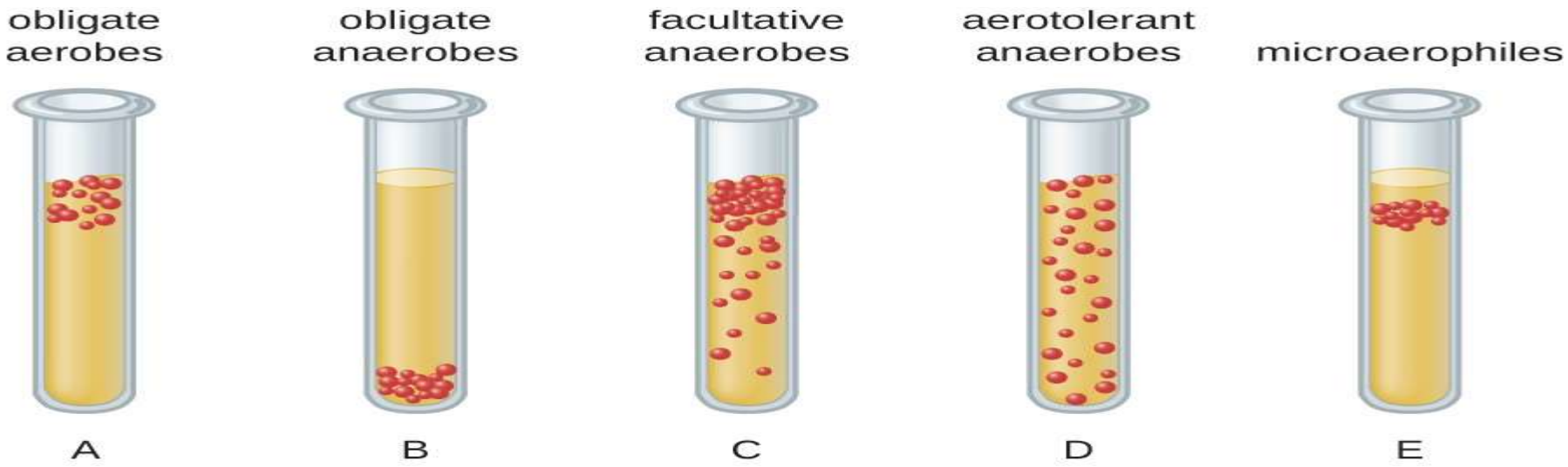
Pseudomonas spp.

Obligate anaerobes: require atmosphere with no O_2 an organic molecule is final electron acceptor in catabolism (like a fermentation pathway)

Clostrida - grow in "Brewer Jar"

Facultative anaerobes: grow with or without O_2, usually are also fermenters, like E. coli

Microaerophile: grow best in lower oxygen and higher carbon dioxide, Strep., candle jar



pH

- **Acidophiles** (*Lactobacillus acidophilus*)
  Acid (below pH 4) good preservative for pickles, sauerkraut, cheeses
- **Alkaliphiles** (*Vibrio*)

- **Neutralophiles** (pH 6-8)

Majority of the medically important bacteria grow best at neutral or slightly alkaline reaction (pH 7.2-7.6)

pH

- Many bacteria and viruses survive low pH of stomach to infect intestines
- *Helicobacter pylori* lives in stomach under mucus layer

Physical/Chemical factors that affect bacterial growth; pH: measure acidity and alkalinity of media

Bacteria grow best at pH range of 6.5 to 7.5

Fungi grow better at slightly acid condition (5.0 to 5.5)

Sabaraud dextrose and Potato dextrose agars

One pathogen, ***Helicobacter pylori***, is adapted to and survives in stomach acid (cause of ulcers)

Hydrostatic pressure: some bacteria grow really well deep in the ocean at pressures that crush submarines like and "egg"

Physical/Chemical factors that affect bacterial growth; pH: measure acidity and alkalinity of media

Osmotic pressure; relative salt concentrations in water solutions

Hypertonic: higher salt concentrations, slows or stops bacterial growth; salt preservative in meat

some prefer higher salt: Halophiles

some survive and thrive, Vibrio bacteria, V. cholera

Hypotonic: lower salt, fresh water, net flow water into cells, bacteria have rigid cell wall resist rupture

