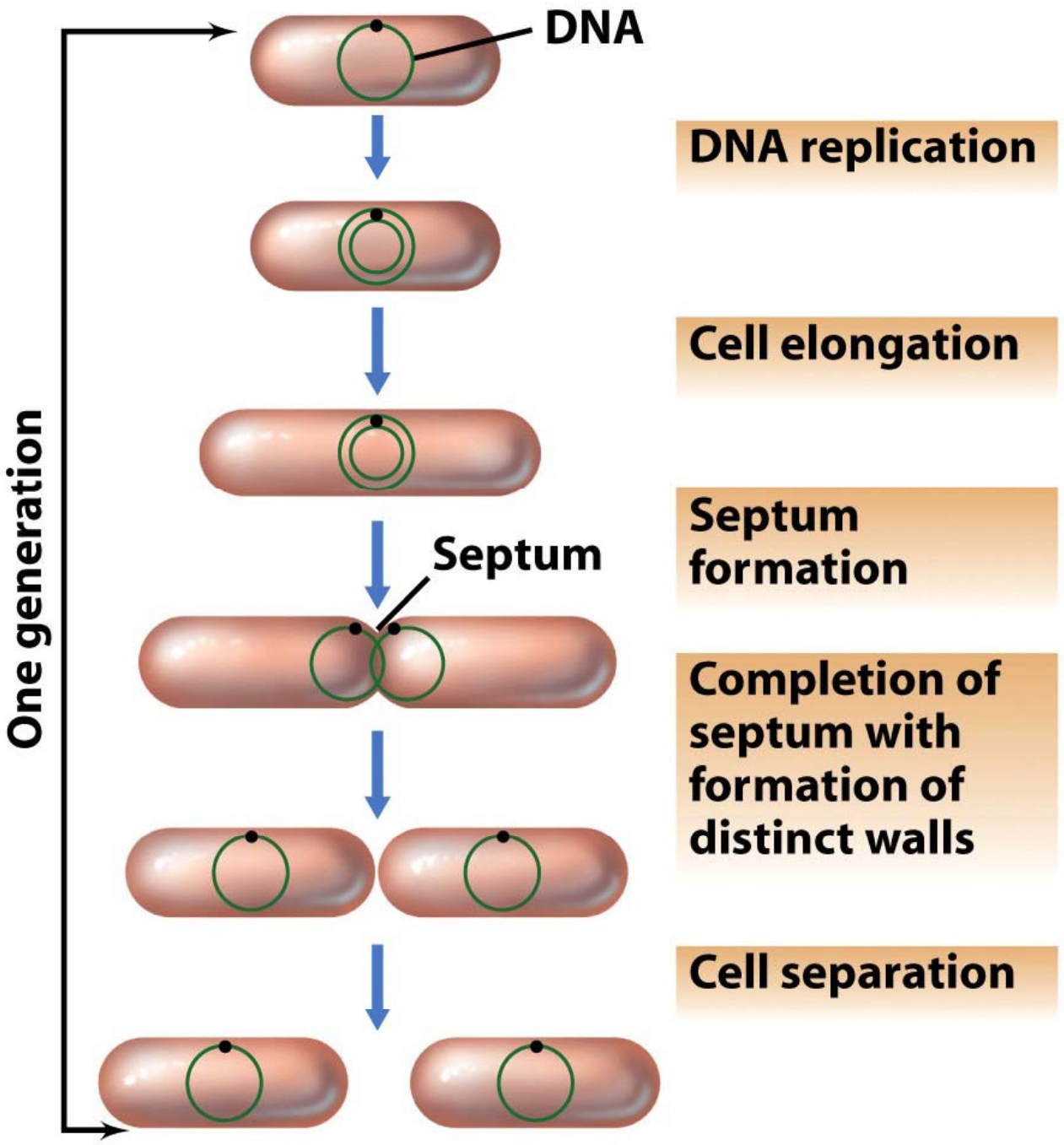
The Process of Growth

- 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 nonculturable, morphology changes (life cycle)

- Divide via Binary Fission: 3 mechanisms involved!
  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

