Microbial Growth

Environmental Forcing Functions:

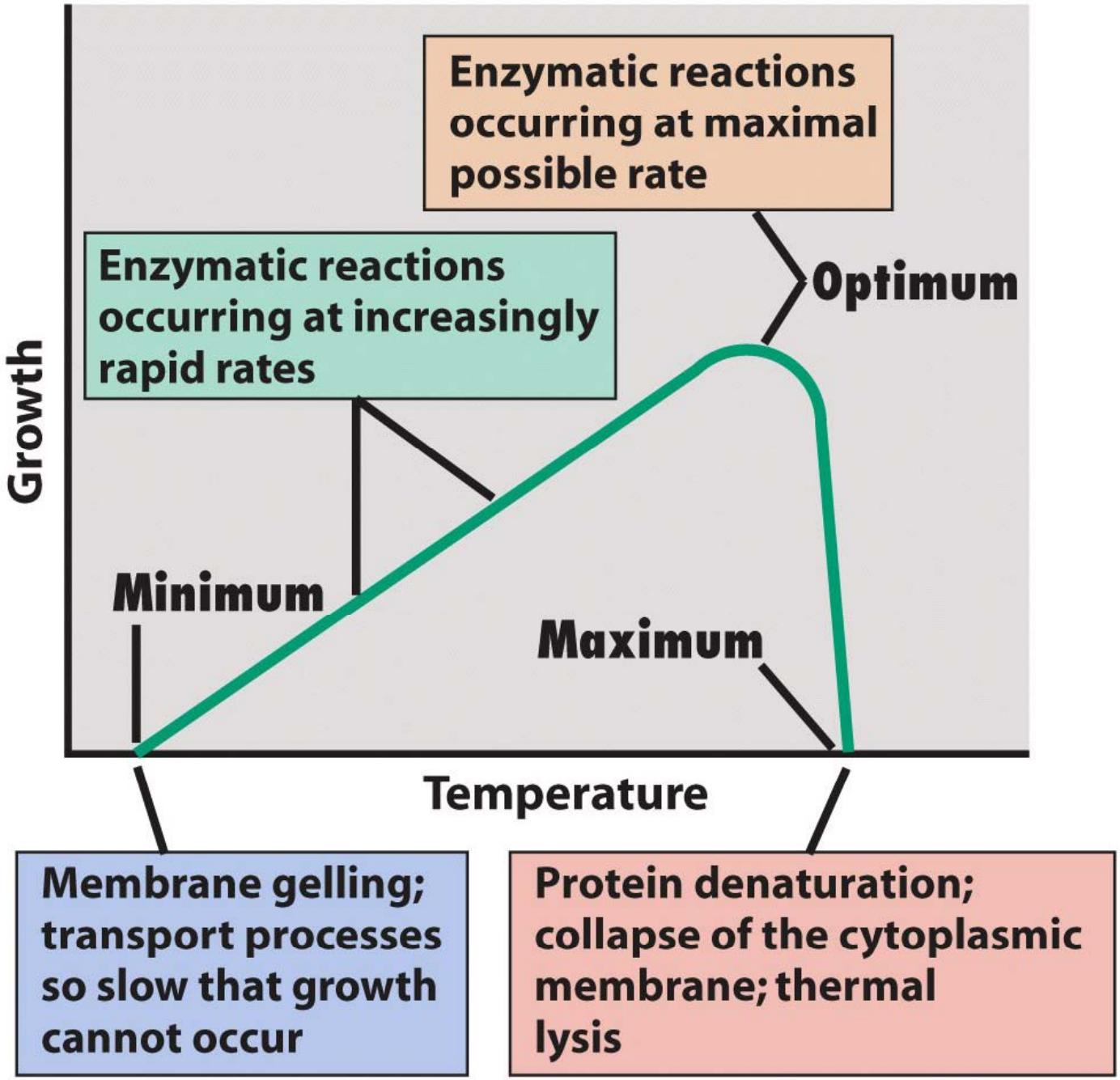
- Temperature:
  - Psychrophile, Mesophile, Thermophile & Hyperthermophile

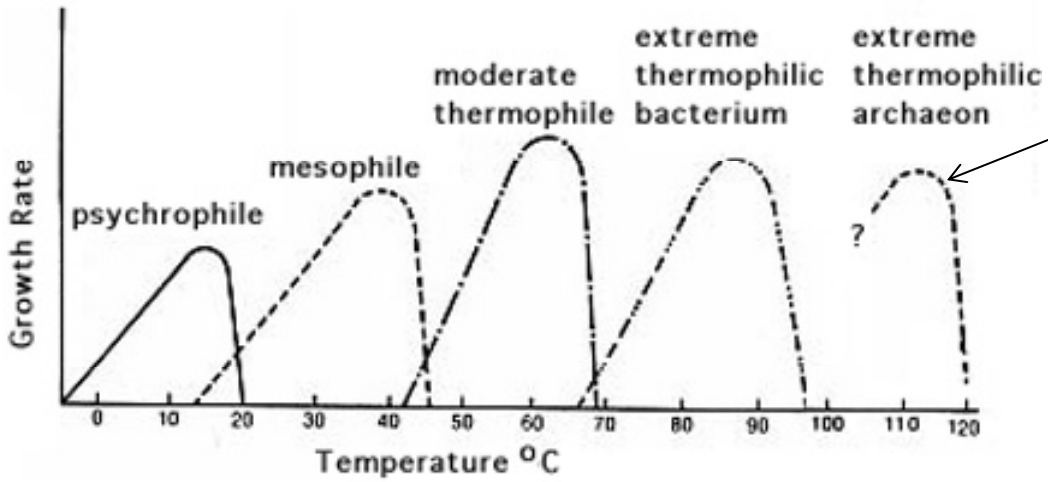
  Cardinal Temps: Min*, Max, & Optimal*

  Q_{10} Rule: 10°C rise will double the growth rate*

- Pressure: Barophiles (Most are also psychrophiles!)

  Found only in the deep ocean.....so far


Enzymatic reactions occurring at maximal possible rate
Optimum
Enzymatic reactions occurring at increasingly rapid rates
Growth
Minimum
Maximum
Temperature
Membrane gelling; transport processes so slow that growth cannot occur
Protein denaturation; collapse of the cytoplasmic membrane; thermal lysis



Methanopyrus kandleri 116
Takai, *et. al.* 2008 PNAS

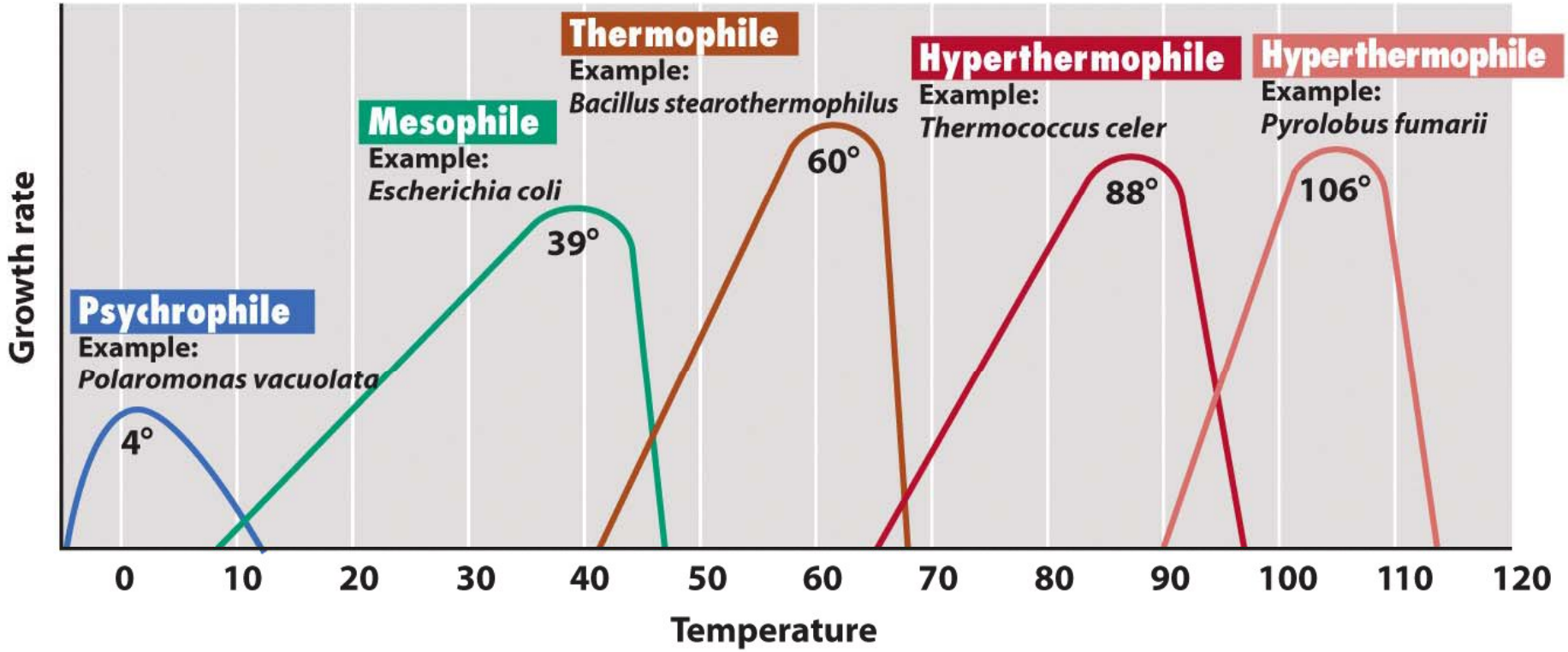
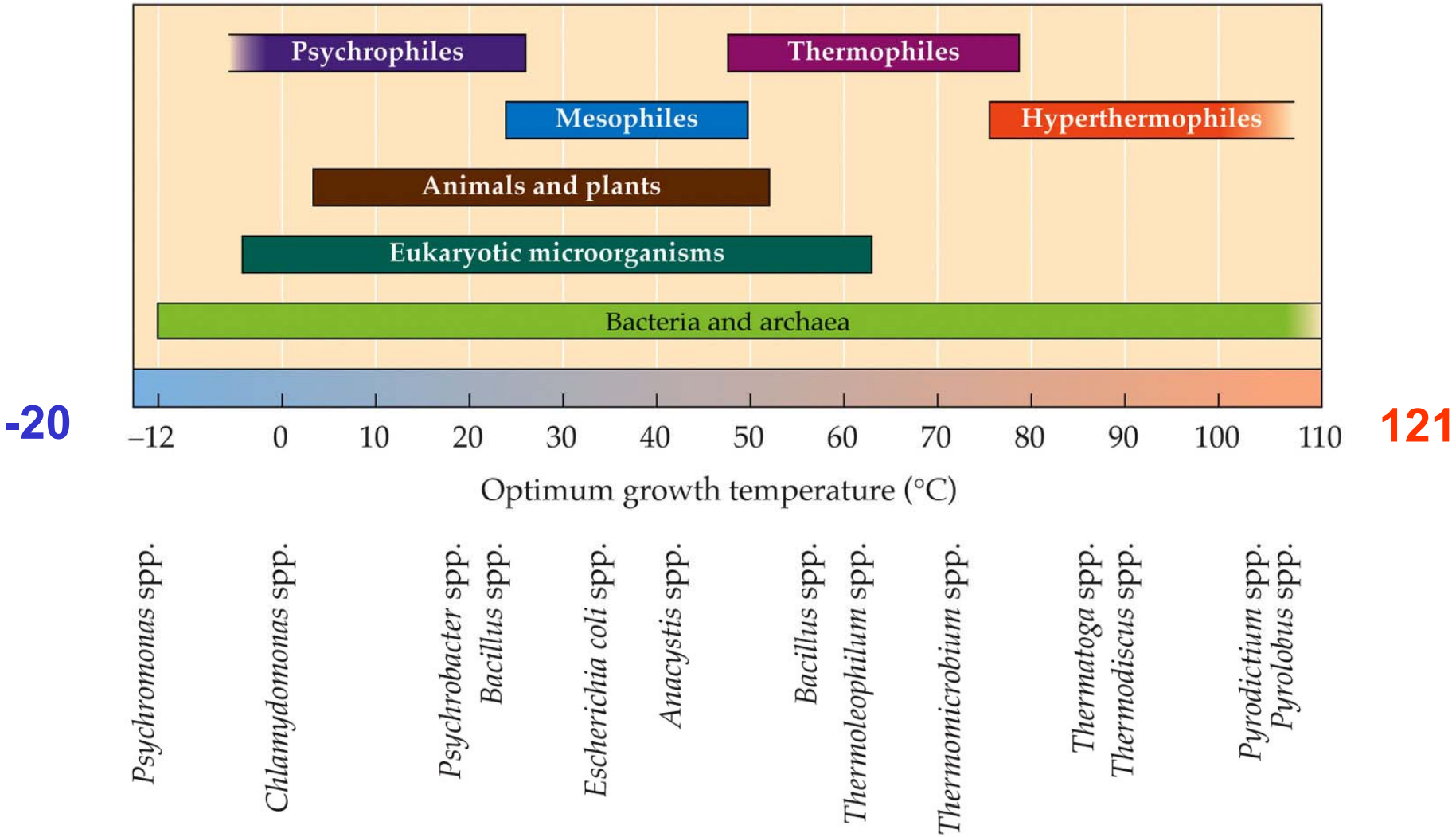
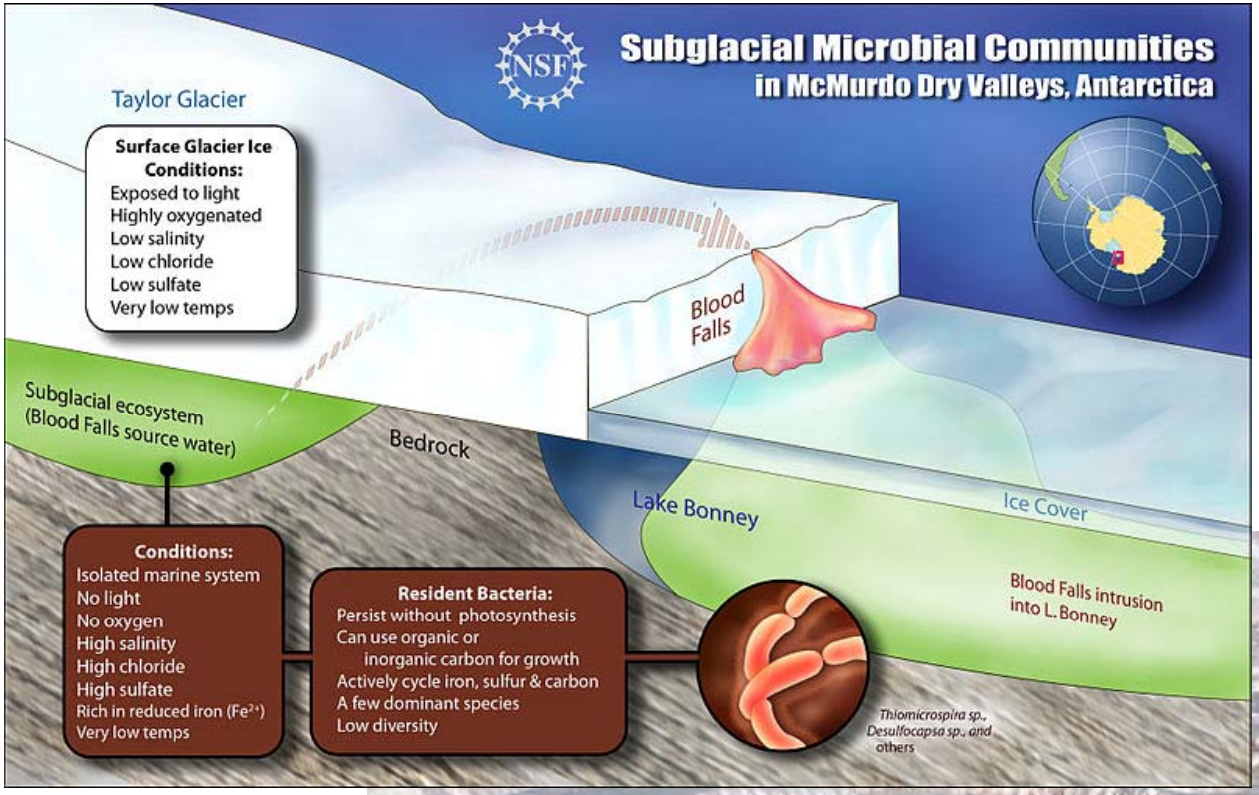


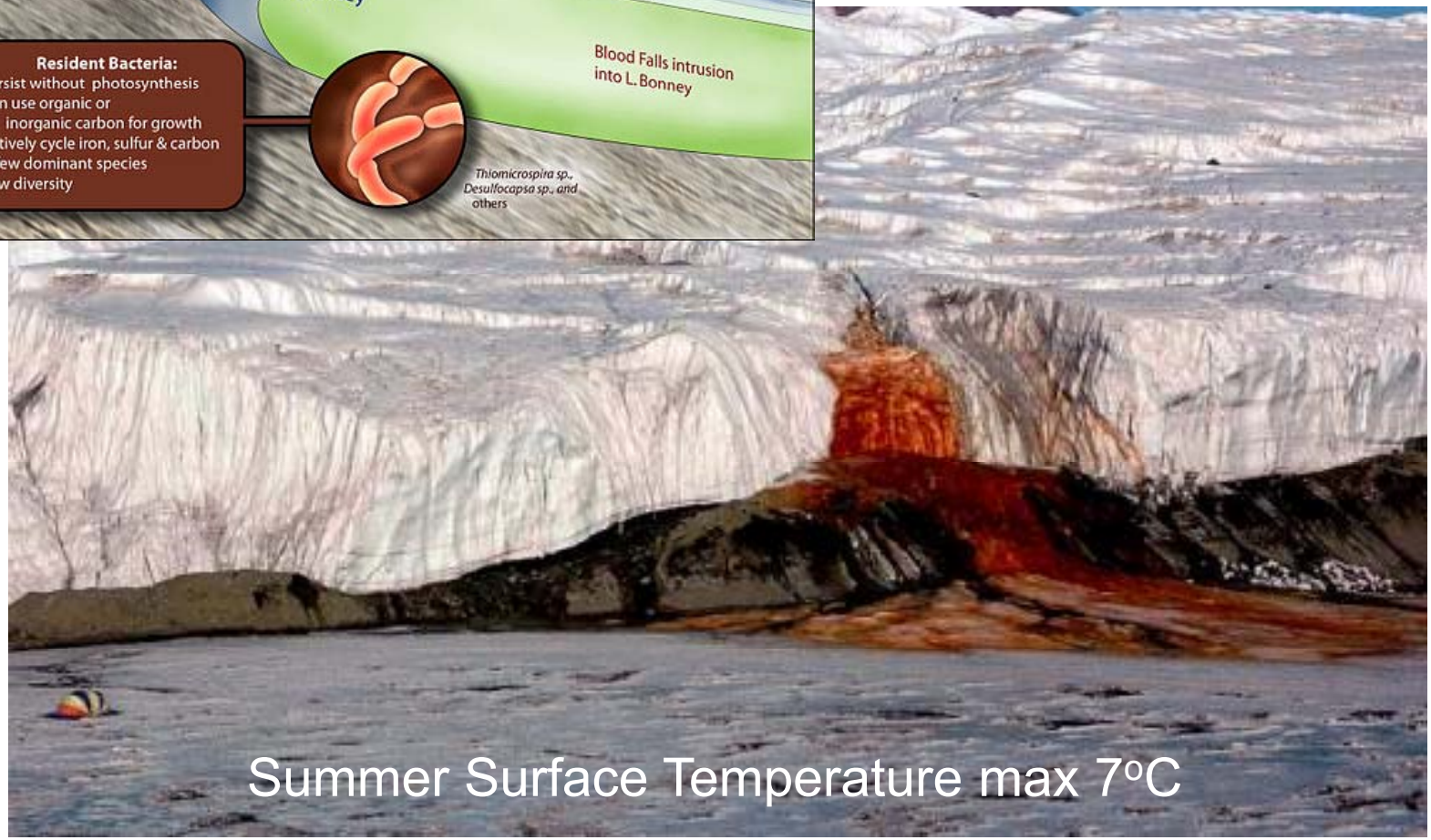
Table 6.3 Temperature ranges for growth of *Bacteria* and *Archaea*

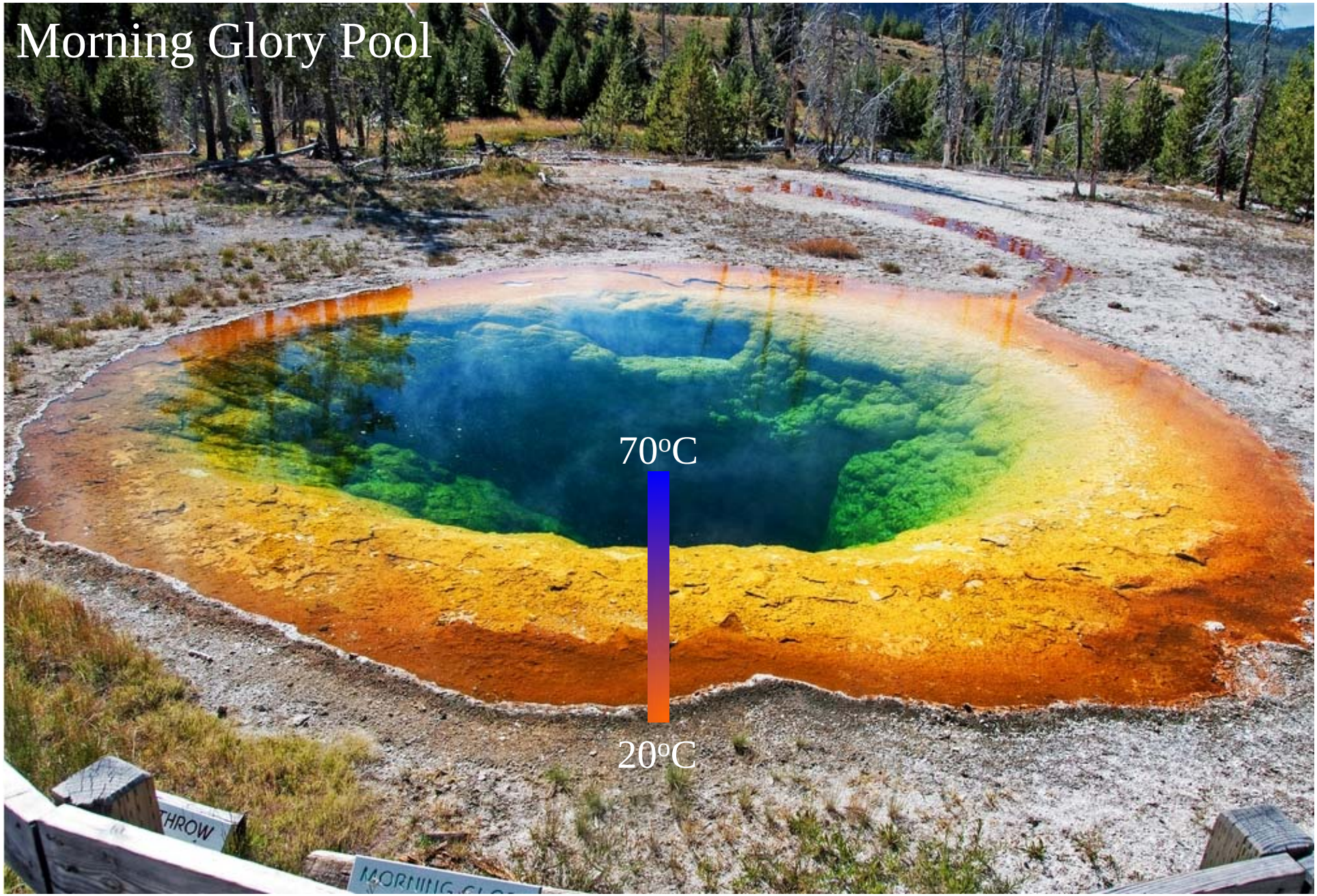
Species	Range (°C)
Psychrophiles	
Cytophaga psychrophila	4–20
Bacillus insolitus	<0–25
Aquaspirillum psychrophilum	2–26
Mesophiles	
Escherichia coli	10–40
Lactobacillus lactis	18–42
Bacillus subtilis	22–40
Pseudomonas fluorescens	4–40
Thermophiles	
Bacillus thermoleovorans	42–75
Thermoleophilum album	45–70
Thermus aquaticus	40–79
Chloroflexus aurantiacus	45–70
Hyperthermophiles (*Archaea*)	
Hyperthermus butylicus	85–108
Methanothermus fervidus	65–97
Pyrodictium occultum	80–110
Thermococcus celer	70–95