Isotonic: equal solute (salt) no net flow, preferable

Measuring Bacterial Growth

Bacterial Division

- Bacteria divide by binary fission
- Alternative means
  - Budding
  - Conidiospores (filamentous bacteria)
  - Fragmentation

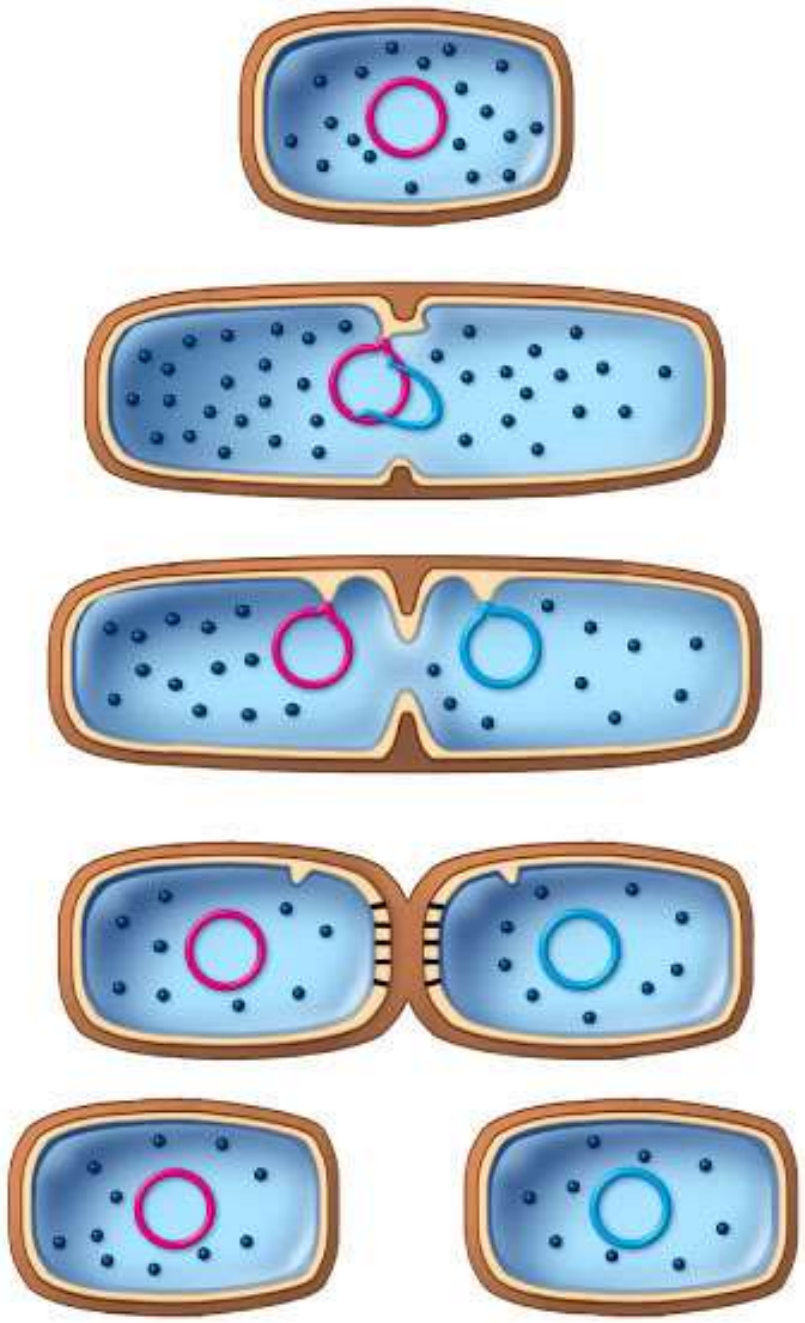
(a) A young cell at early phase of cycle.

(b) A parent cell prepares for division by enlarging its cell wall, cell membrane, and overall volume. Midway in the cell, the wall develops notches that will eventually form the transverse septum, and the duplicated chromosome becomes affixed to a special membrane site.