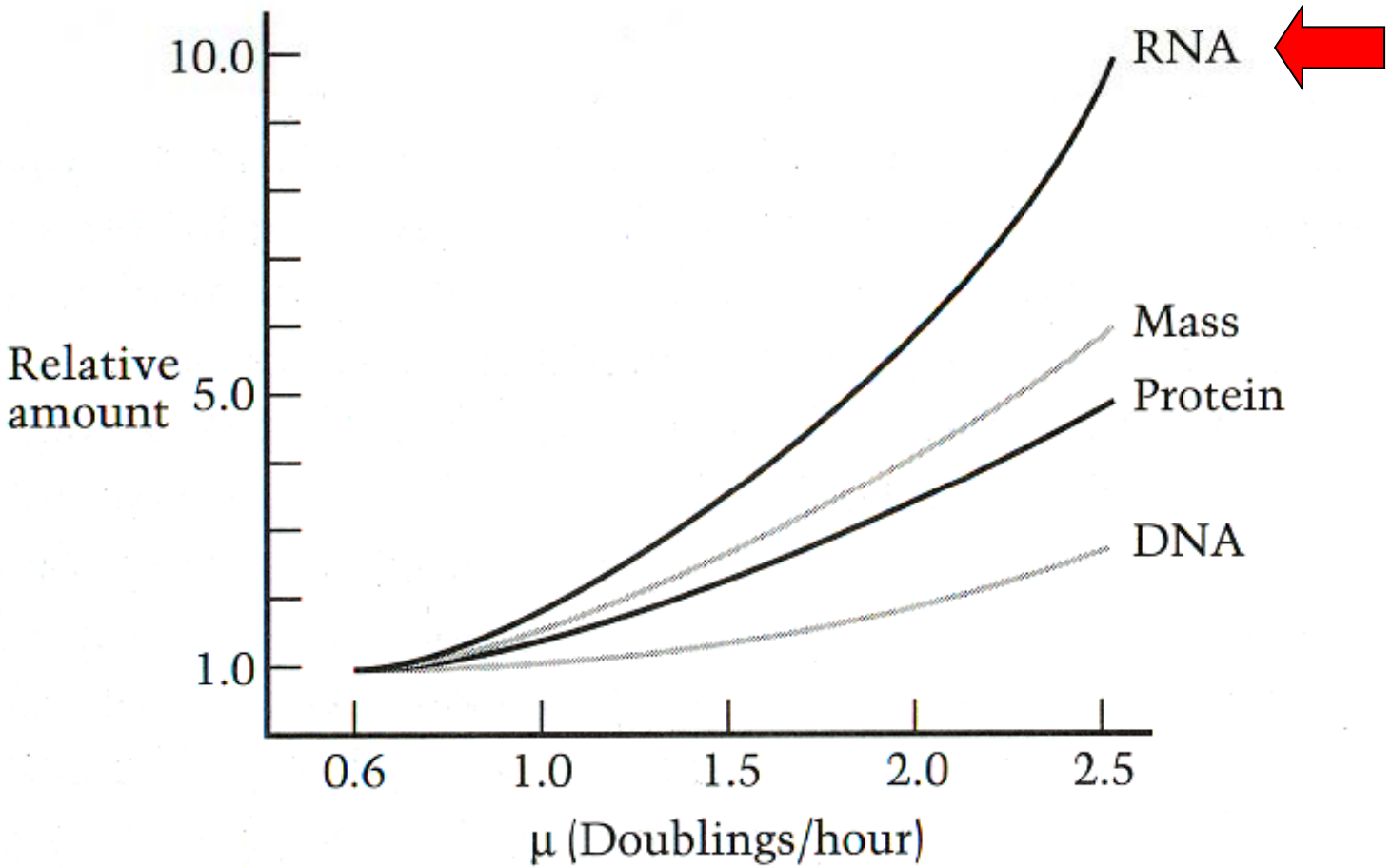


Figure 1

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.)