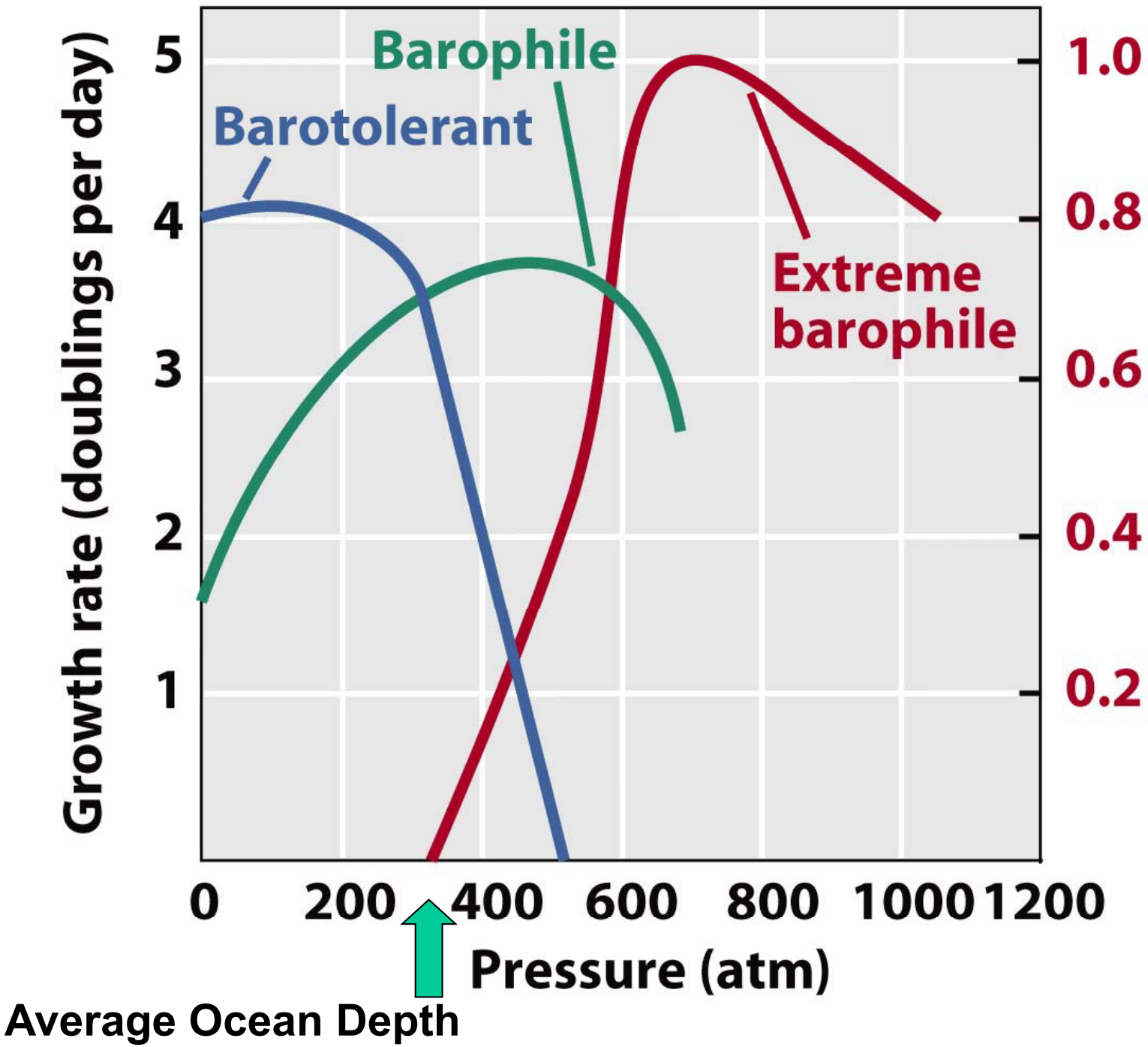
Growth temperature ranges for various life forms




NSF
Subglacial Microbial Communities
in McMurdo Dry Valleys, Antarctica
Taylor Glacier
Surface Glacier Ice Conditions:
Exposed to light
Highly oxygenated
Low salinity
Low chloride
Low sulfate
Very low temps
Blood Falls
Subglacial ecosystem (Blood Falls source water)
Bedrock
Lake Bonney
Ice Cover
Blood Falls intrusion into L. Bonney
Conditions:
Isolated marine system
No light
No oxygen
High salinity
High chloride
High sulfate
Rich in reduced iron (Fe^{2+})
Very low temps
Resident Bacteria:
Persist without photosynthesis
Can use organic or inorganic carbon for growth
Actively cycle iron, sulfur & carbon
A few dominant species
Low diversity
Thiomicrospira sp., Desulfocapsa sp., and others


Summer Surface Temperature max 7°C


Morning Glory Pool
70°C
20°C

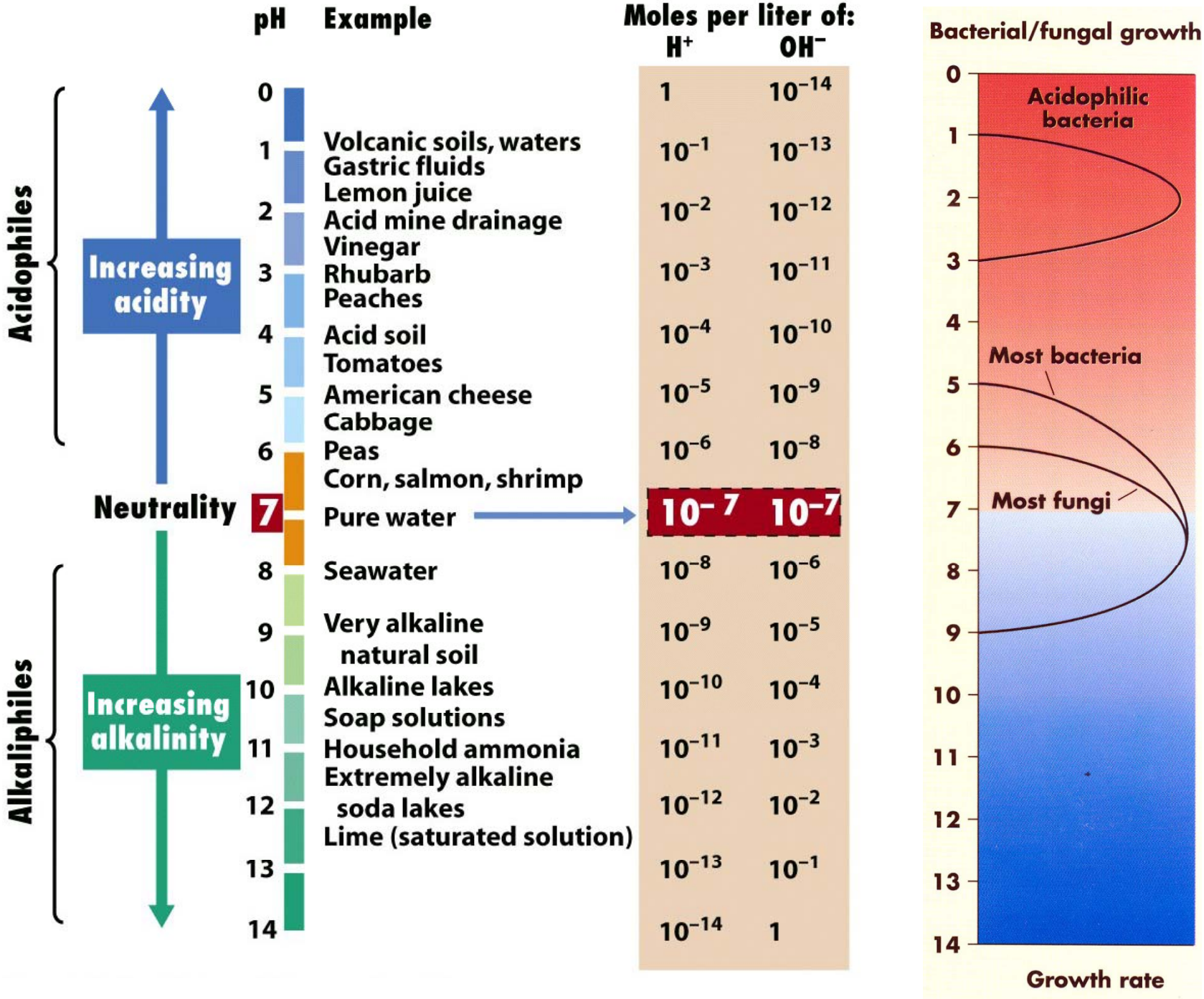

Barophile
Barotolerant
Extreme barophile
Growth rate (doublings per day)
5
4
3
2
1
1.0
0.8
0.6
0.4
0.2
0
200
400
600
800
1000
1200
Pressure (atm)
Average Ocean Depth

Microbial Growth

Environmental Forcing Functions:

- pH: acidophiles & alkaliphiles
  - Cytoplasm still near neutral

- Eh: available electron donors & terminal electron acceptors affects the chemistry of the environment


pH
Example
Moles per liter of:
H+
OH−
Acidophiles
Increasing acidity
Neutrality
Alkaliphiles
Increasing alkalinity
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
Volcanic soils, waters
Gastric fluids
Lemon juice
Acid mine drainage
Vinegar
Rhubarb
Peaches
Acid soil
Tomatoes
American cheese
Cabbage
Peas
Corn, salmon, shrimp
Pure water
Seawater
Very alkaline natural soil
Alkaline lakes
Soap solutions
Household ammonia
Extremely alkaline soda lakes
Lime (saturated solution)
1 10−14
10−1 10−13
10−2 10−12
10−3 10−11
10−4 10−10
10−5 10−9
10−6 10−8
10−7 10−7
10−8 10−6
10−9 10−5
10−10 10−4
10−11 10−3
10−12 10−2
10−13 10−1
10−14 1
Bacterial/fungal growth
Acidophilic bacteria
Most bacteria
Most fungi
Growth rate

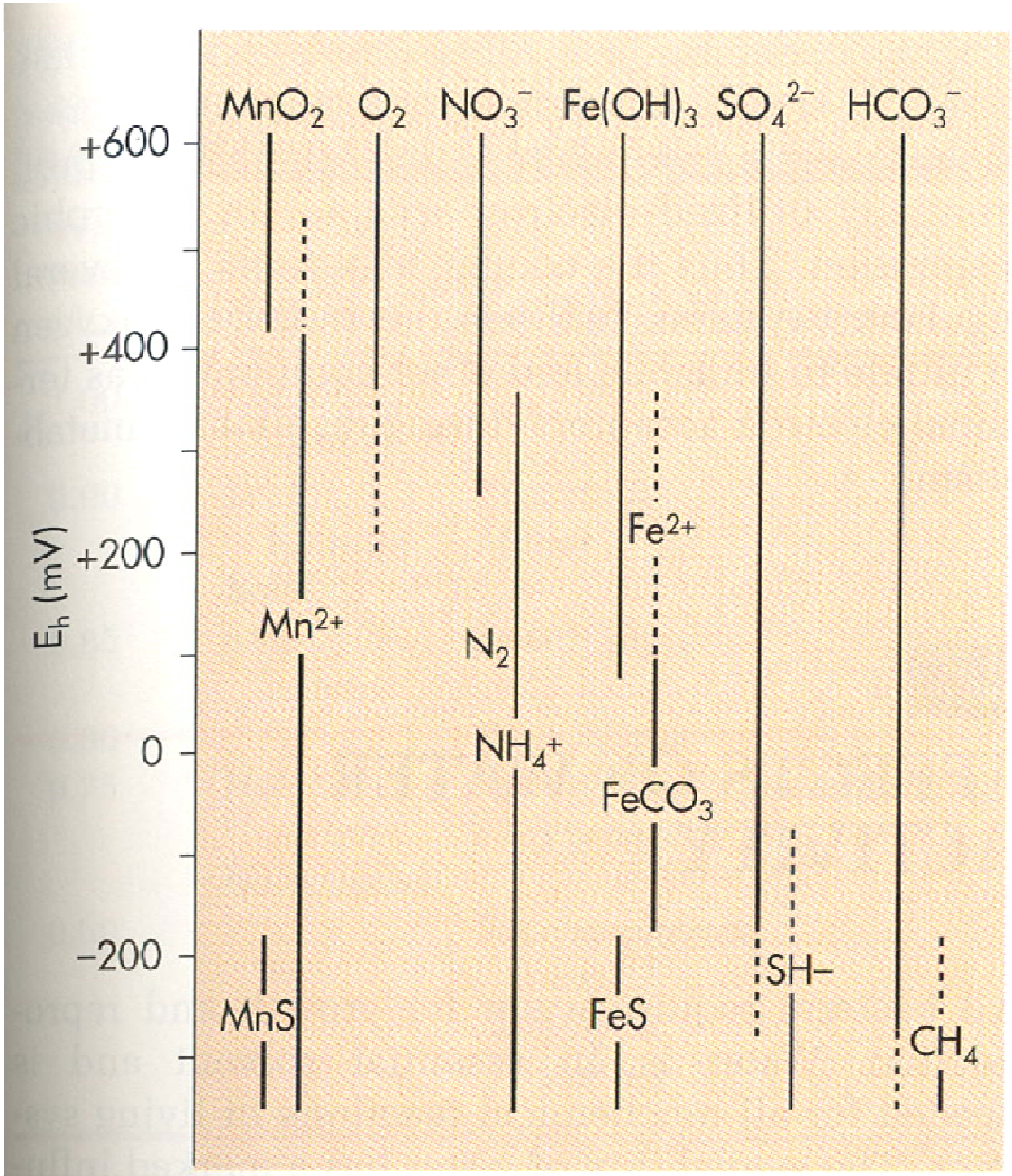
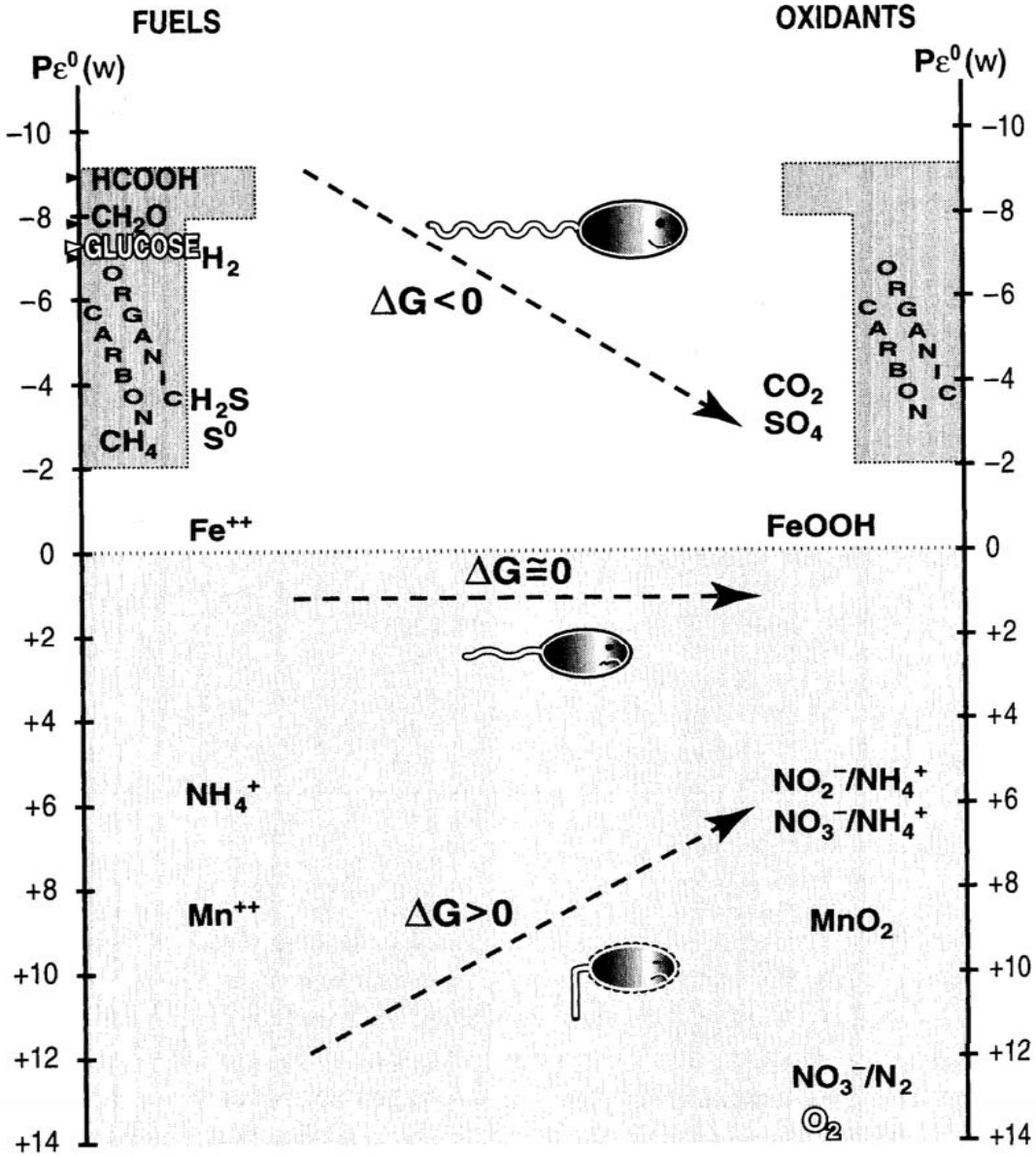


Fig. 9-26 E_h Values. Ranges of E_h values for various substances. In complex systems the reduction potential is influenced by the strongest oxidant, or reductant, in that system.

Thermodynamics: The Chemical Fuels and Oxidants of Life

e- source



e- sink

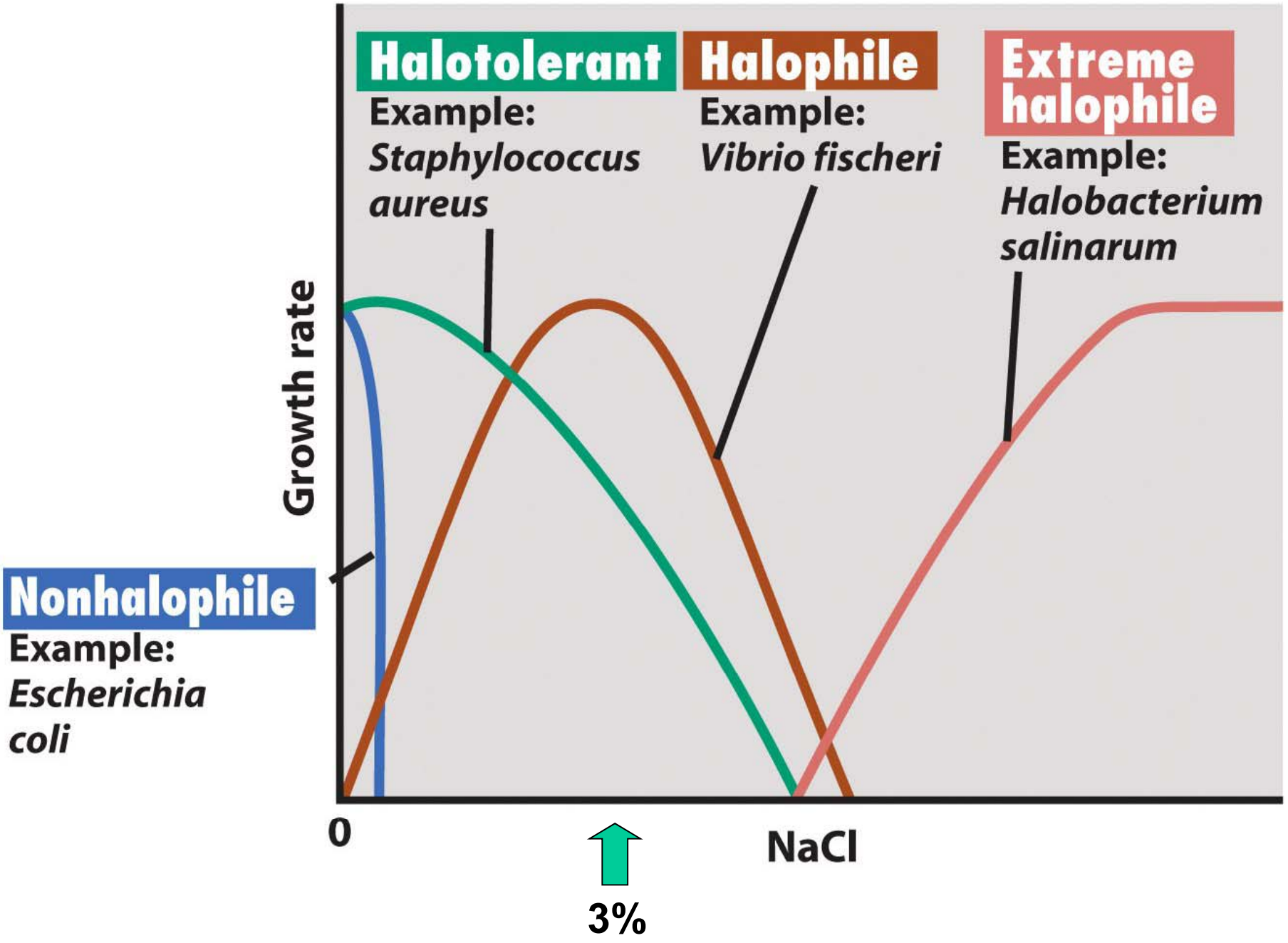
Microbial Growth

Environmental Forcing Functions:

- Salt: Halophiles
  Compatible solutes: amino acid derivatives (e.g., proline & glycine), sugars, & alcohols.