(c) The septum wall grows inward, and the chromosomes are pulled toward opposite cell ends as the membrane enlarges. Other cytoplasmic components are distributed (randomly) to the two developing cells.

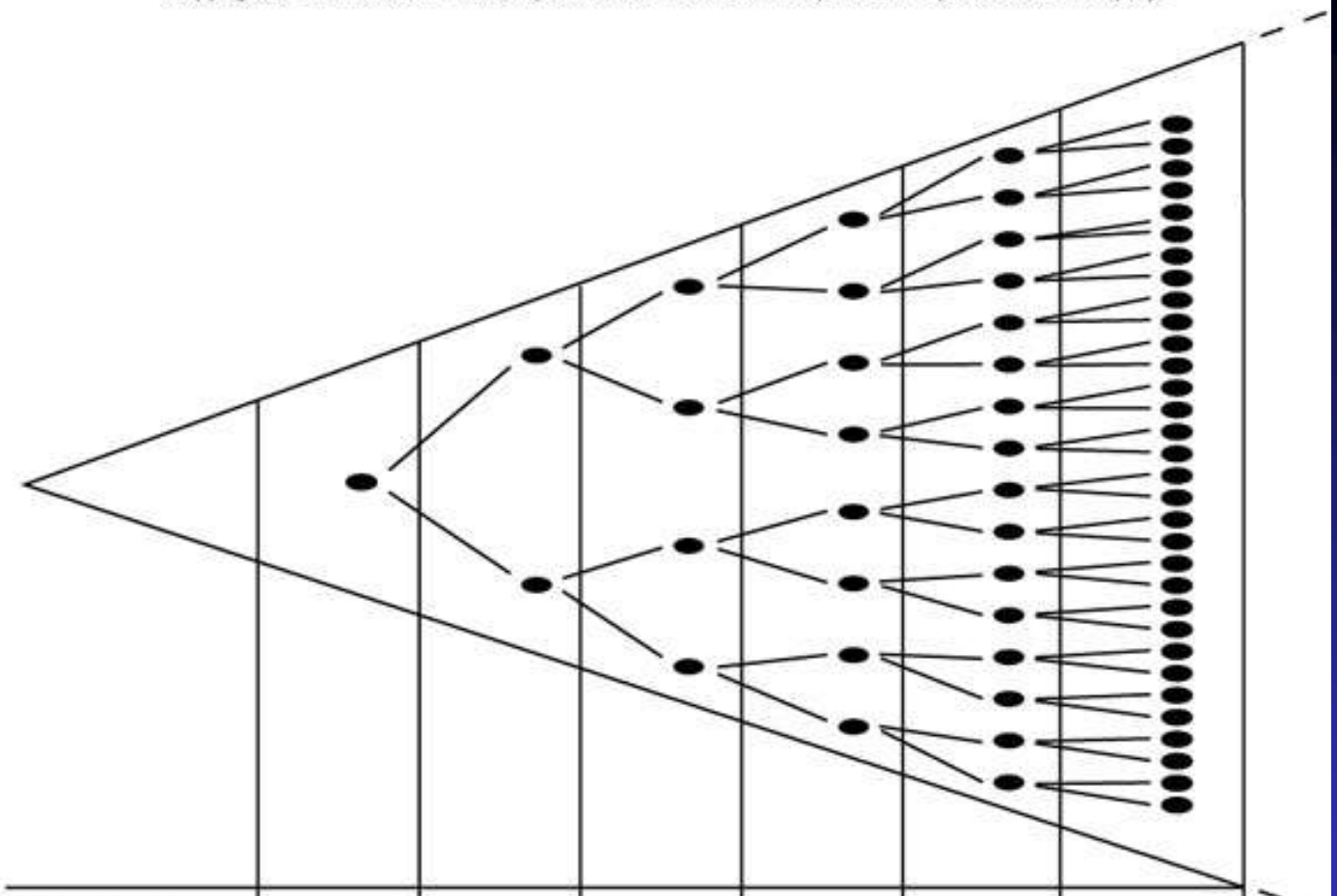
(d) The septum is synthesized completely through the cell center, and the cell membrane patches itself so that there are two separate cell chambers.