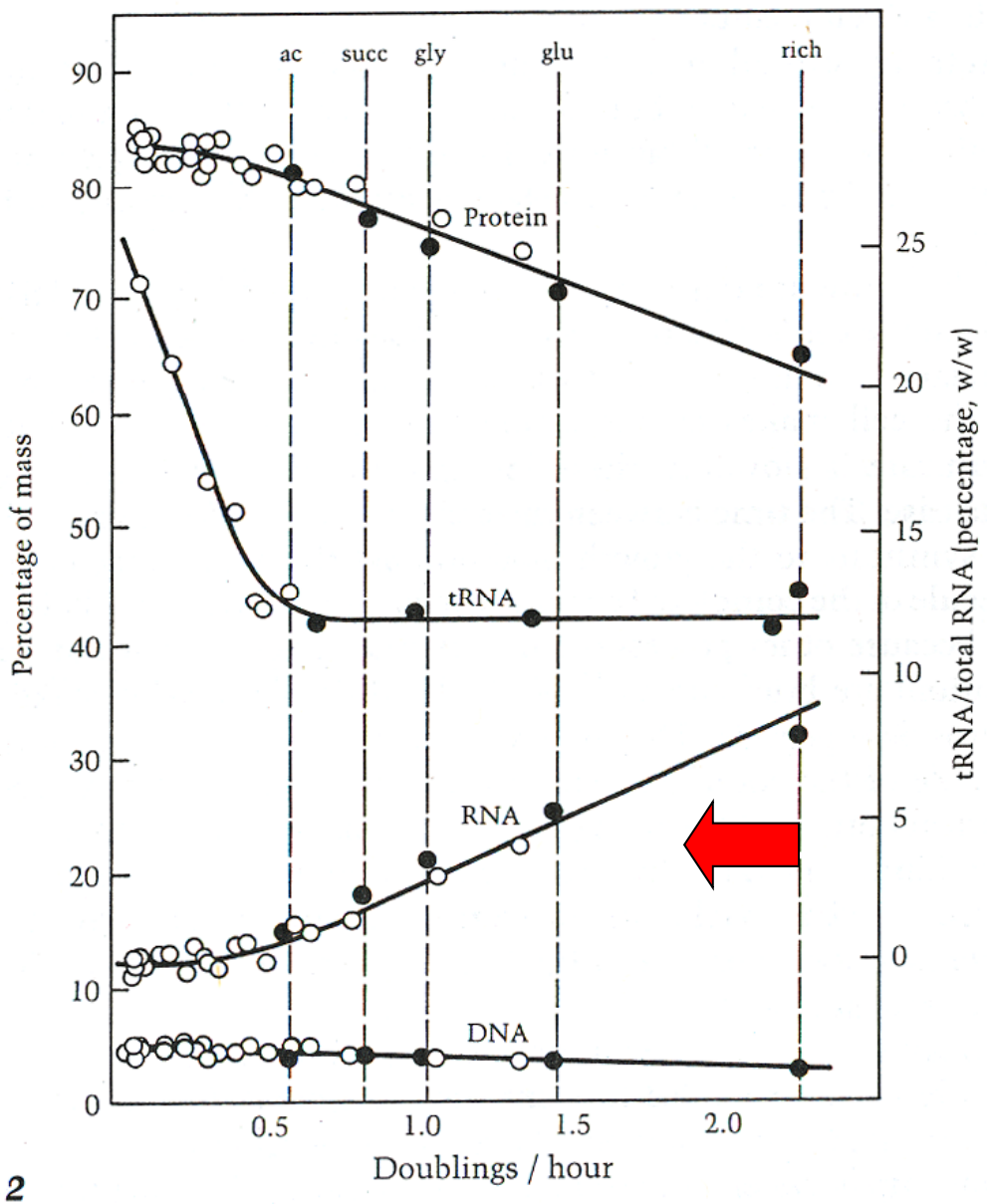
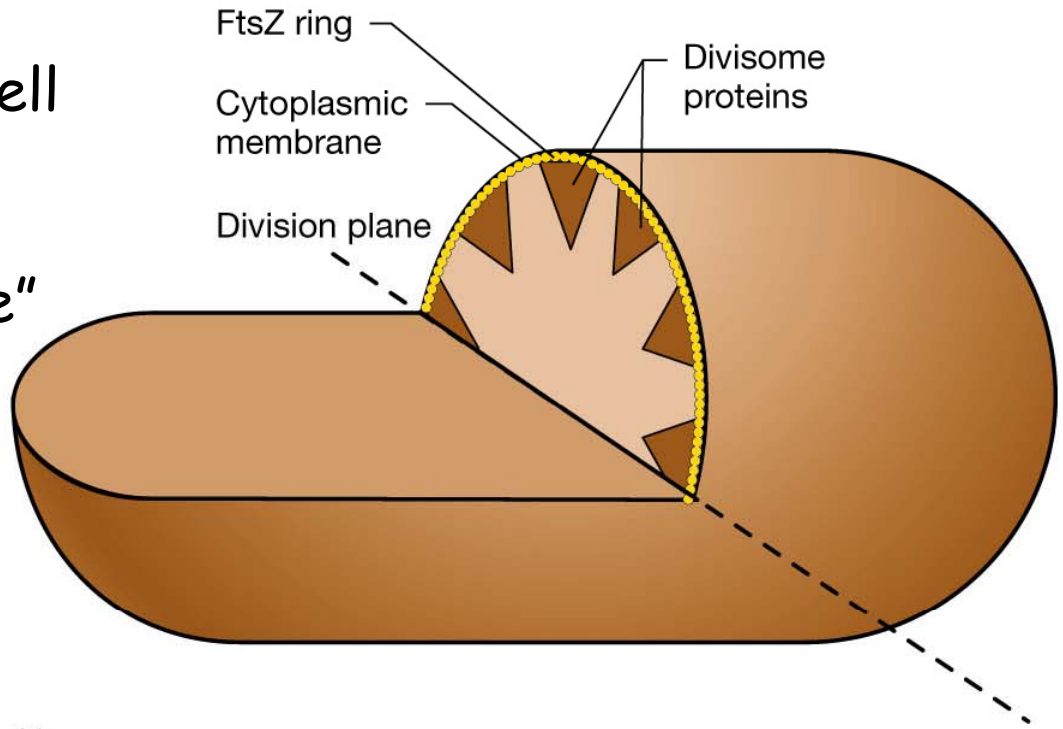