- Water Activity: Xerophiles (live in very dry habitats)
  Rem: All microbes are **osmotrophs**, must use organic material in solution!

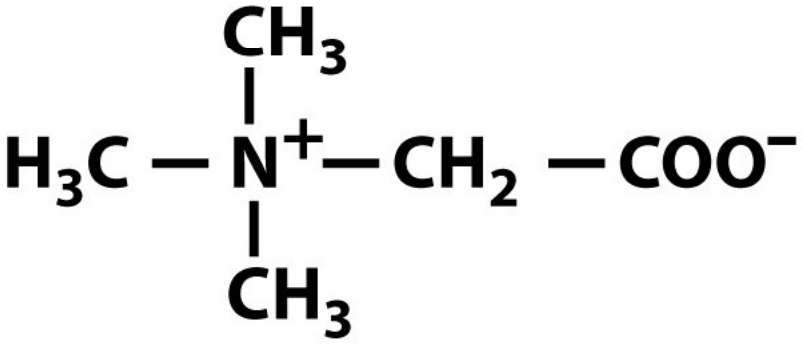
- Oxygen Usage: aerobe, facultative (an)aerobe, microaerophile, obligate anaerobe
  DeTox enzymes: Catalase, Peroxidase, SOD


Halotolerant
Example:
Staphylococcus aureus
Halophile
Example:
Vibrio fischeri
Extreme halophile
Example:
Halobacterium salinarum
Growth rate
Nonhalophile
Example:
Escherichia coli
0
NaCl
3%

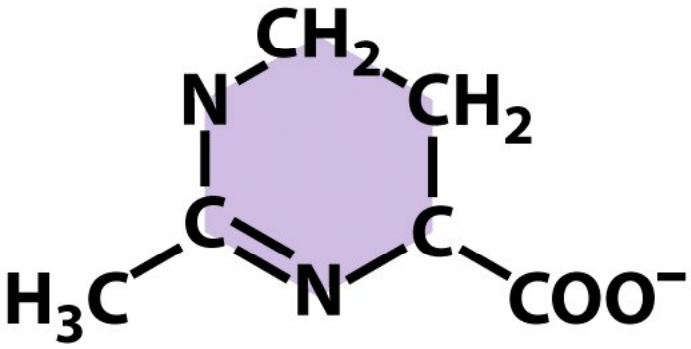
Compatible solutes

1. Amino acid–type and related solutes:

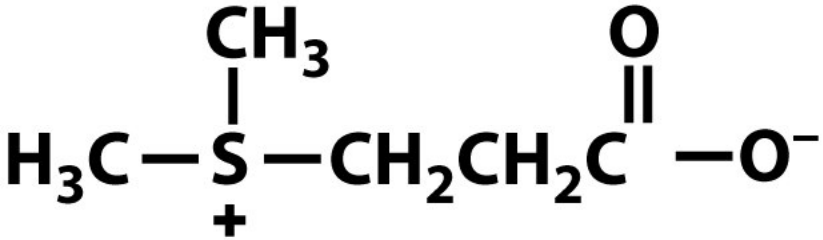
Glycine betaine



Ectoine



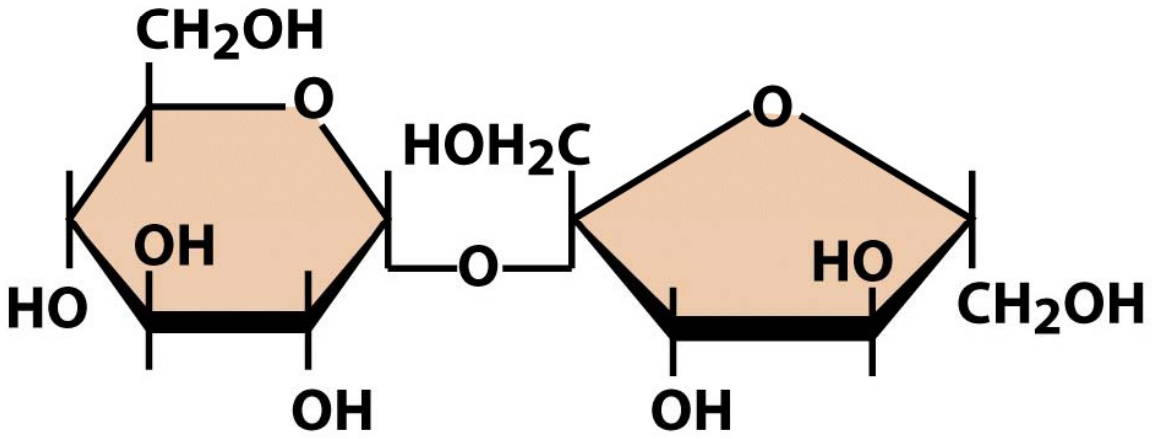
Dimethylsulfoniopropionate



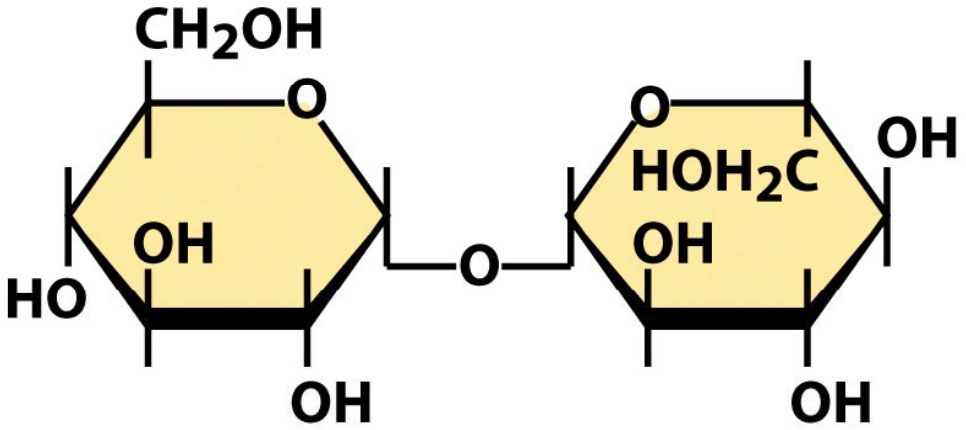
Compatible solutes

2. Carbohydrate-type solutes:

Sucrose



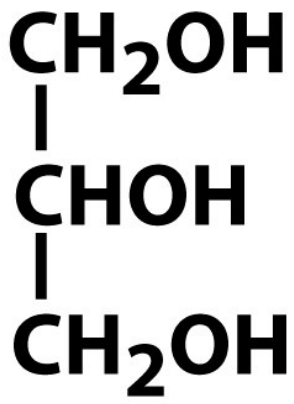
Trehalose



Compatible solutes

3. Alcohol-type solutes:

Glycerol



Mannitol

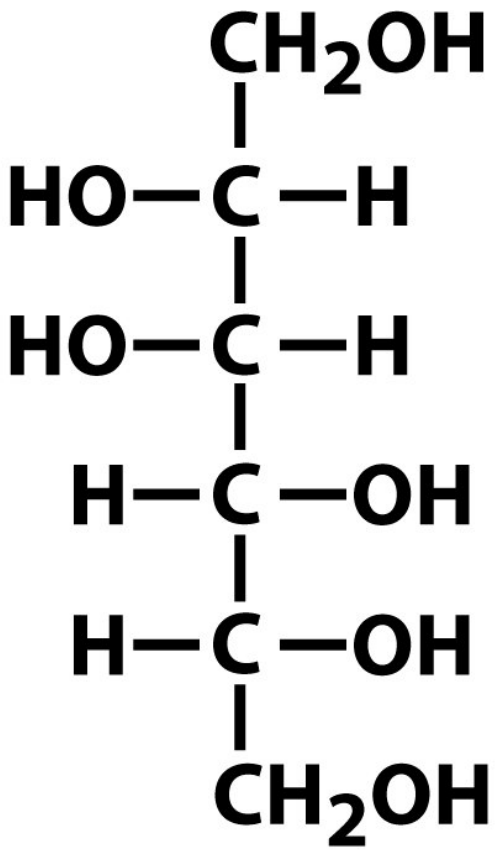


Table 6.4 Tolerance of selected *Bacteria* and *Archaea* for decreased water activity a_w

Type	Organisms	a_w
Nonhalophiles	*Aquaspirillum* and *Caulobacter*	1.00
Marine forms	Pseudomonads and *Alteromonas*	0.98
Moderate halophiles	*Vibrio* species and gram-positive cocci	0.91
Extreme halophiles	*Halobacterium* and *Halococcus*	0.75

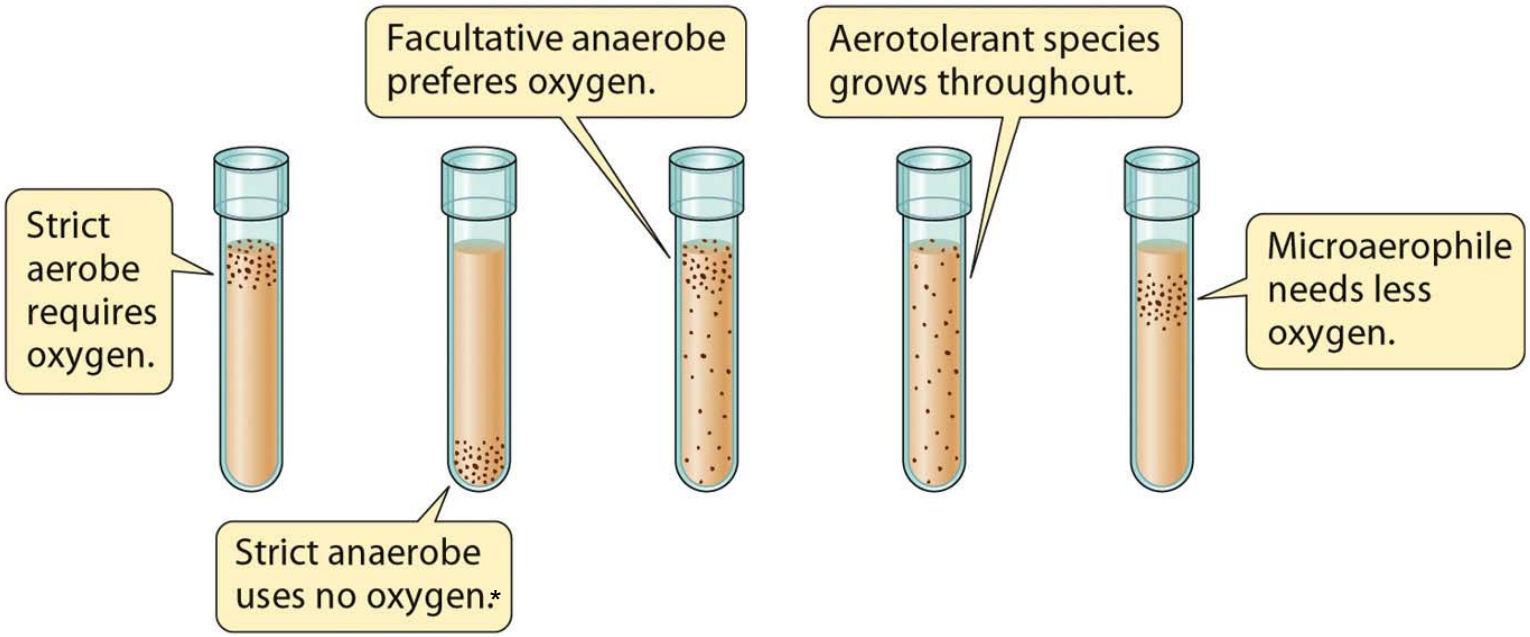
Water activity is a measure of the energy status of the water in a system. It is defined as the vapor pressure of a liquid divided by that of pure water at the same temperature; therefore, pure distilled water has a water activity of exactly one.

$$a_w \equiv p/p_o$$

p = vapor pressure of water in the substance

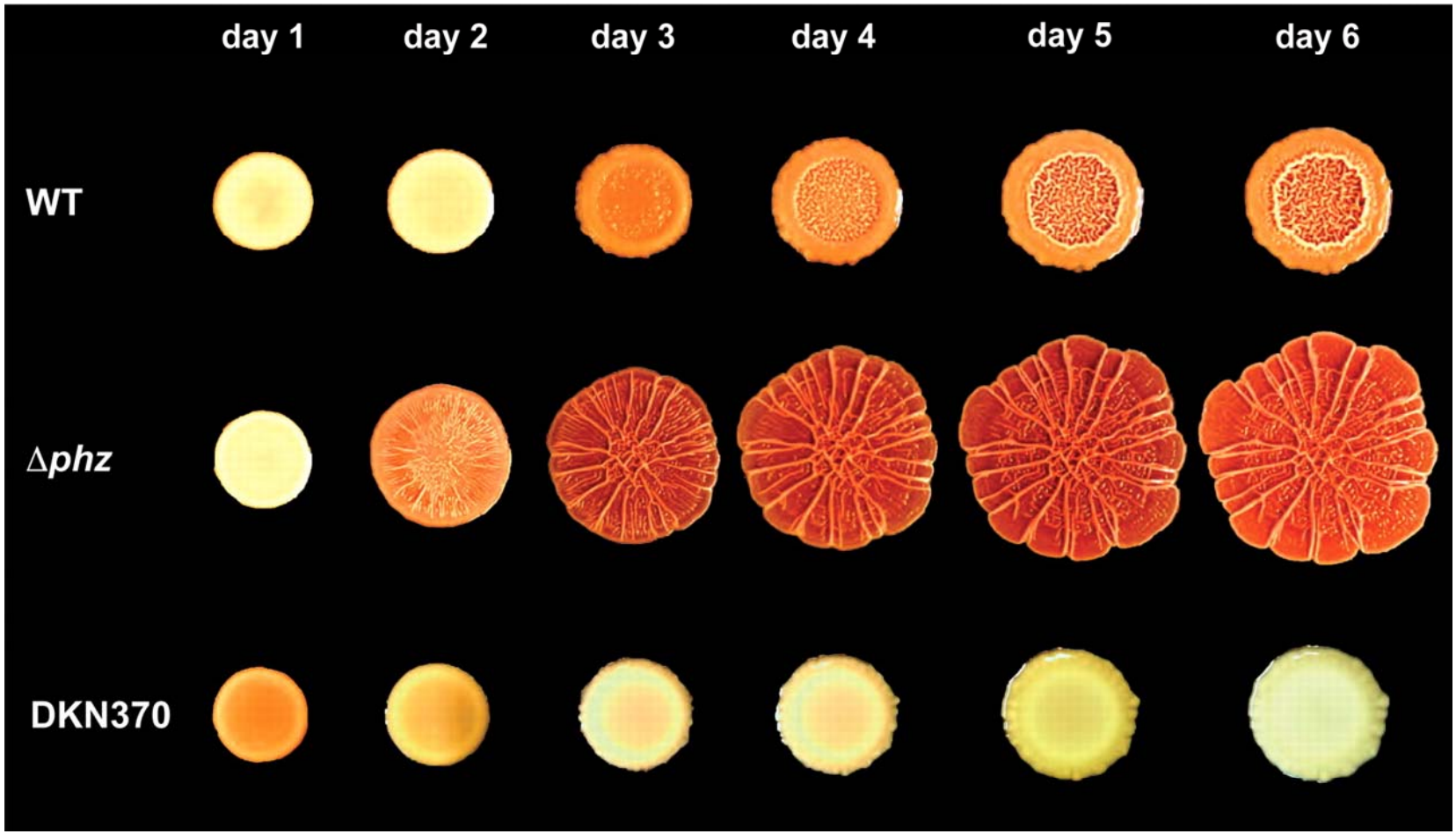
p_o = vapor pressure of pure water

Response of bacterial growth to oxygen availability



*Strict anaerobes will not grow if culture medium is prepared aerobically or if in plastic tubes

Change in Colony Phenotype due to Redox-Active compounds

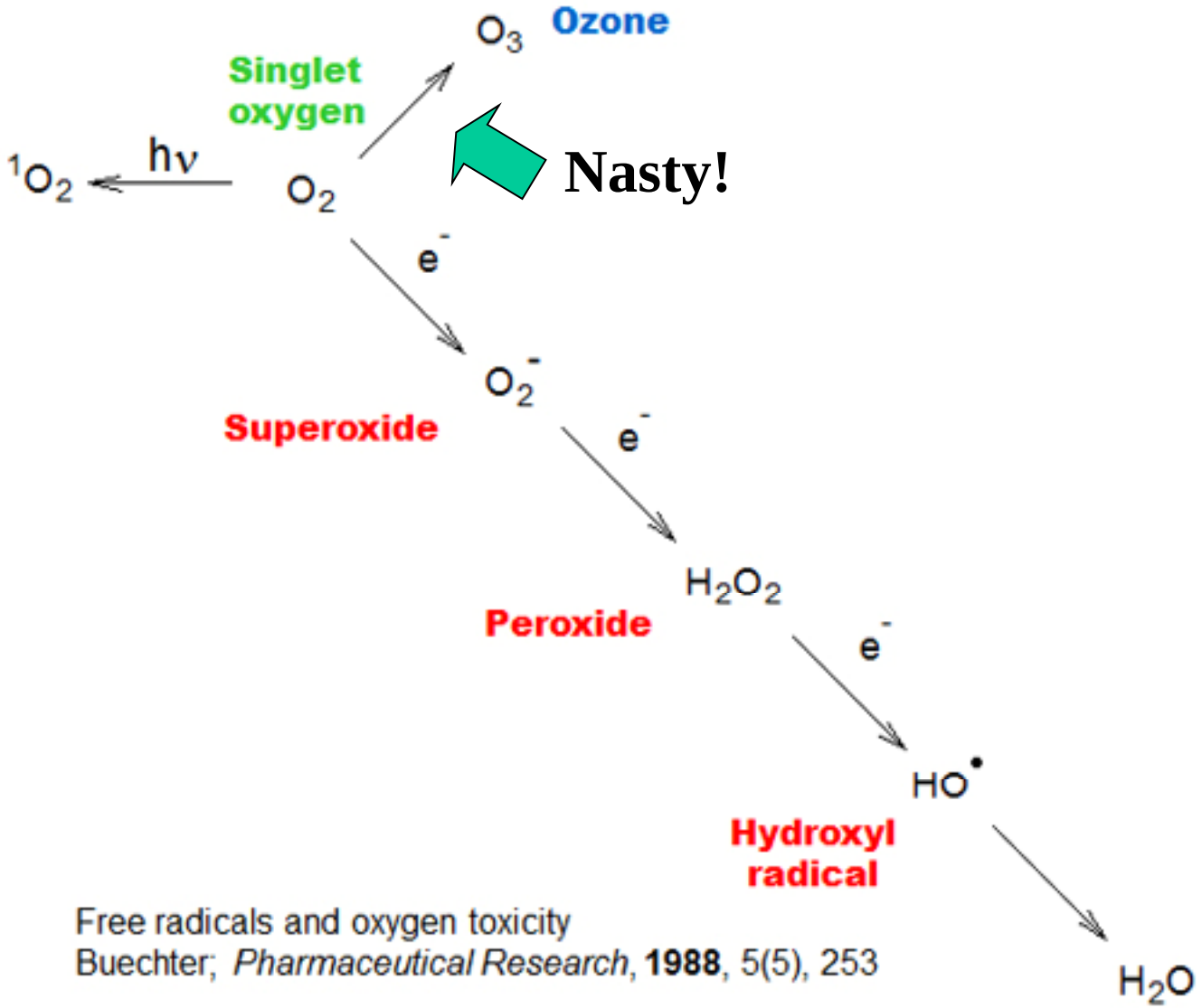


Phenazine production modulates colony morphology in *P. aeruginosa* PA14. Cultures were spotted onto agar plates containing Congo Red and Coomassie Blue, and incubated at 20°C for 6 days. The phenazine null strain (Δphz) started to wrinkle on day 2, the wild type (wt) wrinkled on day 3, whereas a pyocyanin overproducer (DKN370) remained smooth and white after 6 days.