(e) At this point, the daughter cells are divided. Some species will separate completely as shown here, while others will remain attached, forming chains or doublets, for example.



Generation Time

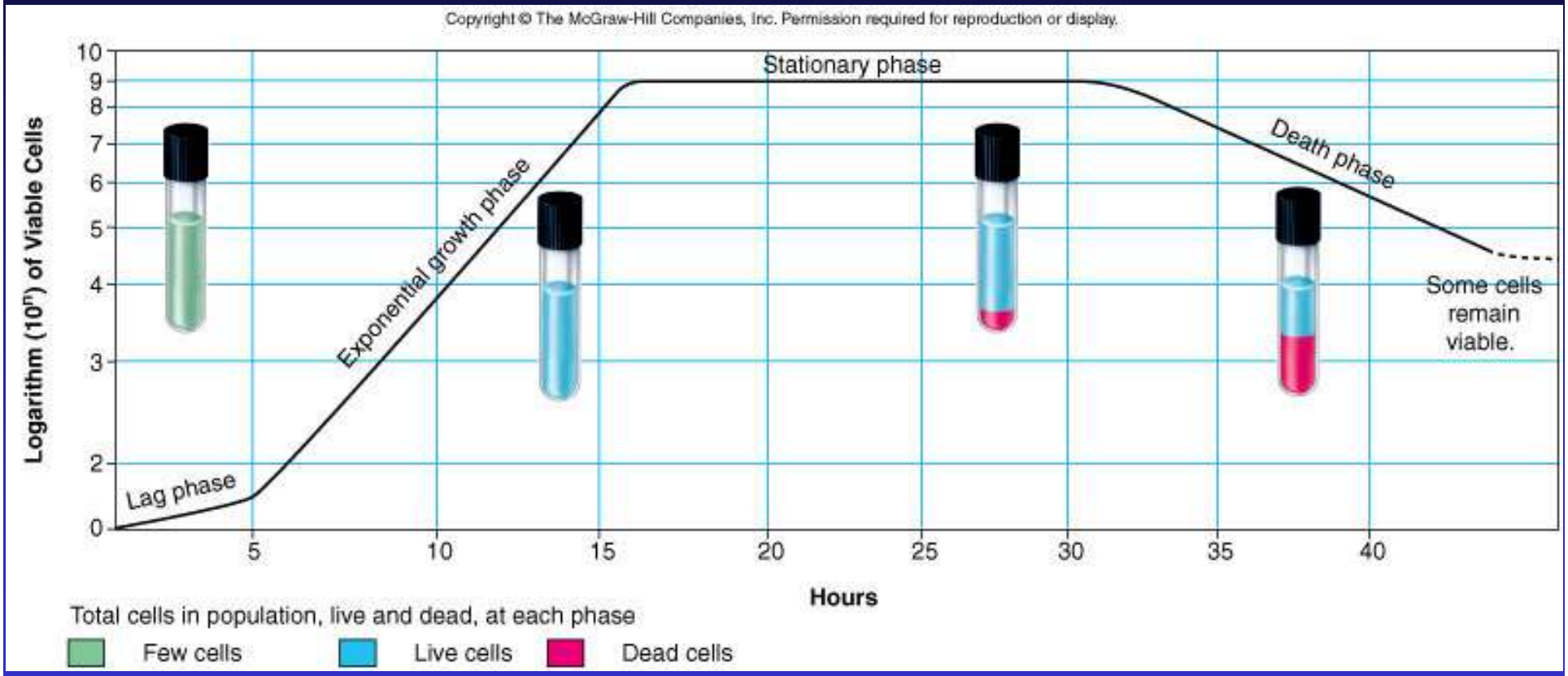
- Time required for cell to divide/for population to double
- Average for bacteria is 1-3 hours
- *E. coli* generation time = 20 min
  - 20 generations (7 hours), 1 cell becomes 1 million cells!