L.E.P. Dietrich *et. al.* 2008 Science


OXYGEN is the #1 Killer
ANAEROBES ARE OUR FRIENDS!
Anaerobic Glove Bag
N_2/H_2 Atmosphere
For Obligate Anaerobes
BBL GasPak Plus
Anaerobic Jar Atmosphere by GasPak

Reactive & Toxic forms of Oxygen



Free radicals and oxygen toxicity
Buechter; *Pharmaceutical Research*, **1988**, 5(5), 253

4 electron reduction of O_2 to water

$$O_2 + e^- \rightarrow O_2^-$$ Superoxide

$$O_2^- + e^- + 2\,H^+ \rightarrow H_2O_2$$ Hydrogen peroxide

$$H_2O_2 + e^- + H^+ \rightarrow H_2O + OH\bullet$$ Hydroxyl radical

$$OH\bullet + e^- + H^+ \rightarrow H_2O$$ Water

Overall: $O_2 + 4\,e^- + 4\,H^+ \rightarrow 2\,H_2O$

Bacterial Enzymes that Protect the Cell Against Toxic Forms of Oxygen

	Catalase	Superoxide Dismutase
Aerobe	+	+
Faculatative anaerobe	+	+
Microaerophile	-	+
Obligate Anaerobe	-	-

Absence of these enzymes leads to Oxygen sensitivity

(a) **Catalase:**

$$H_2O_2 + H_2O_2 \rightarrow 2\,H_2O + O_2$$

(b) **Peroxidase:**

$$H_2O_2 + NADH + H^+ \rightarrow 2\,H_2O + NAD^+$$

(c) **Superoxide dismutase:**

$$O_2^- + O_2^- + 2\,H^+ \rightarrow H_2O_2 + O_2$$

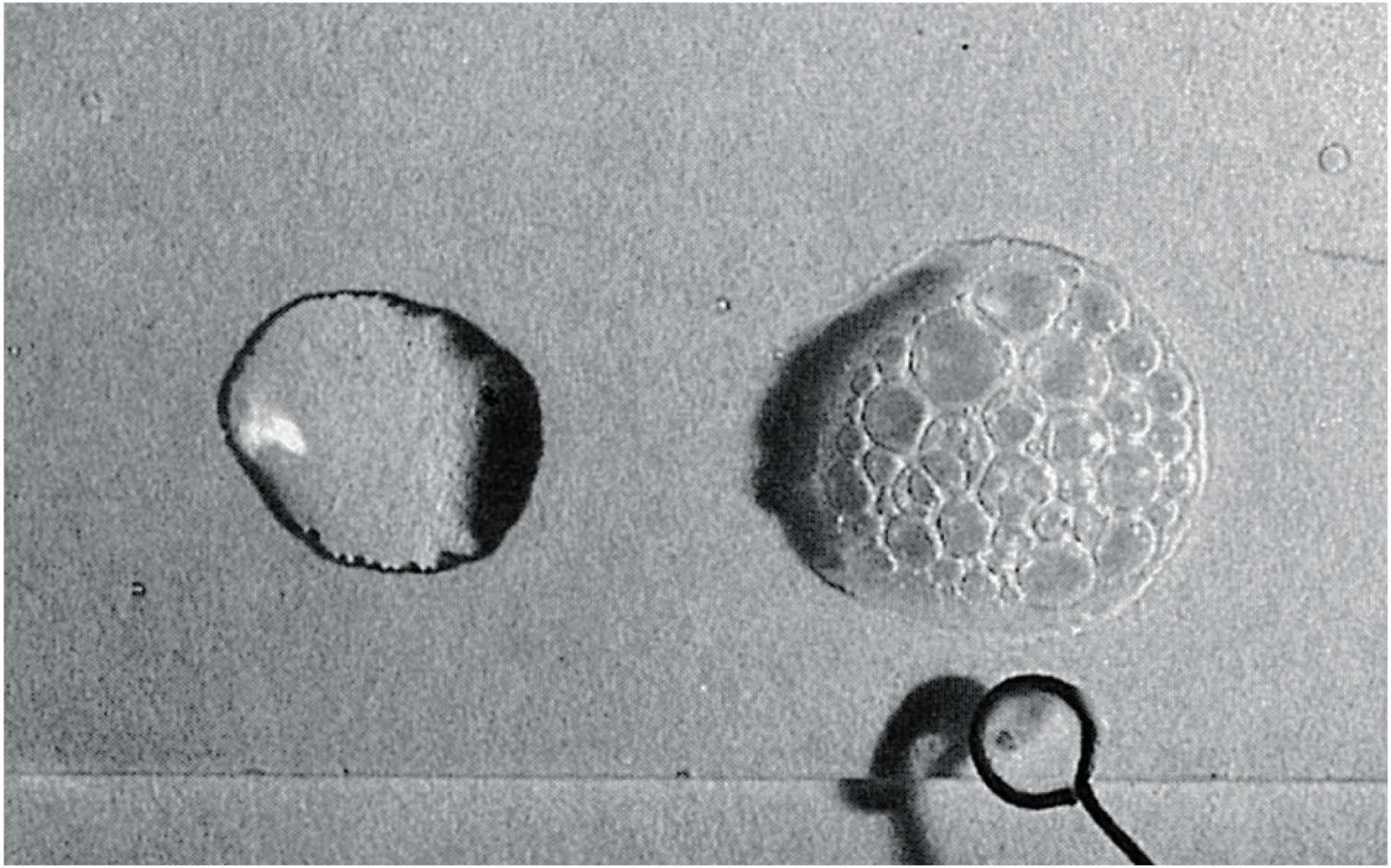
(d) **Superoxide dismutase/catalase in combination:**

$$4\,O_2^- + 4\,H^+ \rightarrow 2\,H_2O + 3\,O_2$$

(e) **Superoxide reductase:**

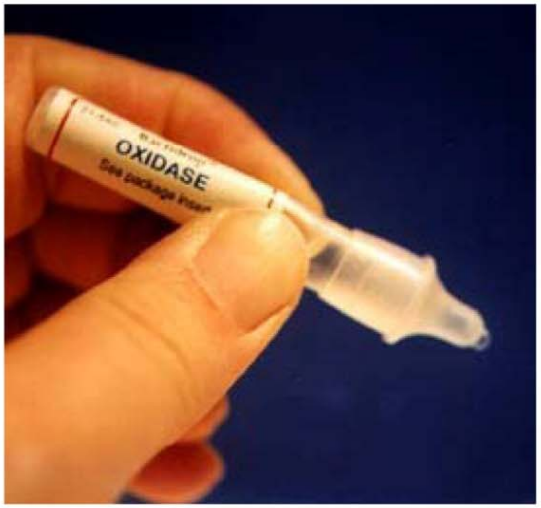
$$O_2^- + 2\,H^+ + \text{cyt } c_{reduced} \rightarrow H_2O_2 + \text{cyt } c_{oxidized}$$

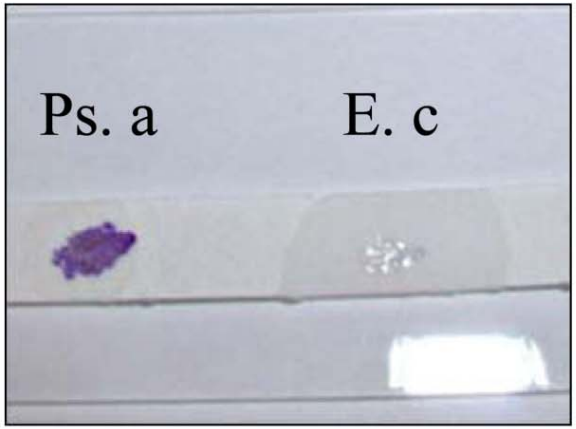
Catalase Test

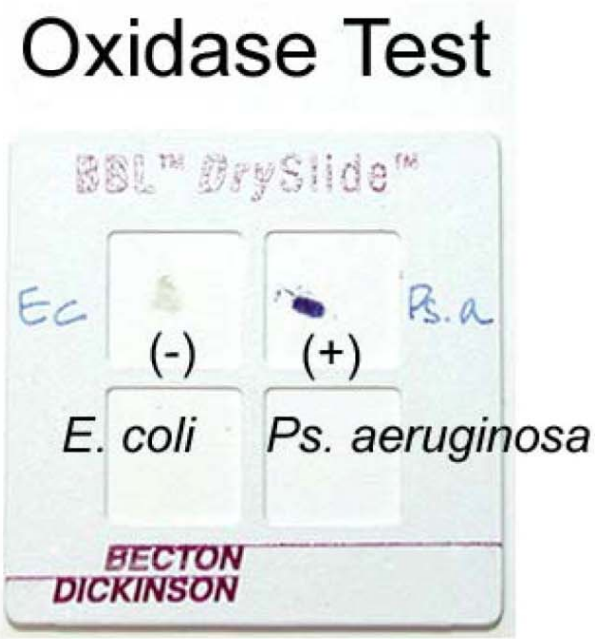


Cytochrome Oxidase Test

An important diagnostic indicator for the ID of *Pseudomonas* and *Neisseria* spp.





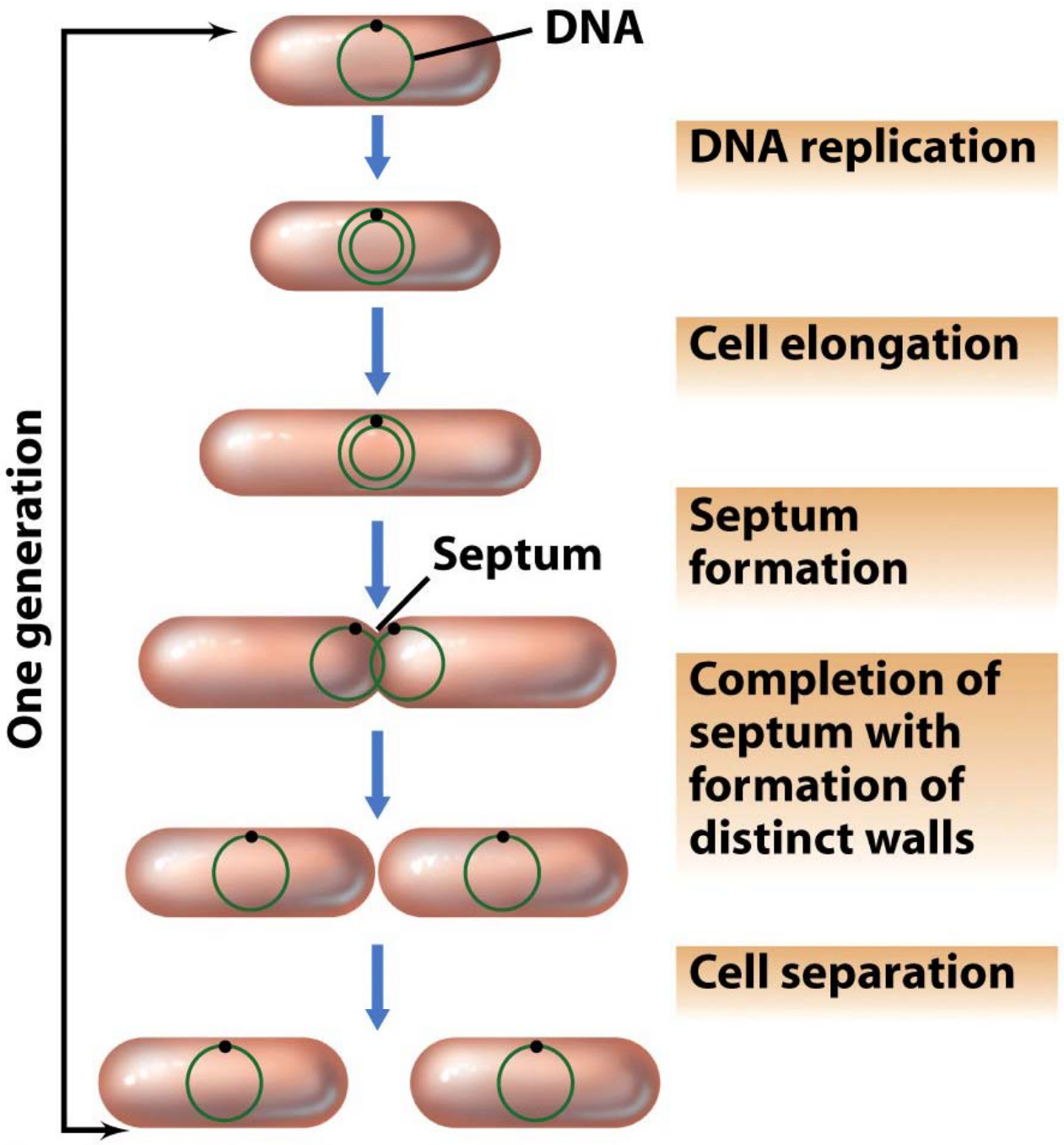


…and now for something completely different

…Microbial Growth

The Process of Growth

- Metabolism required for growth, both anabolic and catabolic. ~2000 reactions!
- Usual Definition: **Increase in cell numbers**
- Other definitions possible – spores, UMC's, respiration, viable but non-culturable, morphology changes (life cycle)
- Divide via Binary Fission: 3 mechanisms
    - Cell Elongation – cell wall
    - DNA Replication – rate limiting step
    - Cell Division – septum formation


DNA
DNA replication
Cell elongation
Septum
Septum formation
Completion of septum with formation of distinct walls
Cell separation
One generation

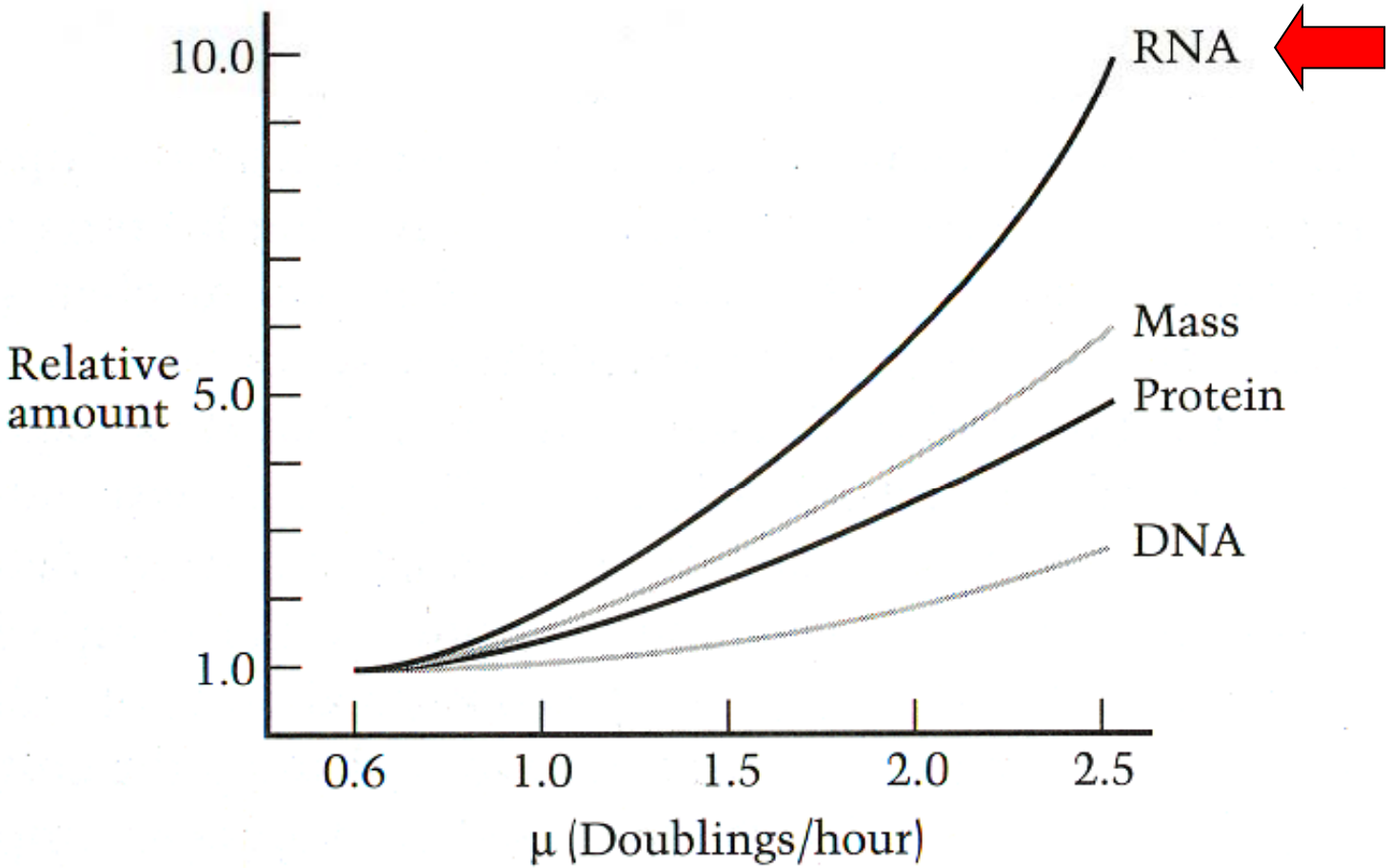


Figure 1

Effect of nutrition-imposed growth rate on the composition of *Escherichia coli* B/r. All values are expressed in amounts per cell normalized to values at $\mu = 0.6$ (mass $= 1.48 \times 10^{-13}$ g; protein $= 1.00 \times 10^{-13}$ g; RNA $= 2.0 \times 10^{-14}$ g; DNA $= 6.3 \times 10^{-15}$ g). (Plotted from data in Bremer and Dennis, 1987.)

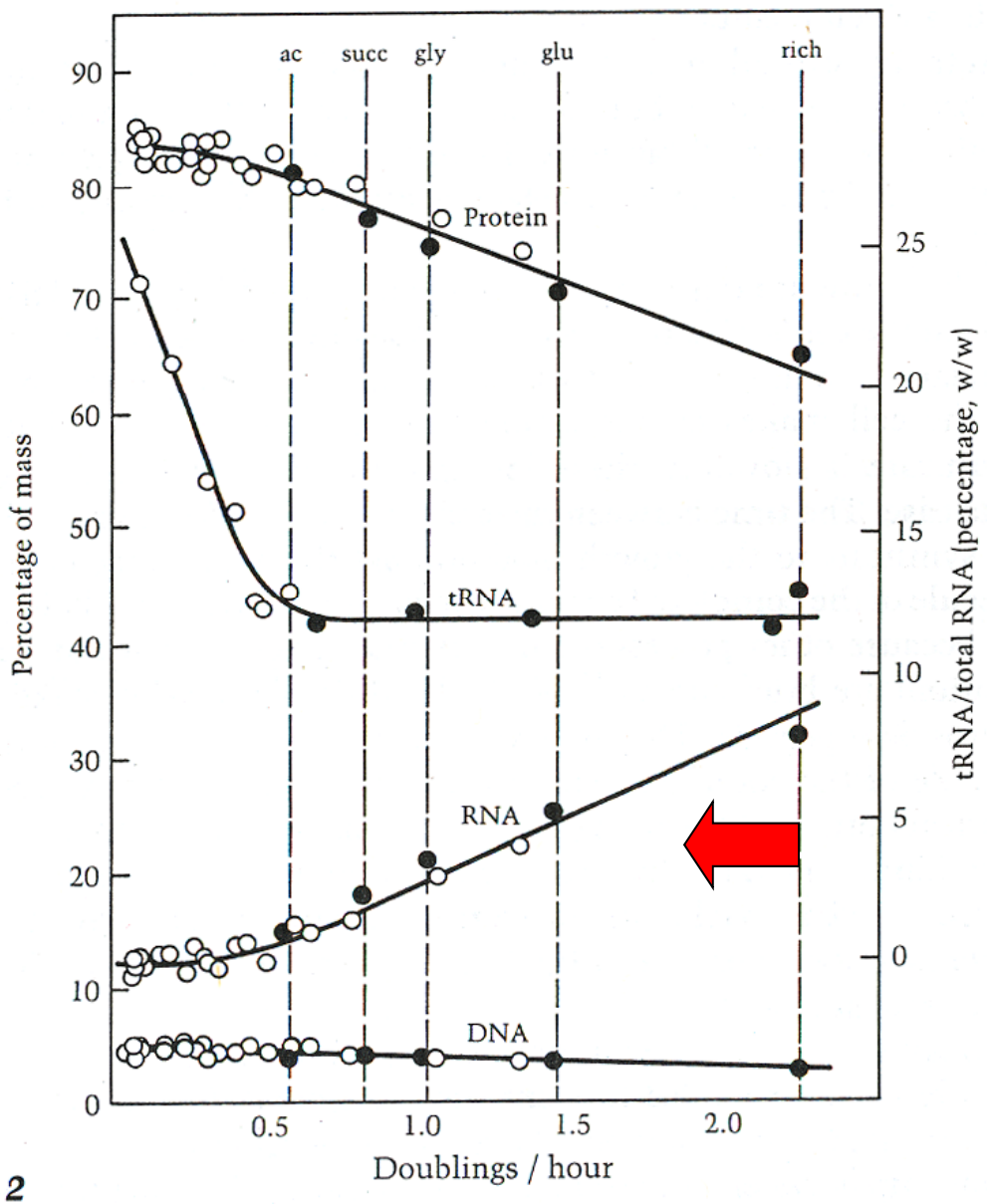
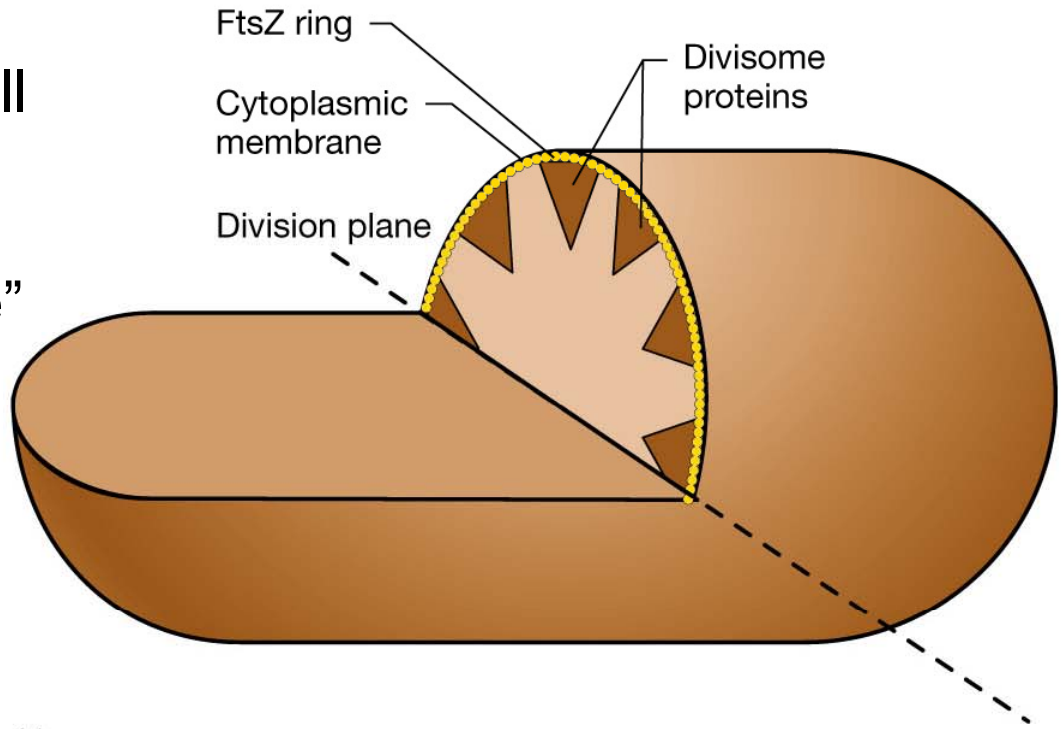


Figure 2

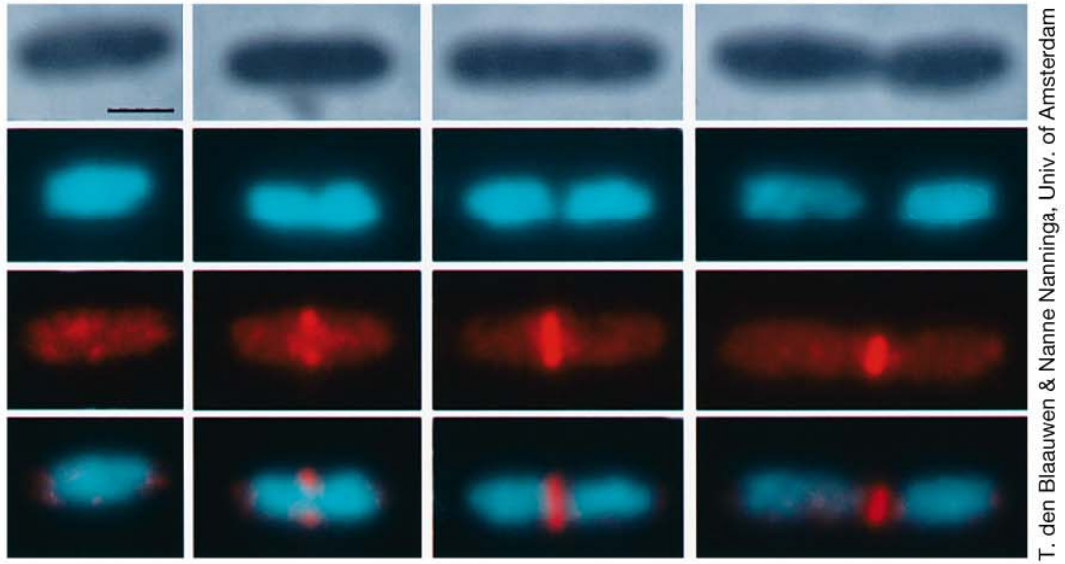
Effect of growth rate on the cellular proportions of protein, RNA, and DNA. Filled circles refer to results from cultures undergoing balanced growth in batch culture in various media; open circles are from cultures growing in a glucose-limited chemostat. (From Jacobsen, 1974.)

FtsZ ring & cell division

The “divisome”



(a)



(b)

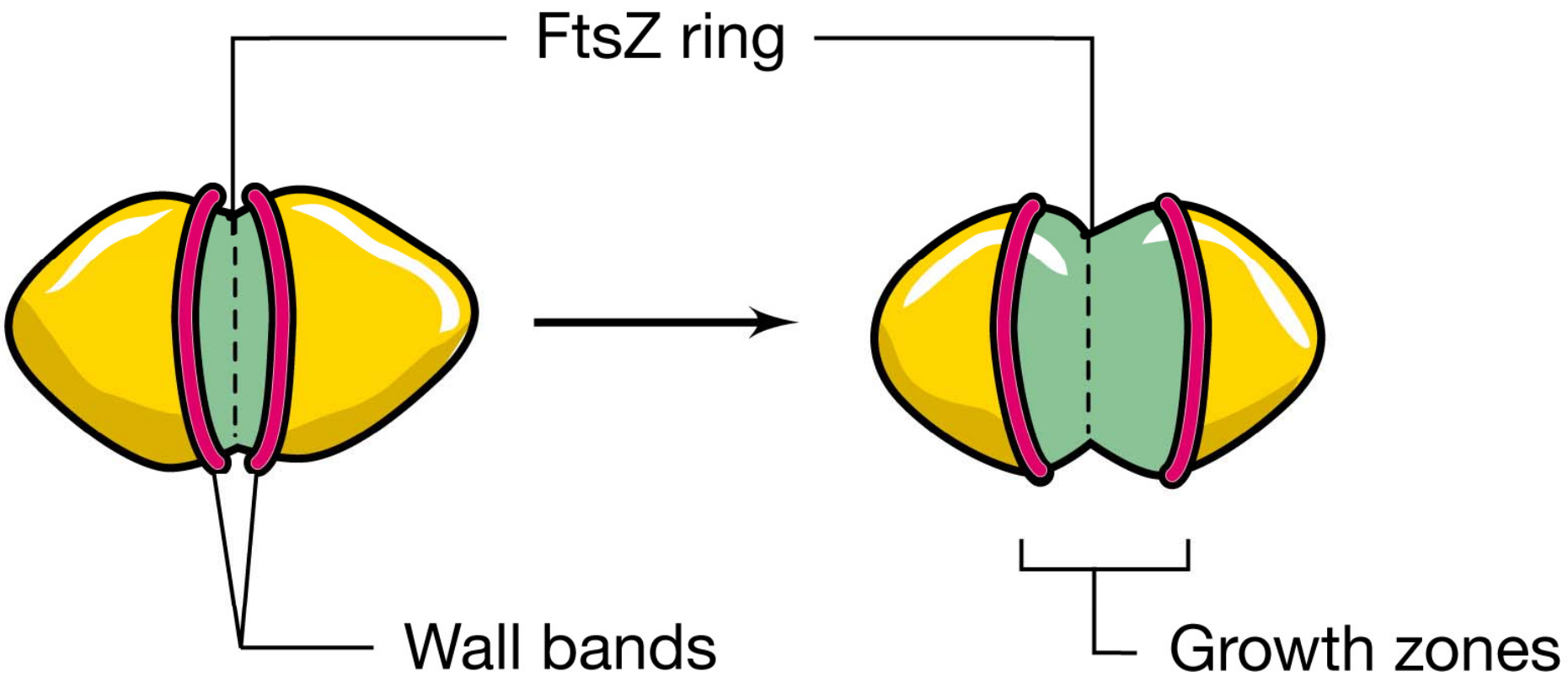
phase

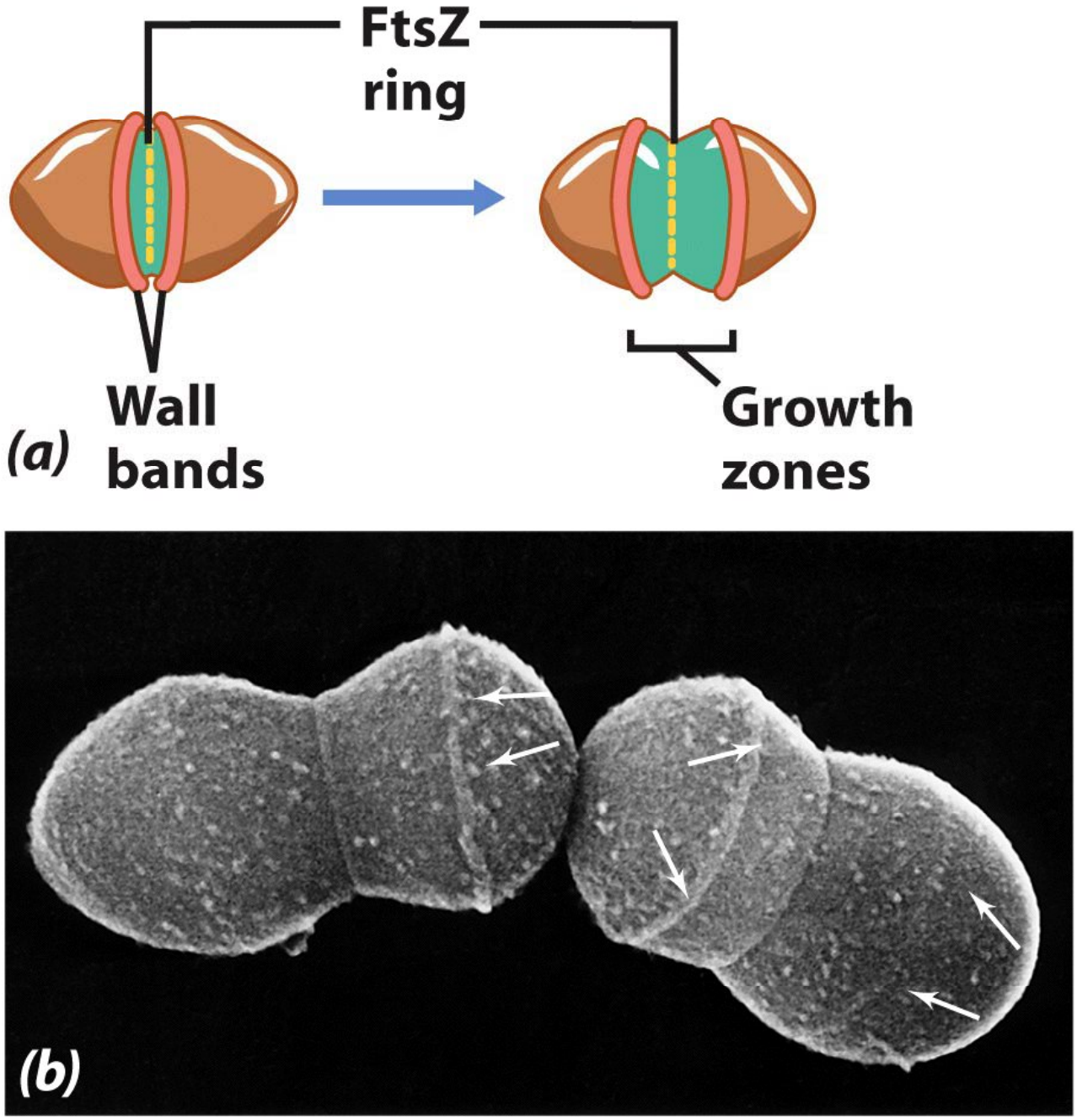
DNA stain

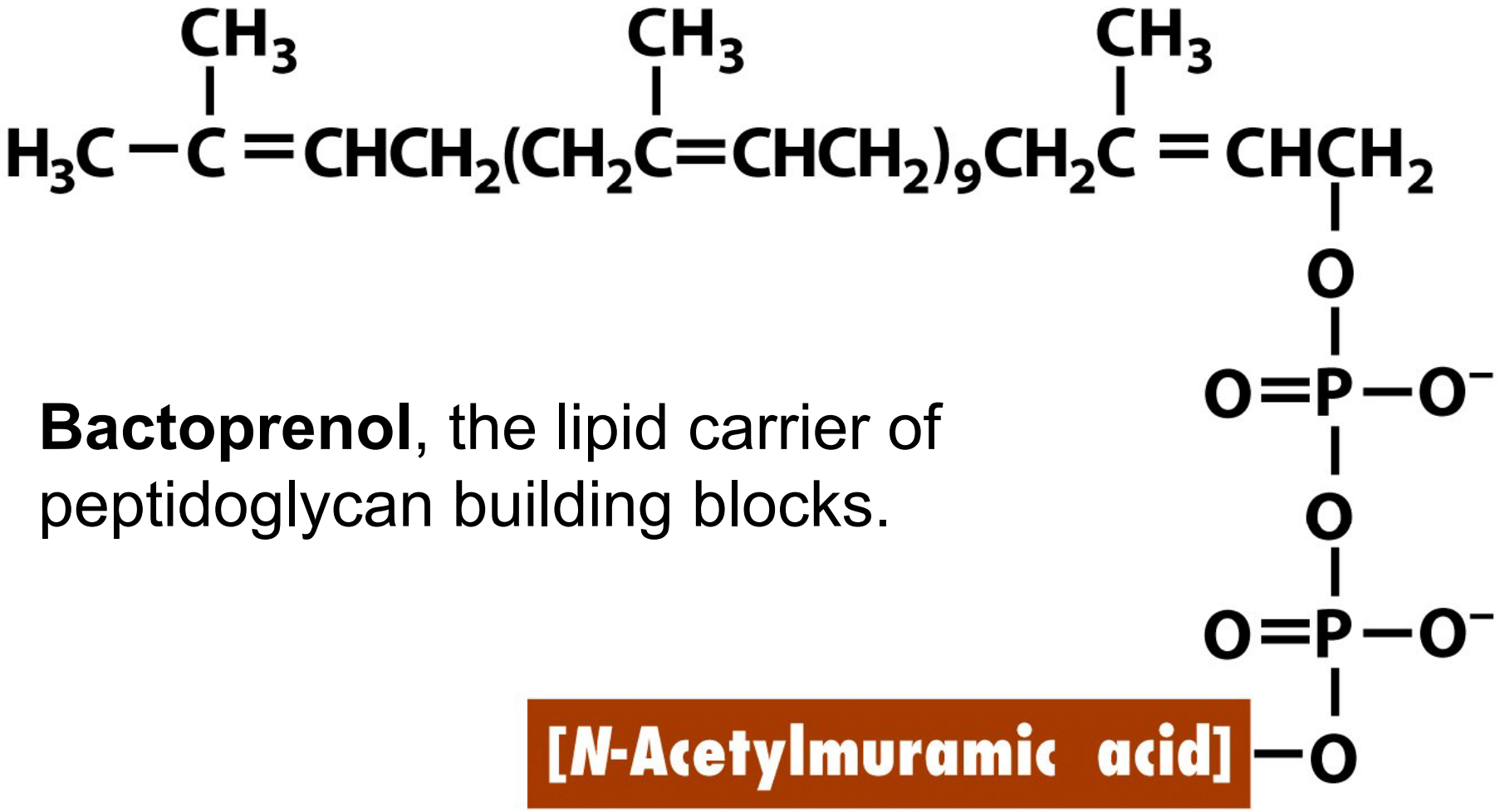
FtsZ stain

DNA & FtsZ

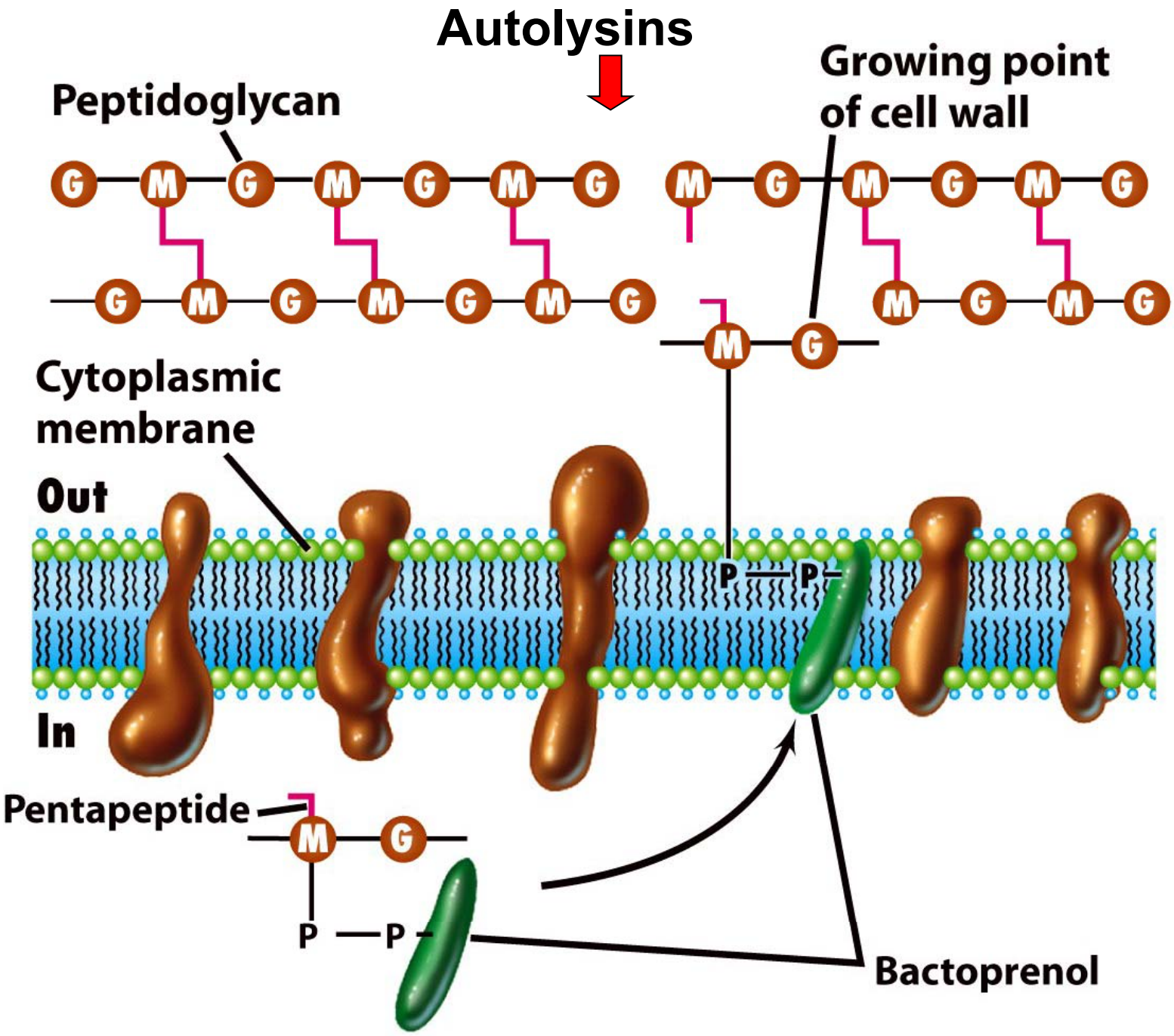
FtsZ similar to Tubulin
MreB similar to Actin



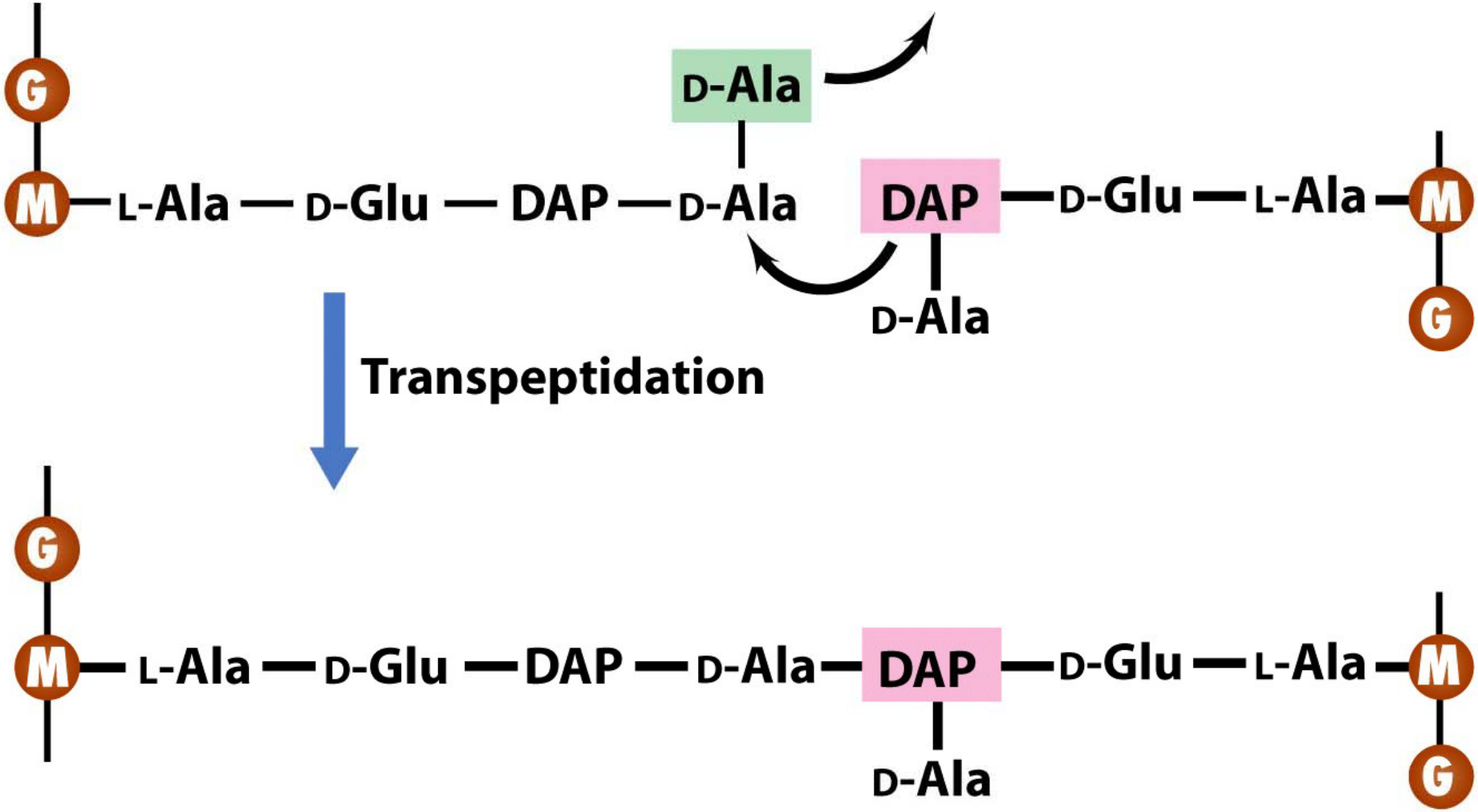

FtsZ
ring
Wall
bands
(a)
Growth
zones
(b)



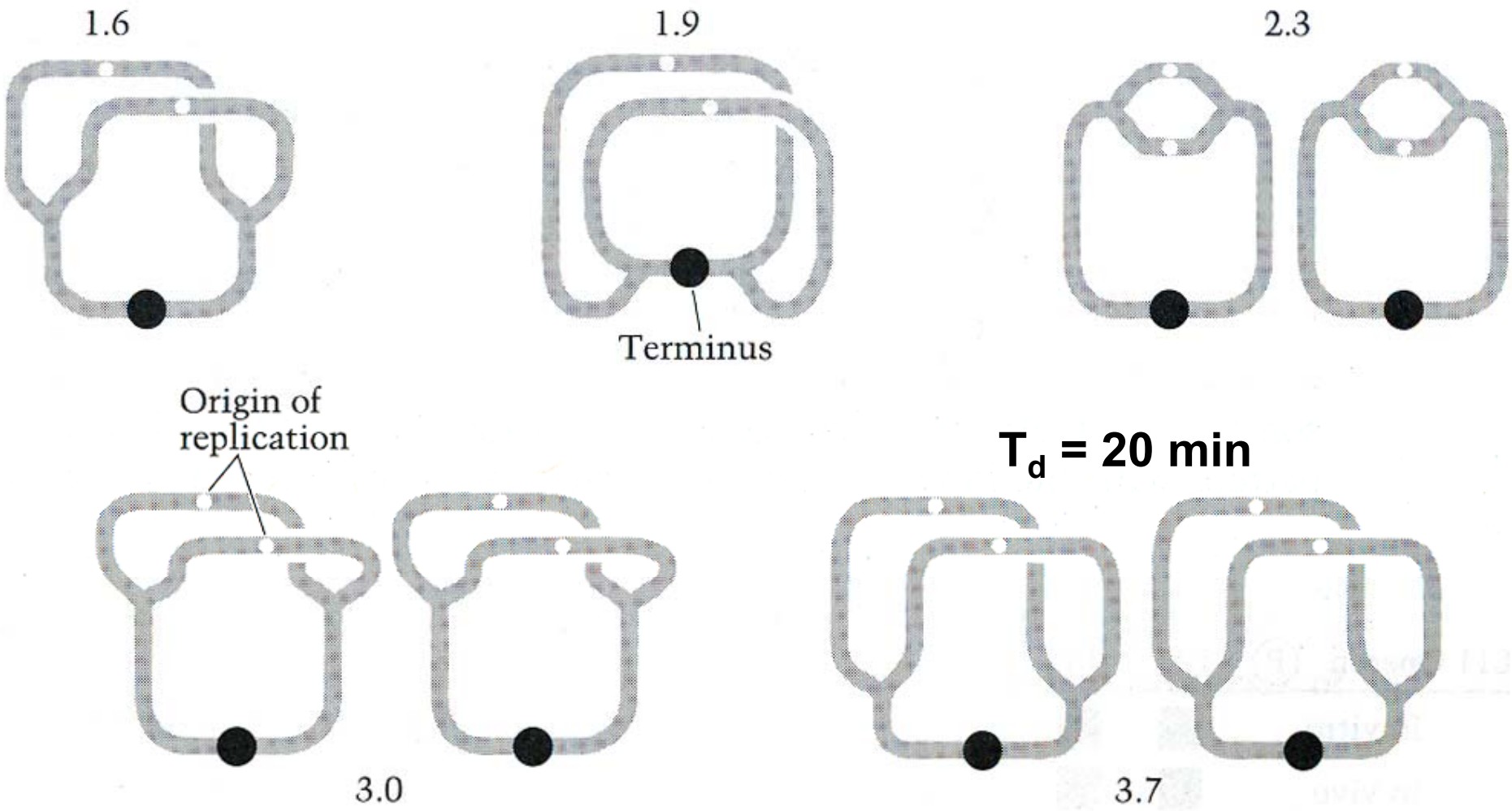
Bactoprenol, the lipid carrier of peptidoglycan building blocks.


Autolysins
Peptidoglycan
Growing point of cell wall
G
M
Cytoplasmic membrane
Out
In
P
Pentapeptide
Bactoprenol

Penicillin blocks this reaction



T_d = 100 min



T_d = 20 min

Figure 10

Chromosome structure and equivalent DNA content of the average cell in culture of *E. coli* B/r growing at various rates. The numbers represent genome equivalents. (From Bremer and Dennis, 1987.)