Number of cells	1	2	4	8	16	32
Number of generations		1	2	3	4	5
Exponential value		2^1 (2×1)	2^2 (2×2)	2^3 (2×2×2)	2^4 (2×2×2×2)	2^5 (2×2×2×2×2)

(a)

Standard Growth Curve



Phases of Growth

- Lag phase – making new enzymes in response to new medium
- Log phase – exponential growth
  - Desired for production of products
  - Most sensitive to drugs and radiation during this period

Phases of Growth

- Stationary phase –
  - –nutrients becoming limiting or waste products becoming toxic
  - – death rate = division rate
- Death phase – death exceeds division

Measuring Growth

- Direct methods – count individual cells
- Indirect Methods – measure effects of bacterial growth

Flask inoculated

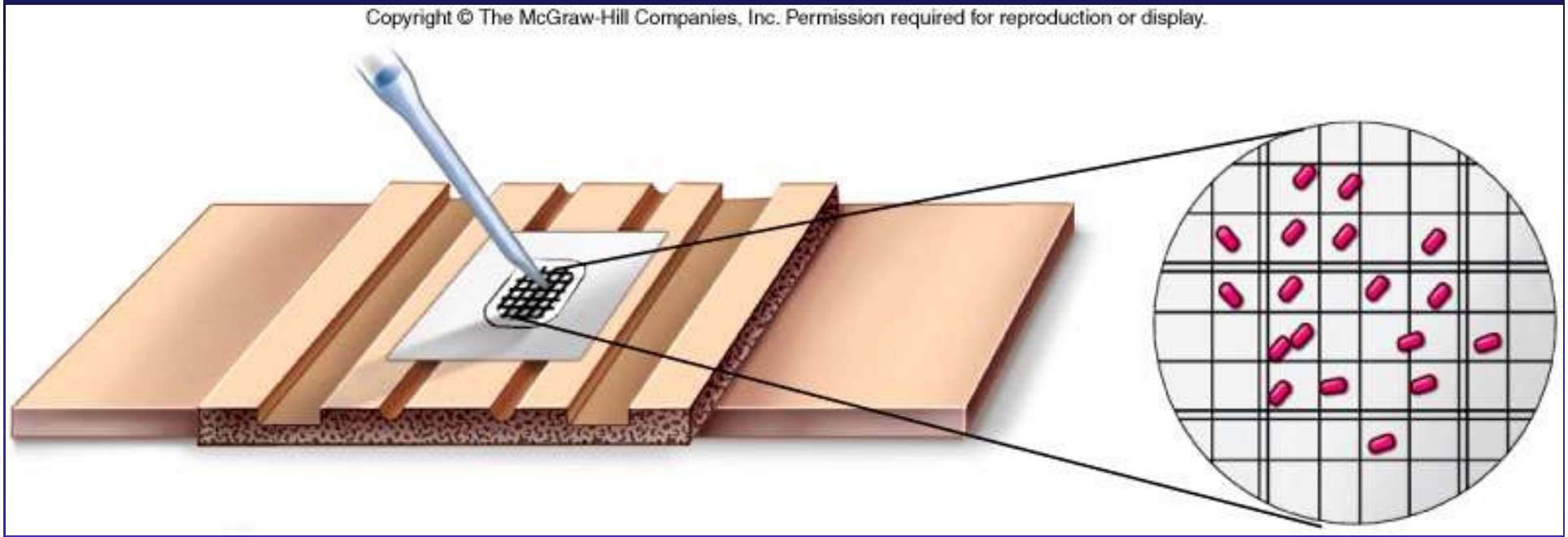
Samples taken at equally spaced intervals (0.1 ml)

0.1 ml

	60 min	120 min	180 min	240 min	300 min	360 min	420 min	480 min	540 min	600 min
Sample is diluted in liquid agar medium and poured or spread over surface of solidified medium										
Plates are incubated, colonies are counted	None									
Number of colonies (CFU) per 0.1 ml	<1*	1	3	7	13	23	45	80	135	230
Total estimated cell population in flask	<5,000	5,000	15,000	35,000	65,000	115,000	225,000	400,000	675,000	1,150,000

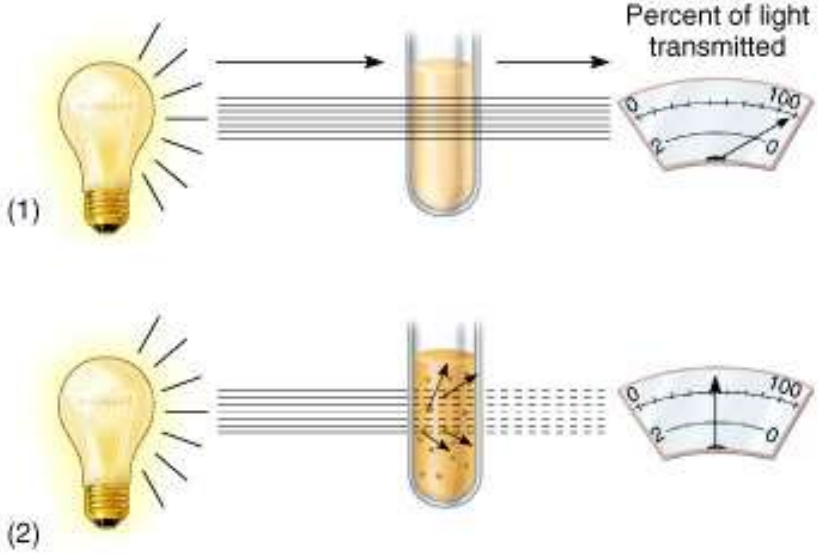
* Only means that too few cells are present to be assayed.

Fig. 7.17



Turbidity

(a)



(b)