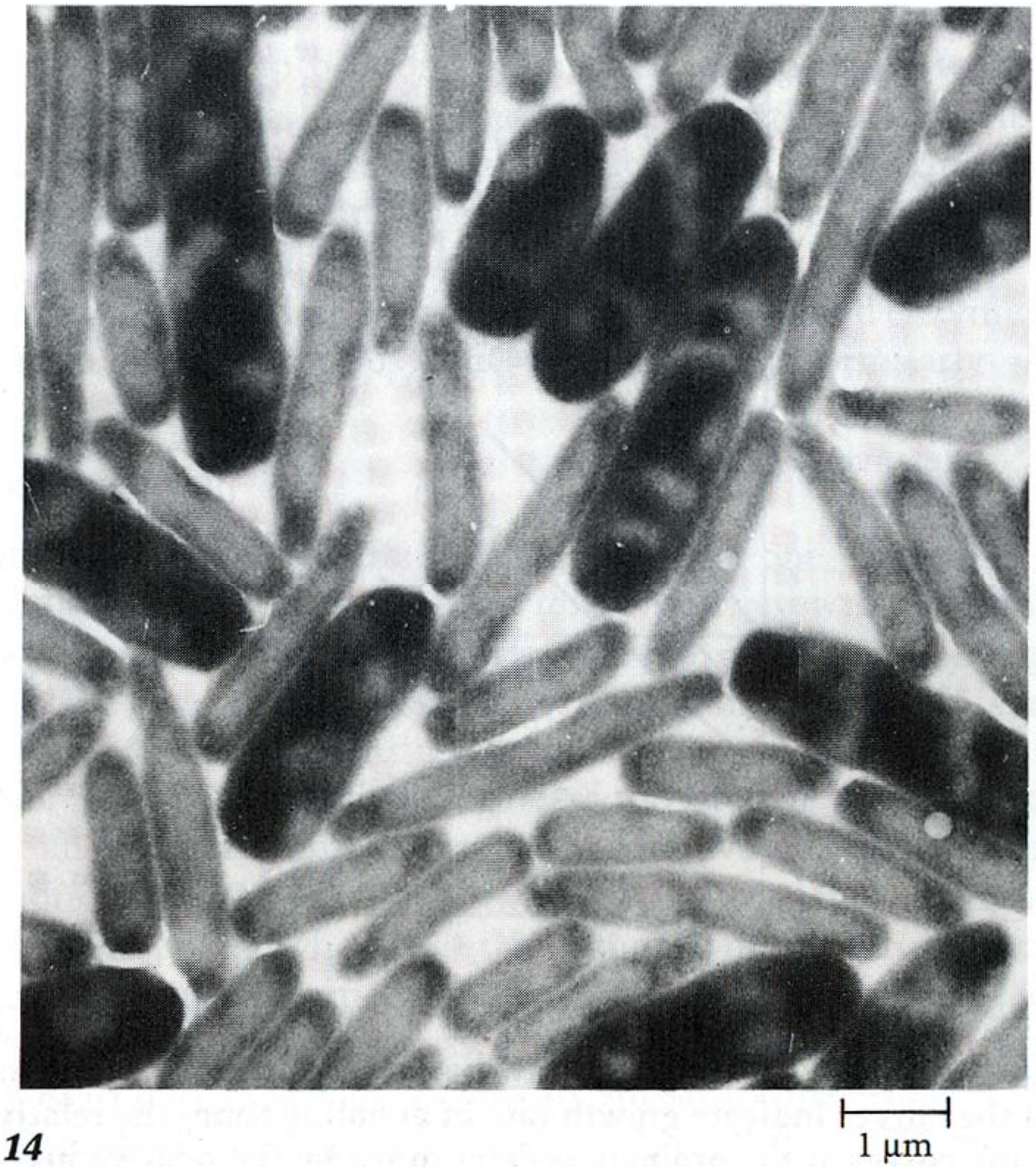


Figure 14

Electron micrograph of a mixture of cells of *E. coli* B/r grown at different rates. The large cells grew with a doubling time of 22 minutes, the small ones with a doubling time of 72 minutes. (From Nanninga and Woldringh, 1985.)

The Process of Growth

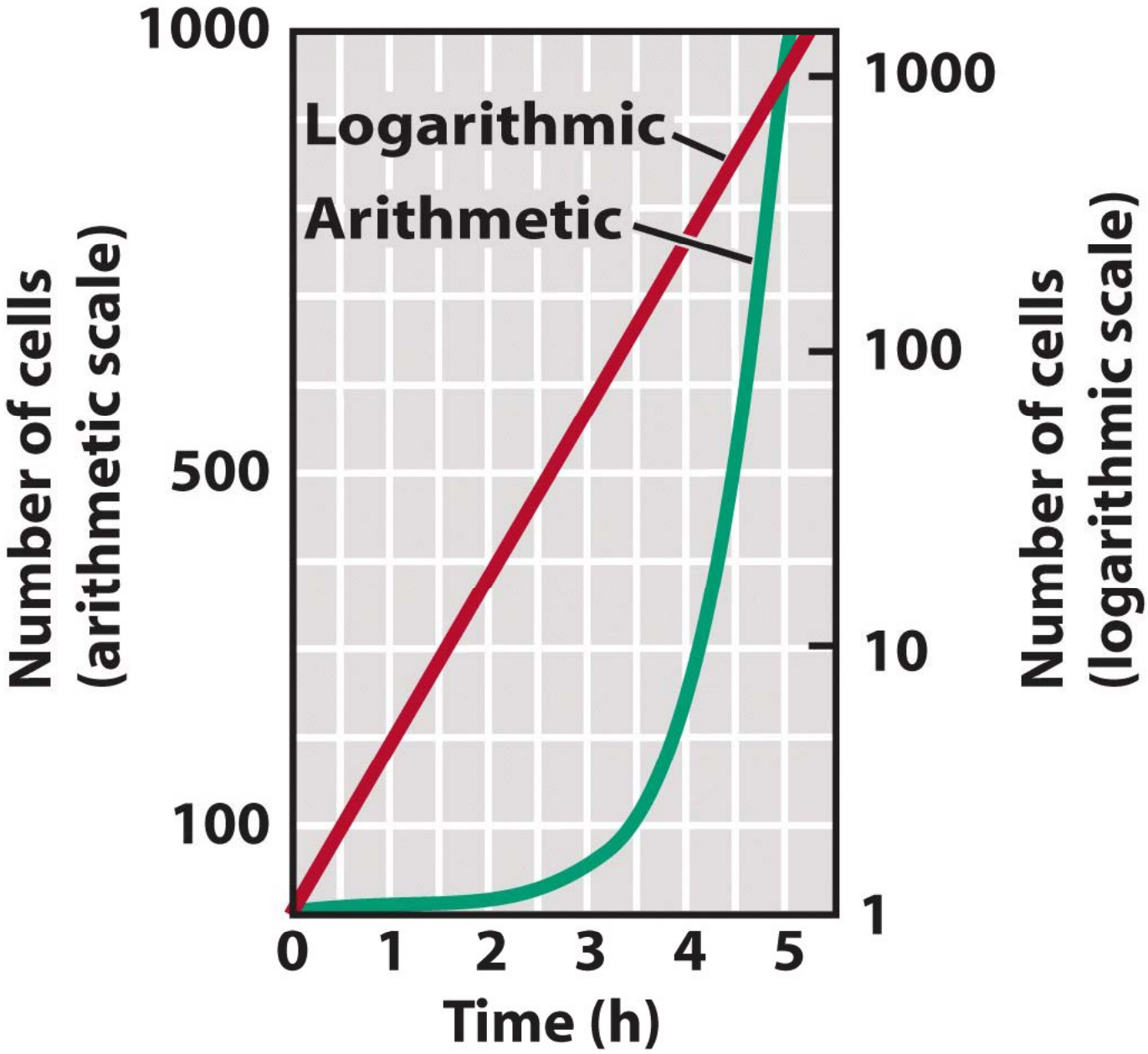
- Growth Rate: Time it takes to reproduce
  $\mathbf{t_{1/2} = \ln 2/\mu = 0.693/\mu}$

- Phases of Growth in Batch culture
  - Lag, Log, Stationary, Death

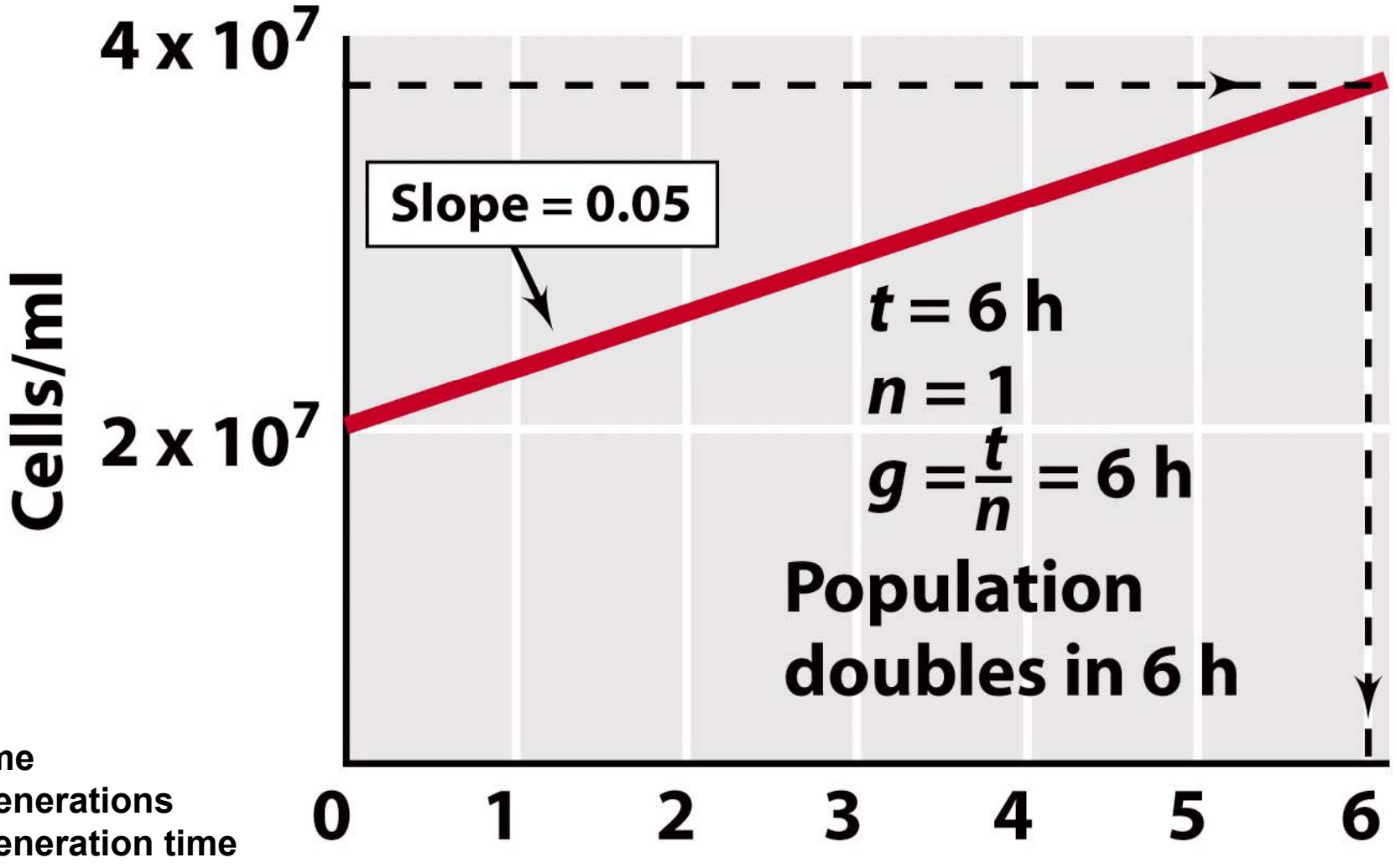
- Measurement of Growth
  - Total cell counts
  - Viable cell counts
  - Turbidity

The growth rate of a microbial culture

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})


1000
500
100
Number of cells
(arithmetic scale)
Logarithmic
Arithmetic
1000
100
10
1
Number of cells
(logarithmic scale)
0
1
2
3
4
5
Time (h)

Estimating the generation time of a microbial culture with semi-log plots in exponential phase



t = time
n = generations
g = generation time

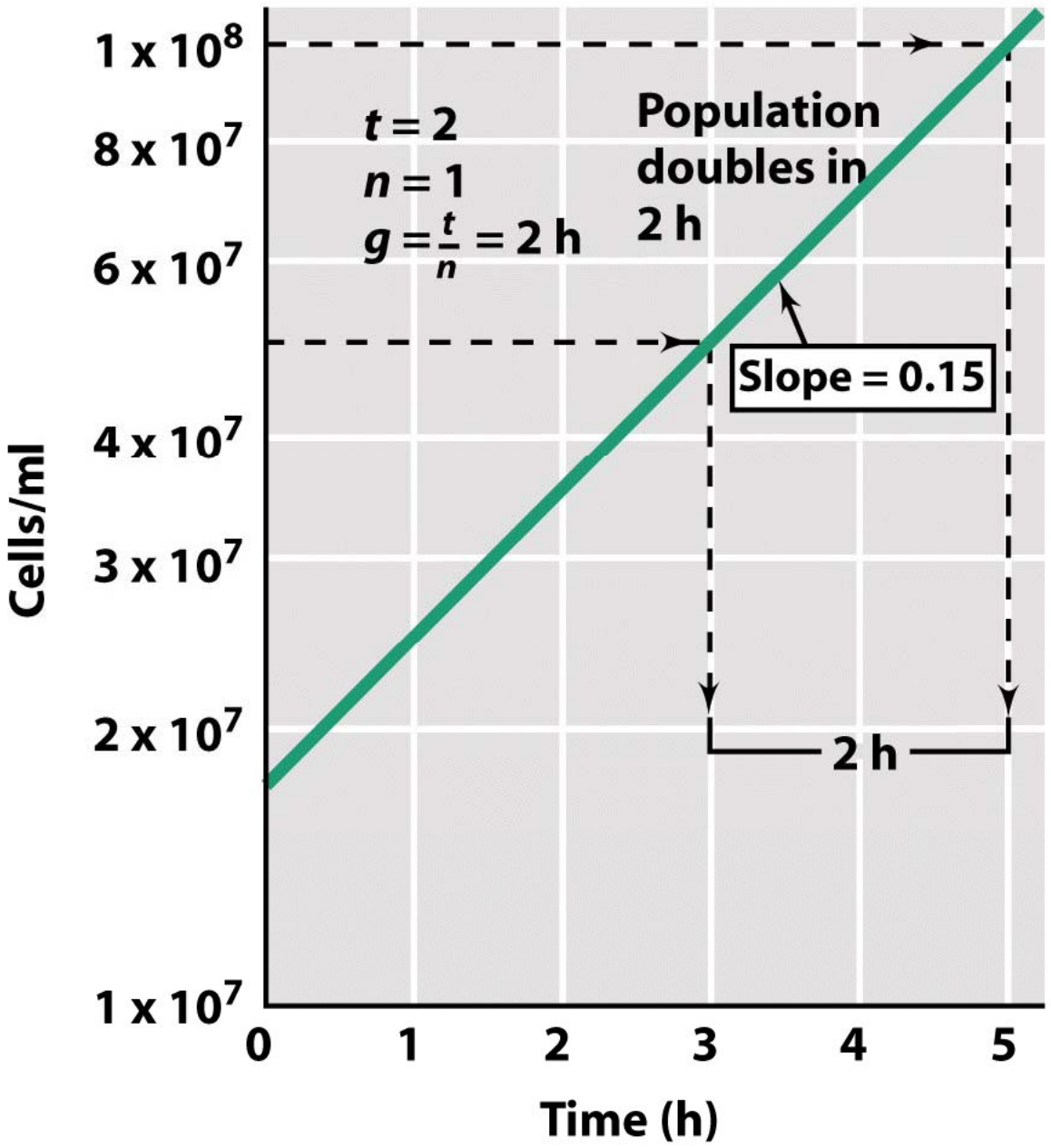

1 x 10^8
8 x 10^7
6 x 10^7
4 x 10^7
3 x 10^7
2 x 10^7
1 x 10^7
Cells/ml
t = 2
n = 1
g = t/n = 2 h
Population doubles in 2 h
Slope = 0.15
2 h
0
1
2
3
4
5
Time (h)

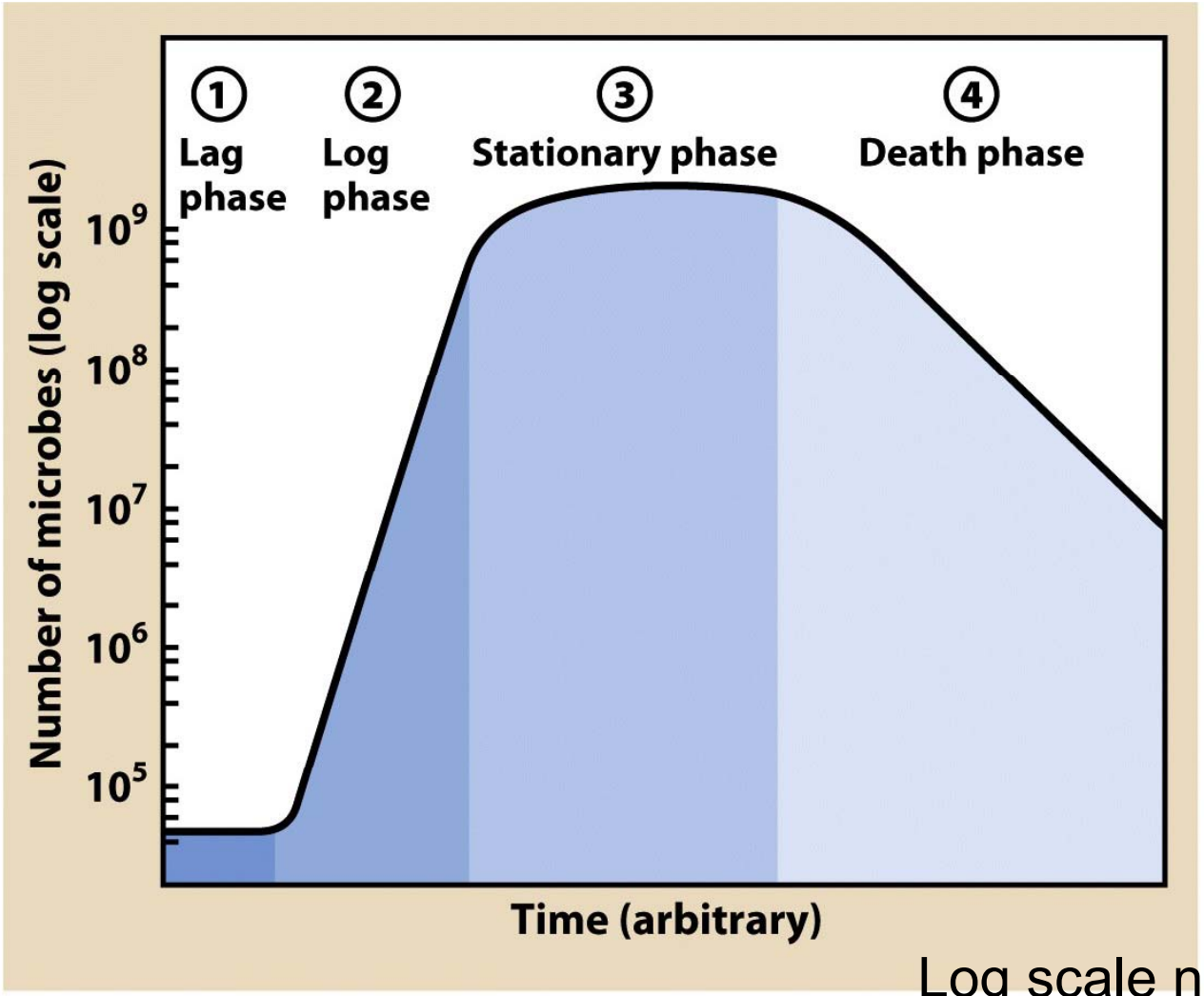
Table 6.1 **Approximate generation times for several organisms growing in media optimal for growth**

Species	Generation Time
Escherichia coli	20 min
Bacillus subtilis	28 min
Staphylococcus aureus	30 min
Pseudomonas aeruginosa	35 min
Thermus aquaticus	50 min
Thermoproteus tenax	1 hr 40 min
Rhodobacter sphaeroides	2 hr 20 min
Sulfolobus acidocaldarius	4 hr
Thermoleophilum album	6 hr
Thermofilum pendens	10 hr
Mycobacterium tuberculosis	13 hr 20 min

The Growth Cycle

- Lag phase
  - Cells synthesizing materials, not dividing
- Log phase = exponential growth
  - $1 \rightarrow 2 \rightarrow 4 \rightarrow 8 \rightarrow 16 \ldots$
    - 10 doublings increases density by ~1000
    - $\log_{10}(N)$ increases linearly
- Stationary phase
  - Cells no longer growing
- Death phase