Metabolic Activity

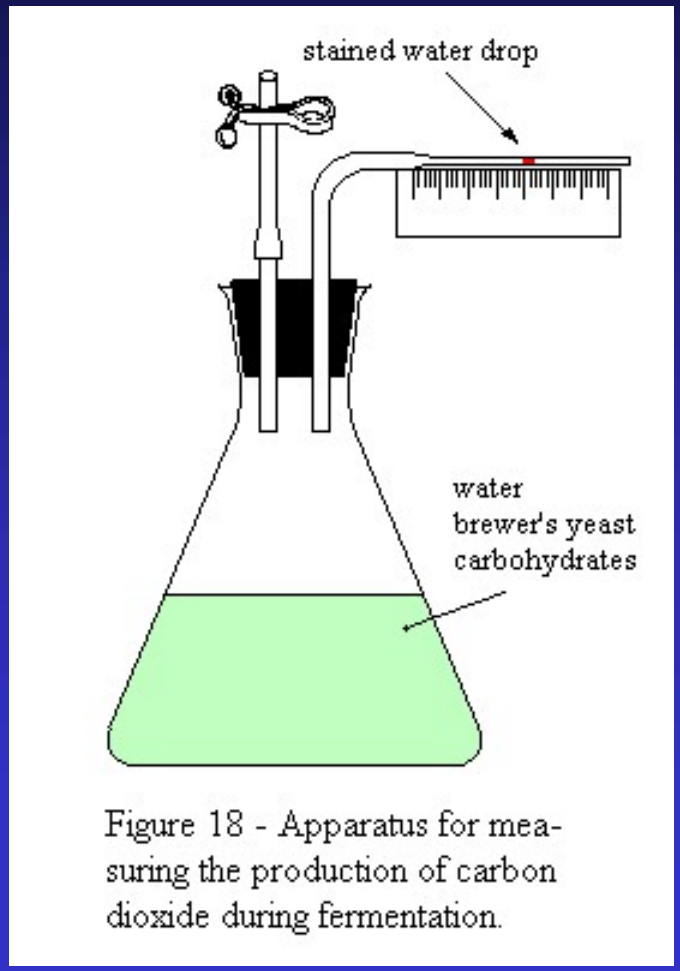
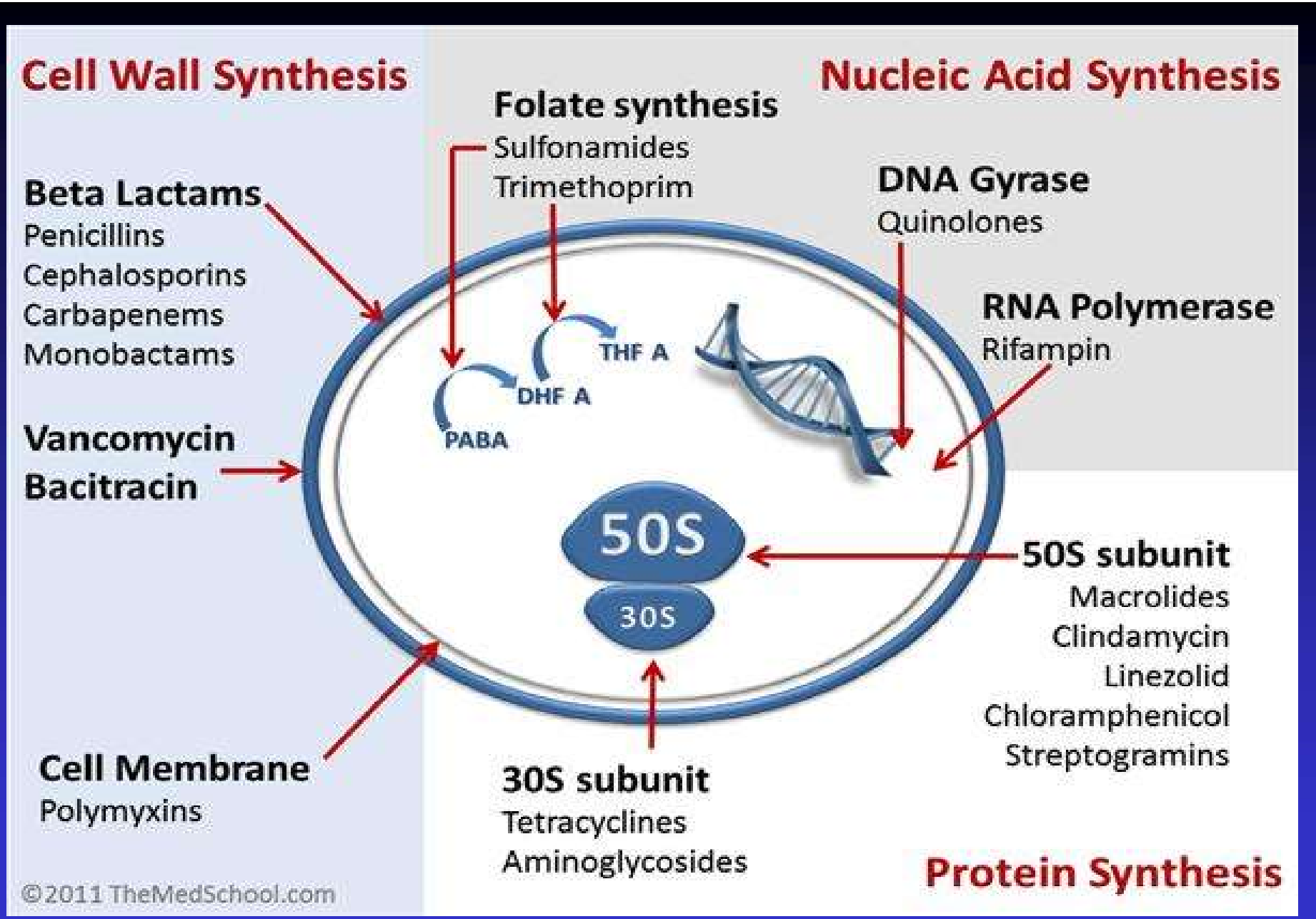


Figure 18 - Apparatus for measuring the production of carbon dioxide during fermentation.


Cell Wall Synthesis
Nucleic Acid Synthesis
Folate synthesis
Sulfonamides
Trimethoprim
Beta Lactams
Penicillins
Cephalosporins
Carbapenems
Monobactams
DNA Gyrase
Quinolones
RNA Polymerase
Rifampin
THF A
DHF A
PABA
Vancomycin
Bacitracin
50S
30S
50S subunit
Macrolides
Clindamycin
Linezolid
Chloramphenicol
Streptogramins
Cell Membrane
Polymyxins
30S subunit
Tetracyclines
Aminoglycosides
Protein Synthesis
©2011 TheMedSchool.com

Dry Weight