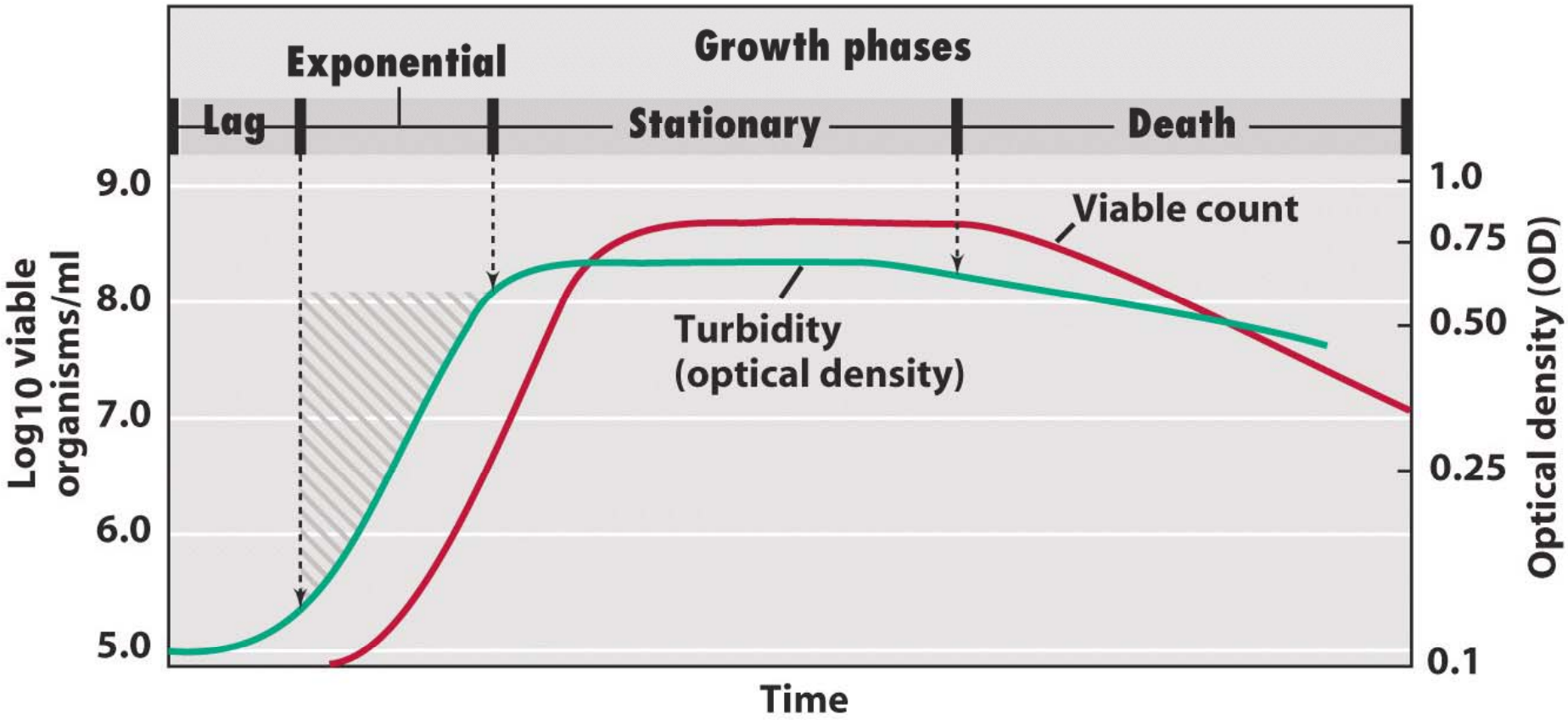
The Growth Cycle



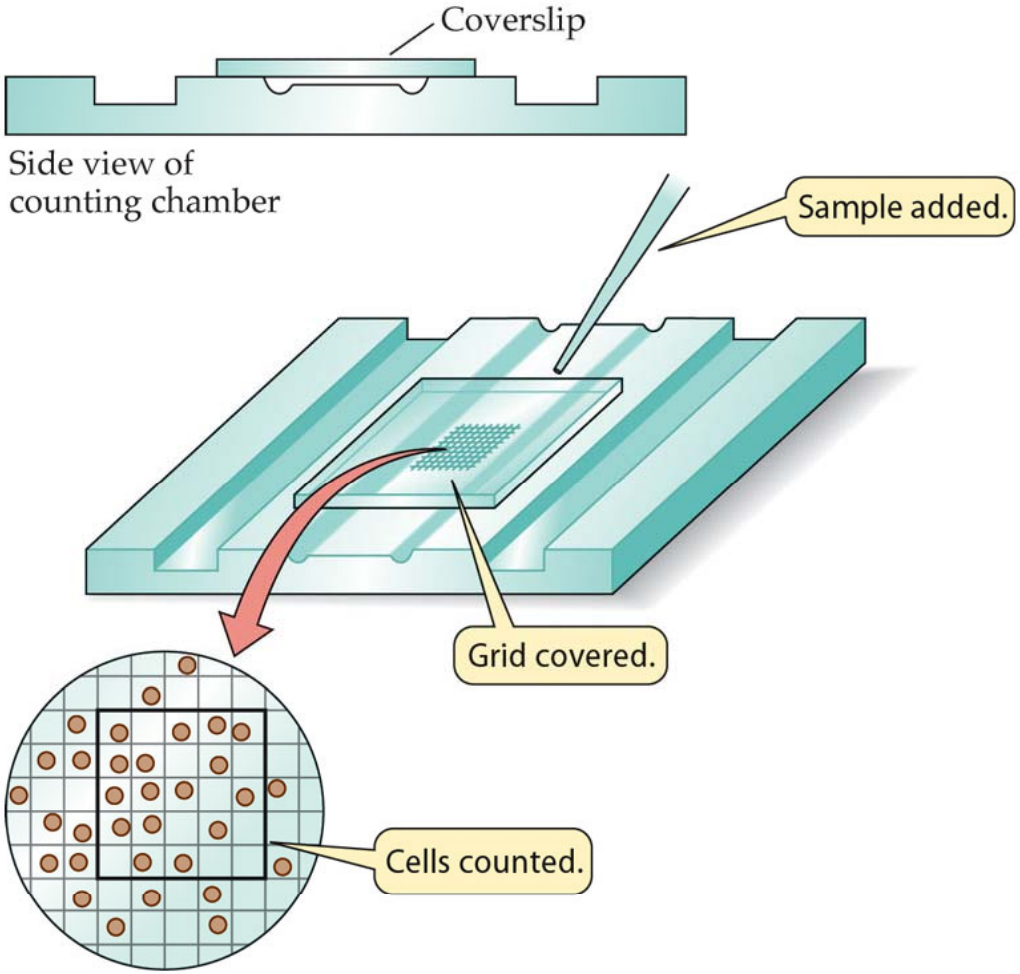
Log scale necessary to show wide range of concentrations

Typical growth curve for an average bacterial population

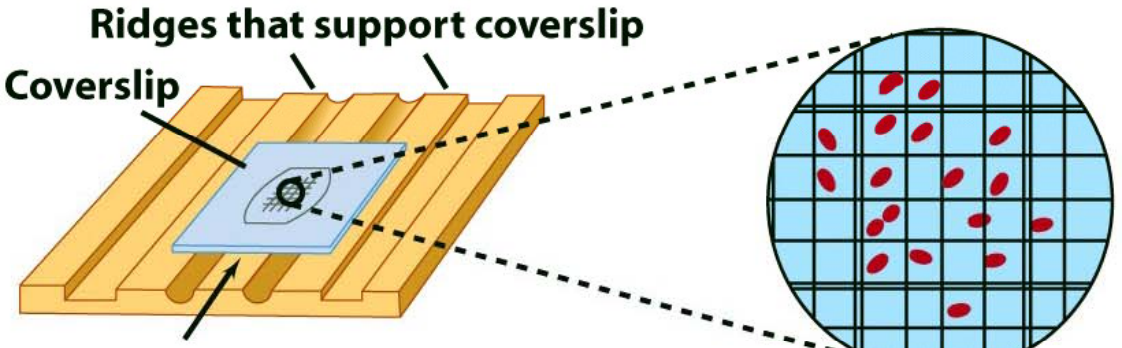
Cryptic Growth



Total Cell counts using the Petroff-Hausser Counter



Total Cell counts using the Petroff-Hausser Counter



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 mm^2 and a total volume of 0.02 mm^3.

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

To calculate number per milliliter of sample:
12 cells x 25 large squares x 50 x 10^3 = 1.5 x 10^7

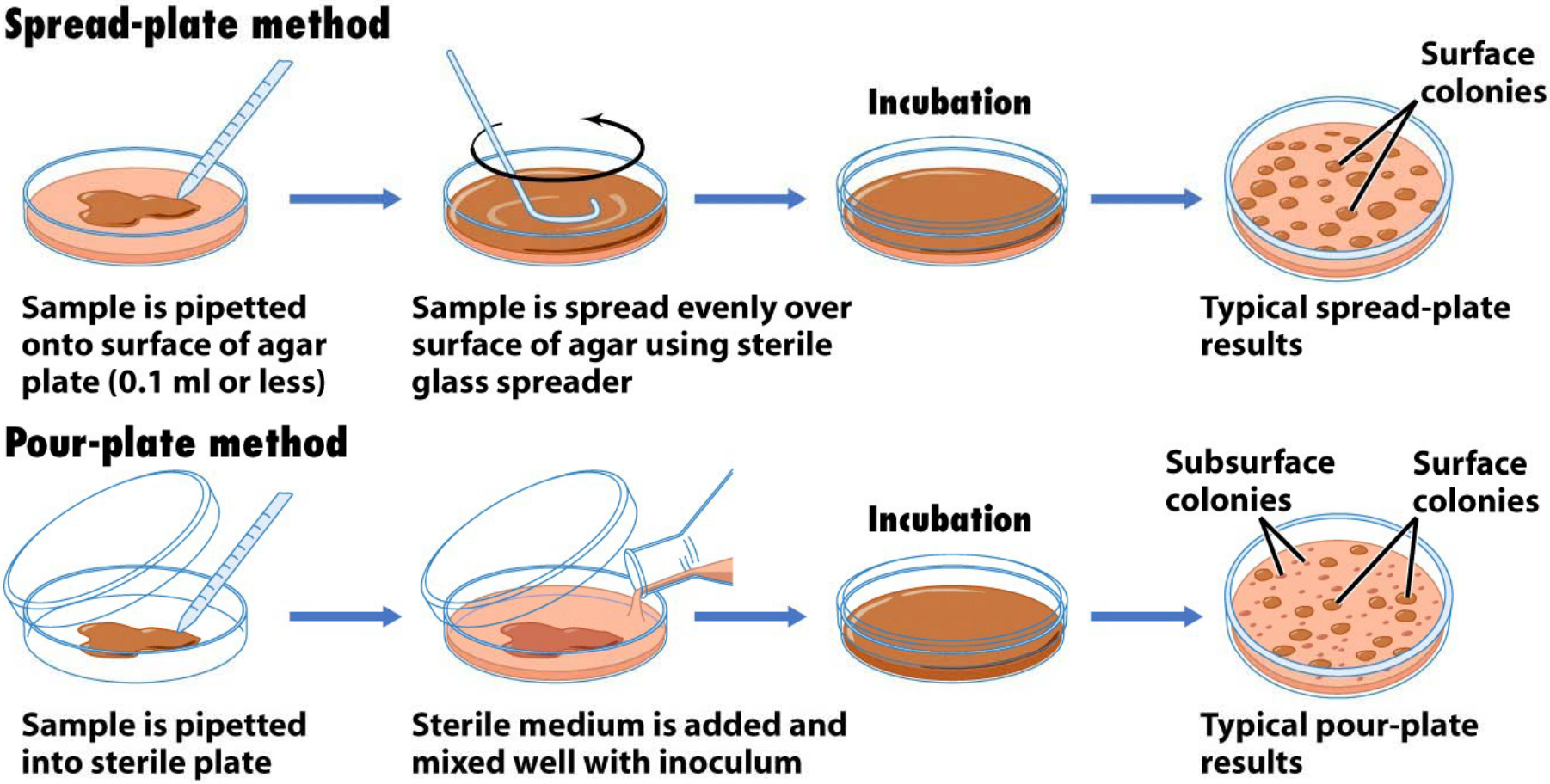
Number /mm^2

Number /mm^3

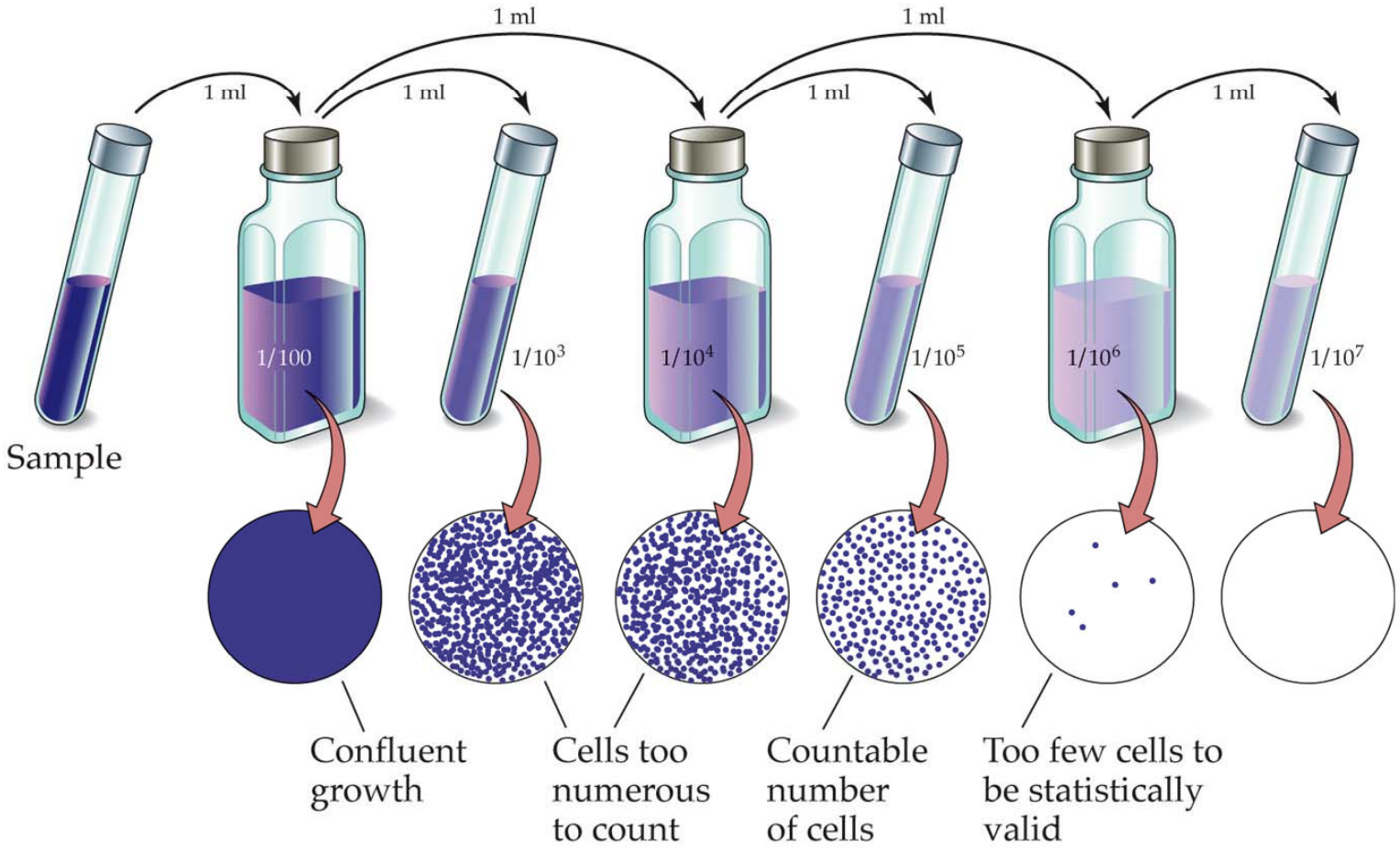
Number /cm^3 (ml)

Viable cell count methods

30-300 on standard Petri Dish

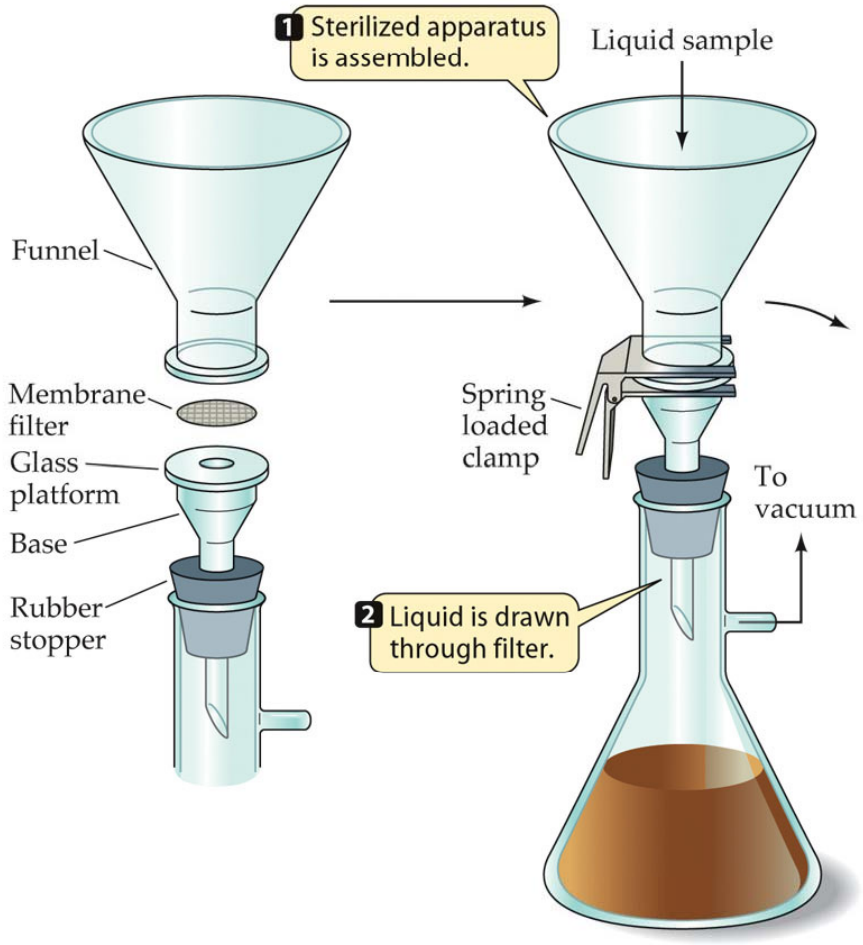


Counting the number of viable cells by serial dilution and plate count

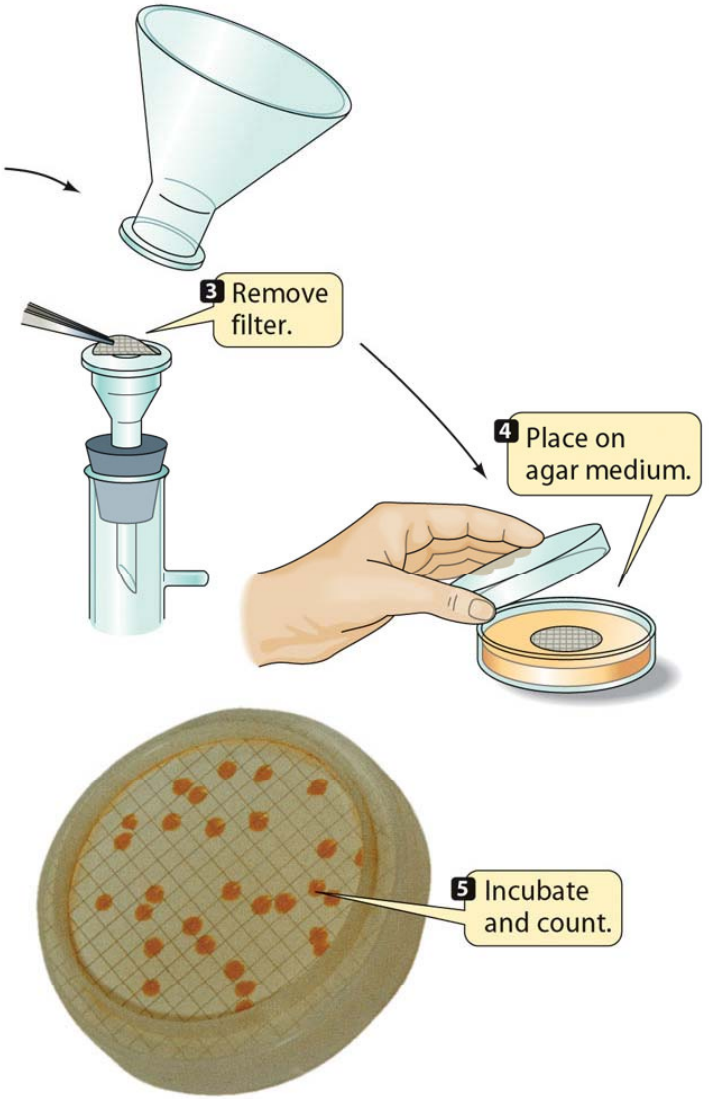


Also known as dilution to extinction.
Used to obtain a pure culture without using solid medium

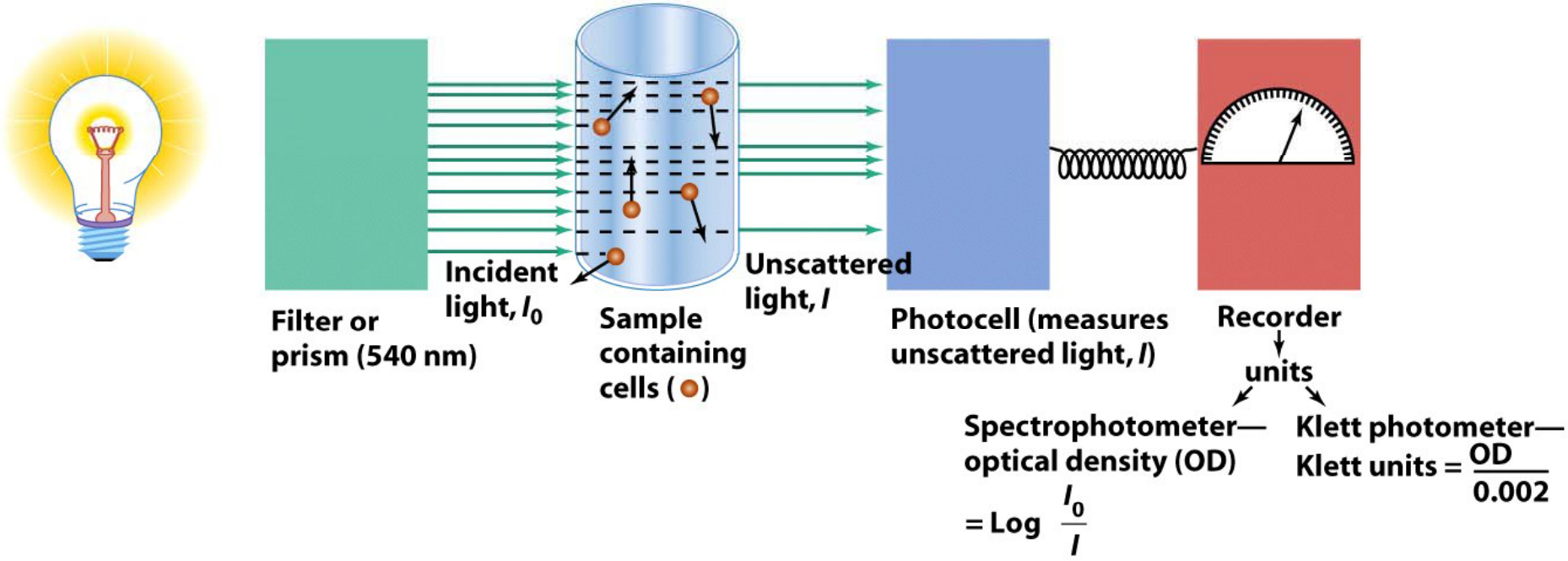
(Part 1) Concentration of cells by membrane filtration



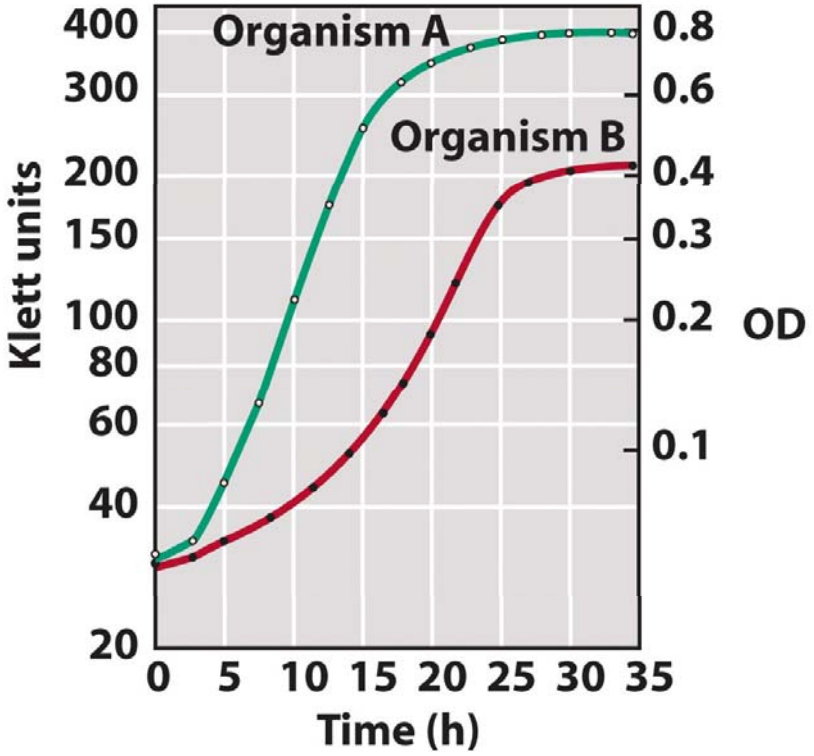
(Part 2) Concentration of cells by membrane filtration

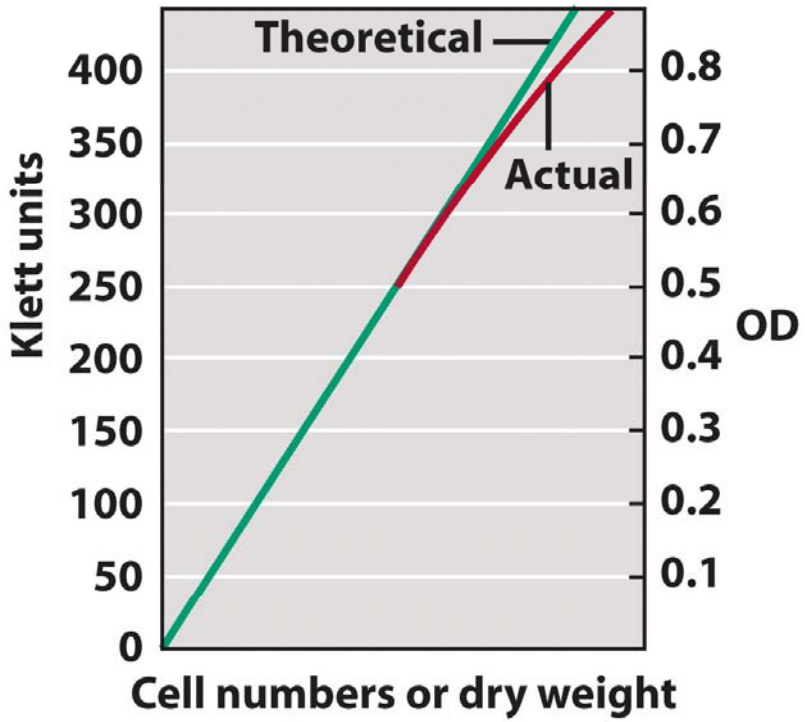


Turbidity measurements of microbial growth



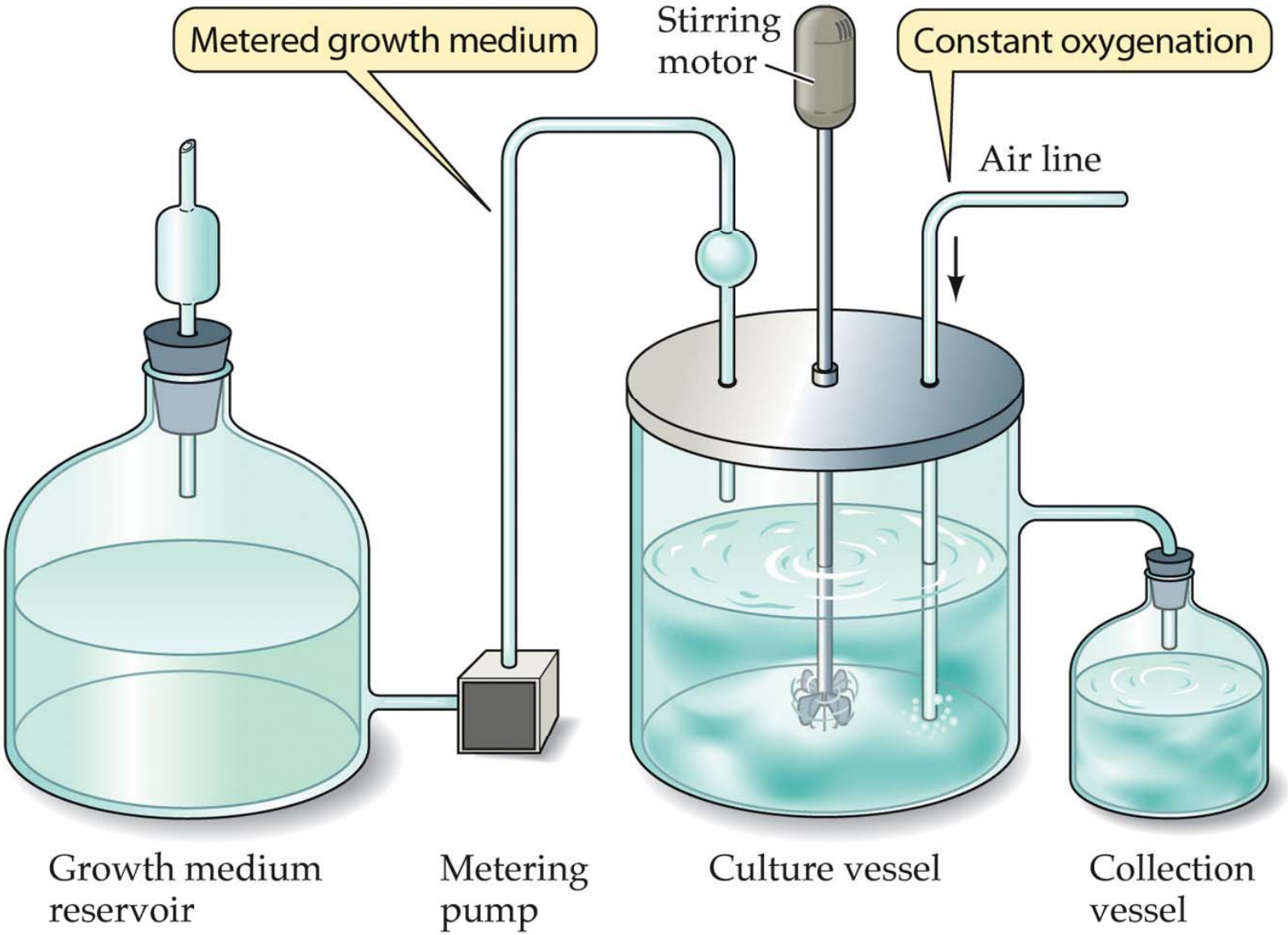
Turbidity measurements of microbial growth





The Process of Growth

- Continuous Culture: **chemostat**
  - Steady State
  - Reproducible Physiology
  - Fine control
  - Key parameters: Ks, μmax, Yield
  - Closed systems vs. Open systems vs. Nature


Metered growth medium
Stirring motor
Constant oxygenation
Air line
Growth medium reservoir
Metering pump
Culture vessel
Collection vessel

$$D = F/V = \mu$$

Where:

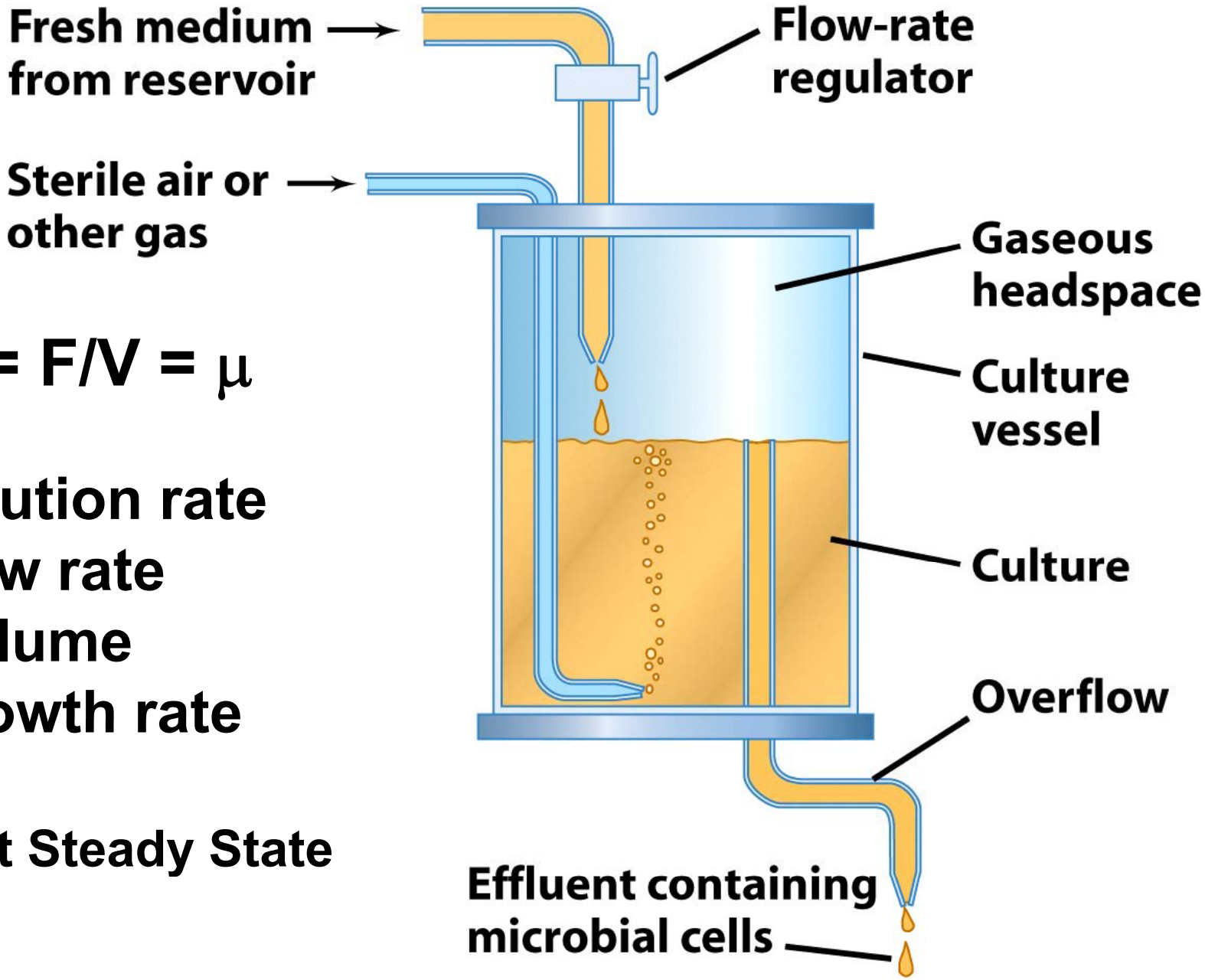
D = dilution rate

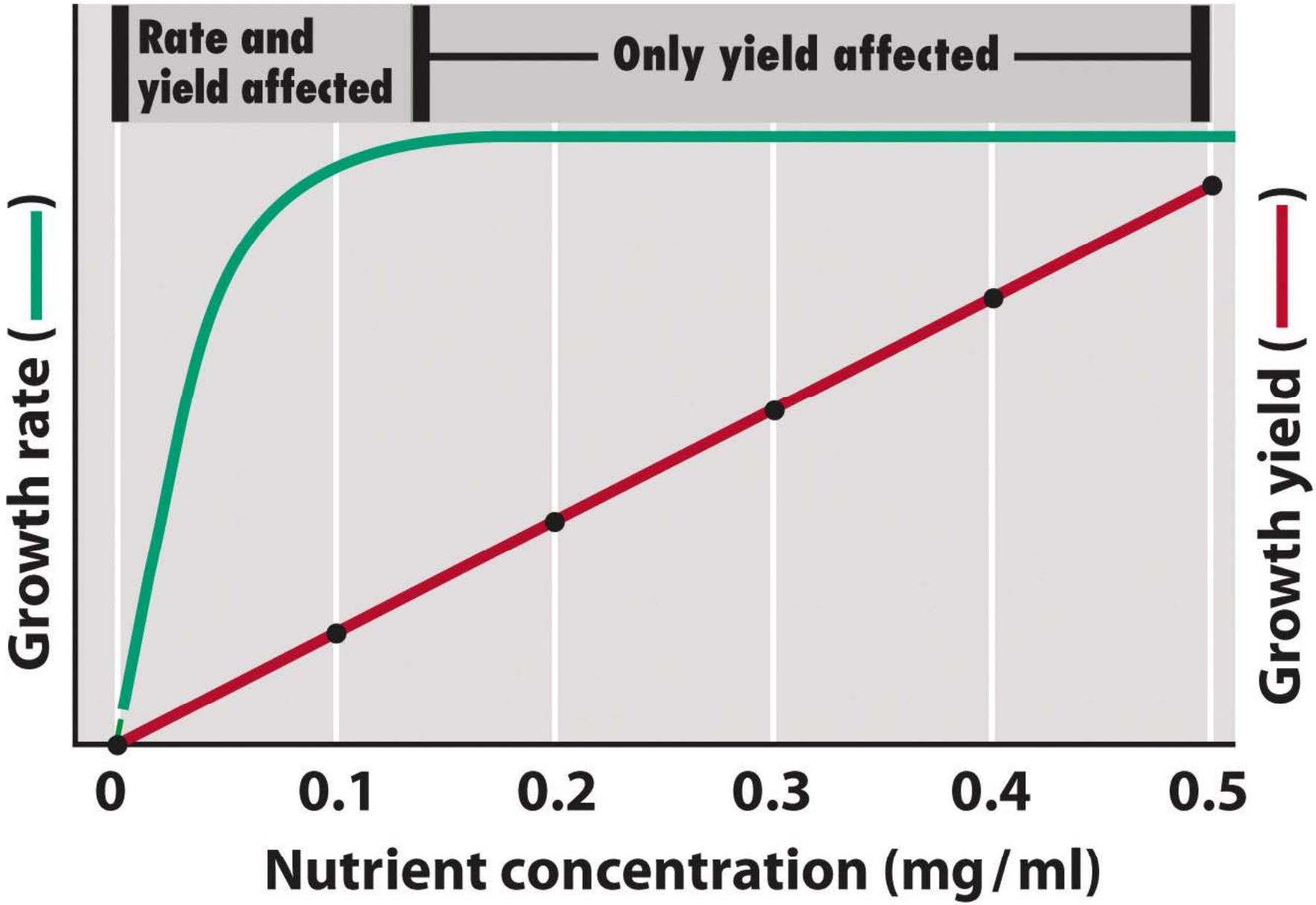
F = flow rate

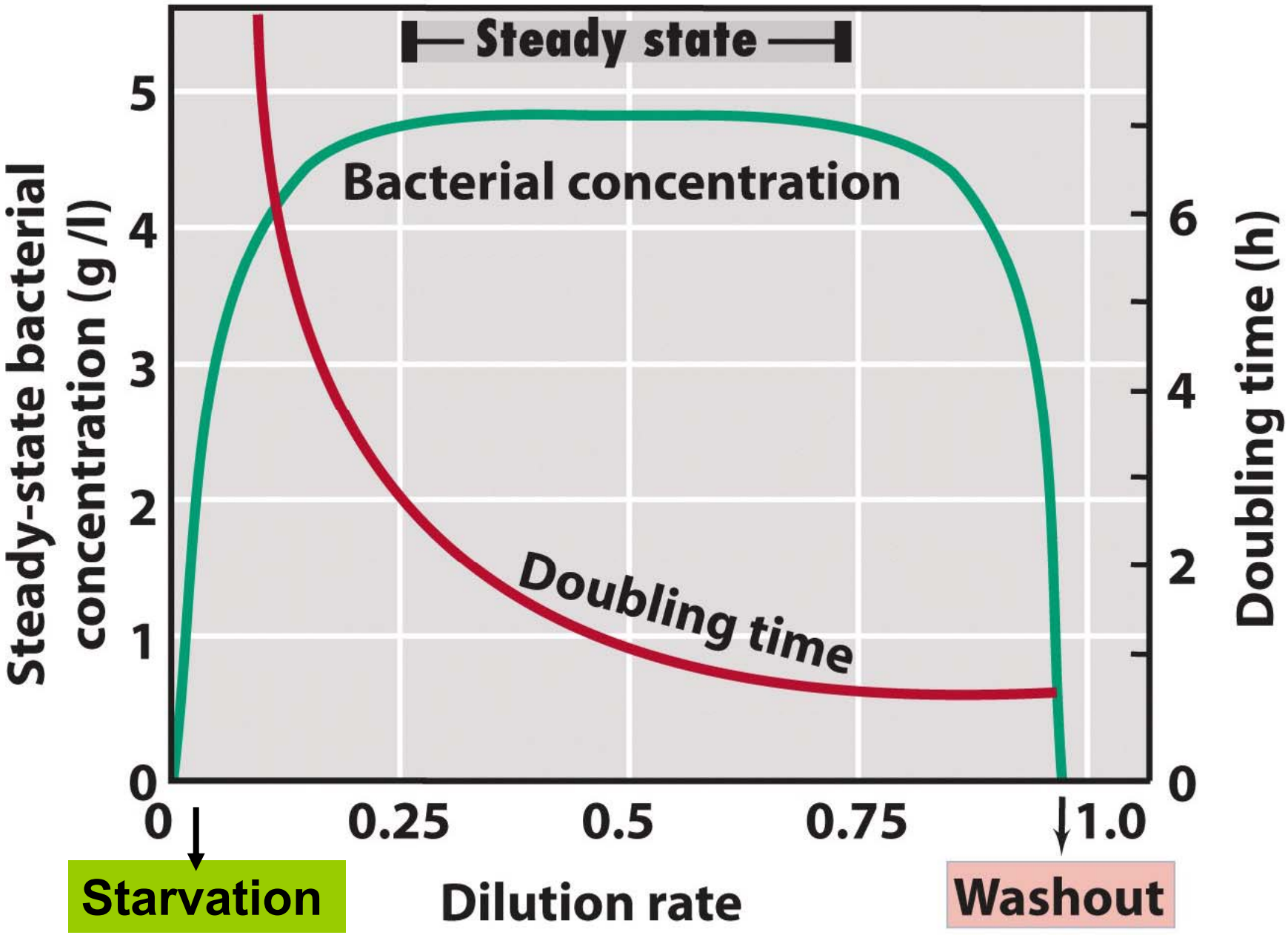
V = volume

μ = growth rate

Rem: At Steady State




Rate and yield affected
Only yield affected
Growth rate (——)
Growth yield (——)
0
0.1
0.2
0.3
0.4
0.5
Nutrient concentration (mg/ml)


Steady state
Bacterial concentration
Doubling time
Steady-state bacterial concentration (g /l)
Doubling time (h)
0
1
2
3
4
5
0
2
4
6
0
0.25
0.5
0.75
1.0
Starvation
Dilution rate
Washout

Steady-state relationship between substrate concentration and output of bacterial mass

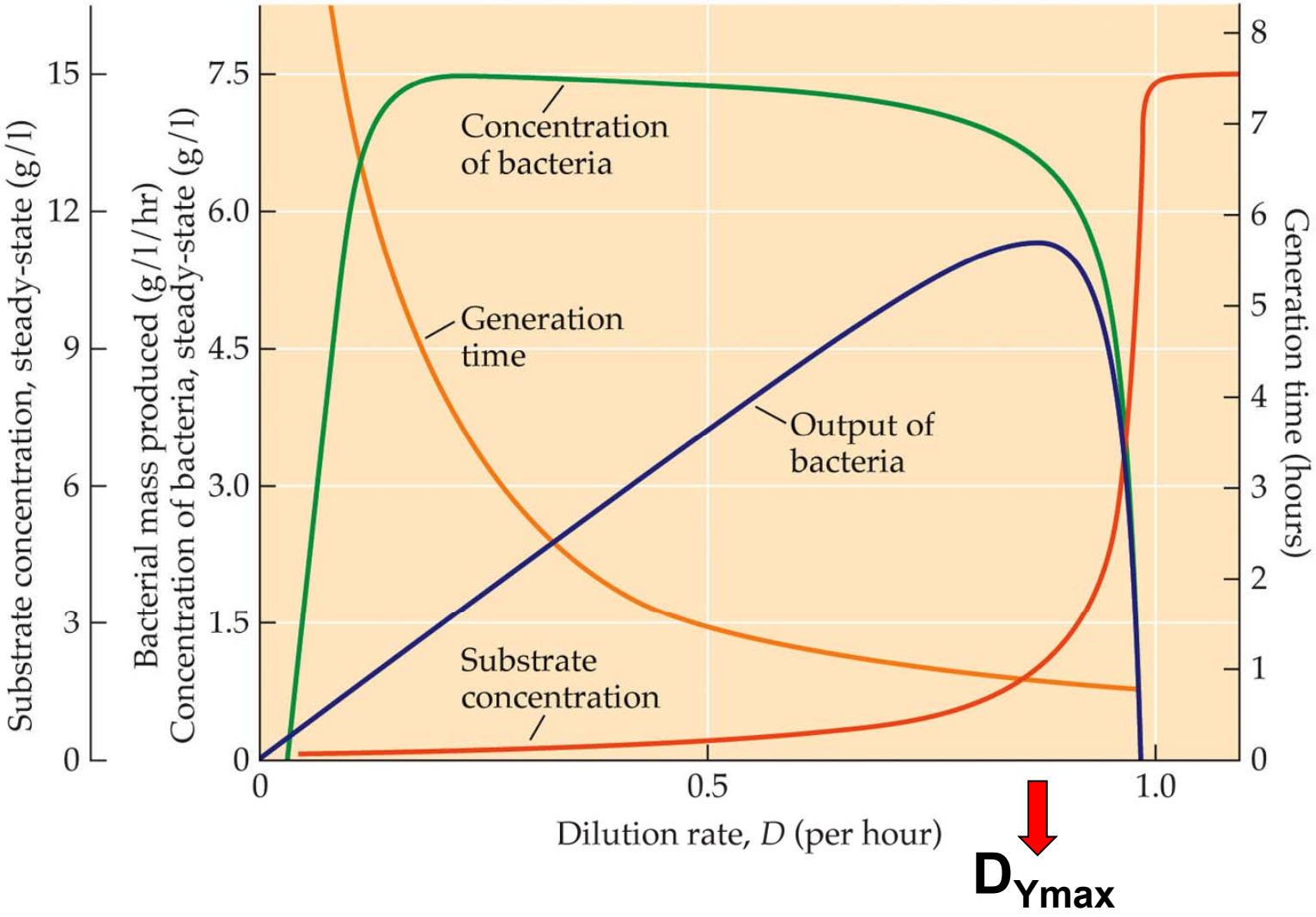


Table 6.2 Growth yields of anaerobic bacteria utilizing glucose as the energy source

	Mol ATP/Mol Glucose	y_{max} (g of cell/mol Glucose)	y_{ATP} (g of cell/mol ATP)
Lactobacillus delbrueckii[a]	2	21	10.5
Enterococcus faecalis[a]	2	20	10
Zymomonas mobilis[b]	1	9	9

[a]Homolactic fermentation, Embden–Meyerhof pathway (see Chapter 10).
[b]Alcoholic fermentation, Entner–Doudoroff pathway (see Chapter 10).