Figure 2

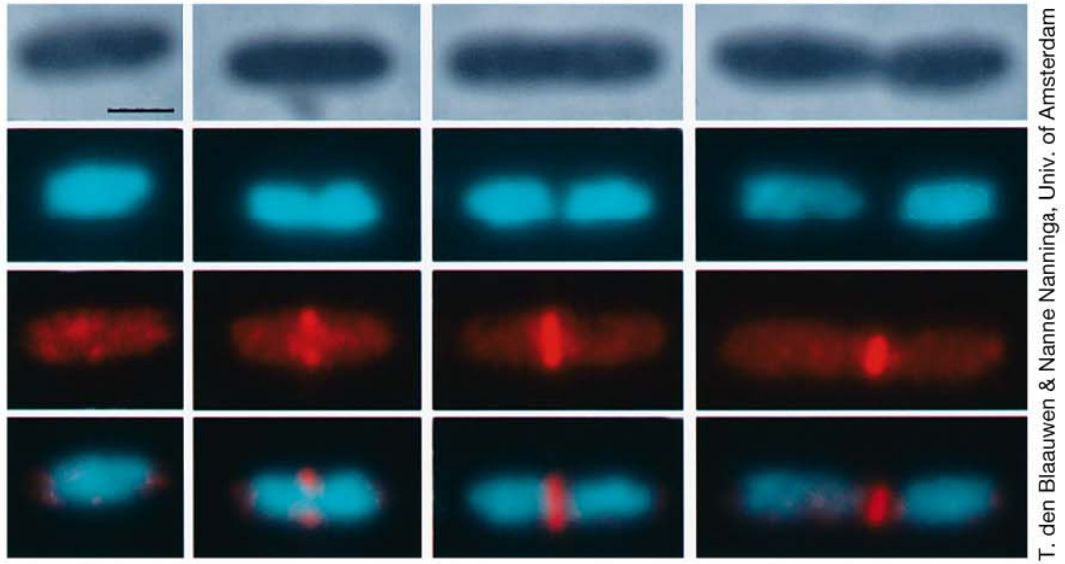
Effect of growth rate on the cellular proportions of protein, RNA, and DNA. Filled circles refer to results from cultures undergoing balanced growth in batch culture in various media; open circles are from cultures growing in a glucose-limited chemostat. (From Jacobsen, 1974.)

FtsZ ring & cell division

The "divisome"



(a)



T. den Blaauwen & Nanne Nanninga, Univ. of Amsterdam

(b)

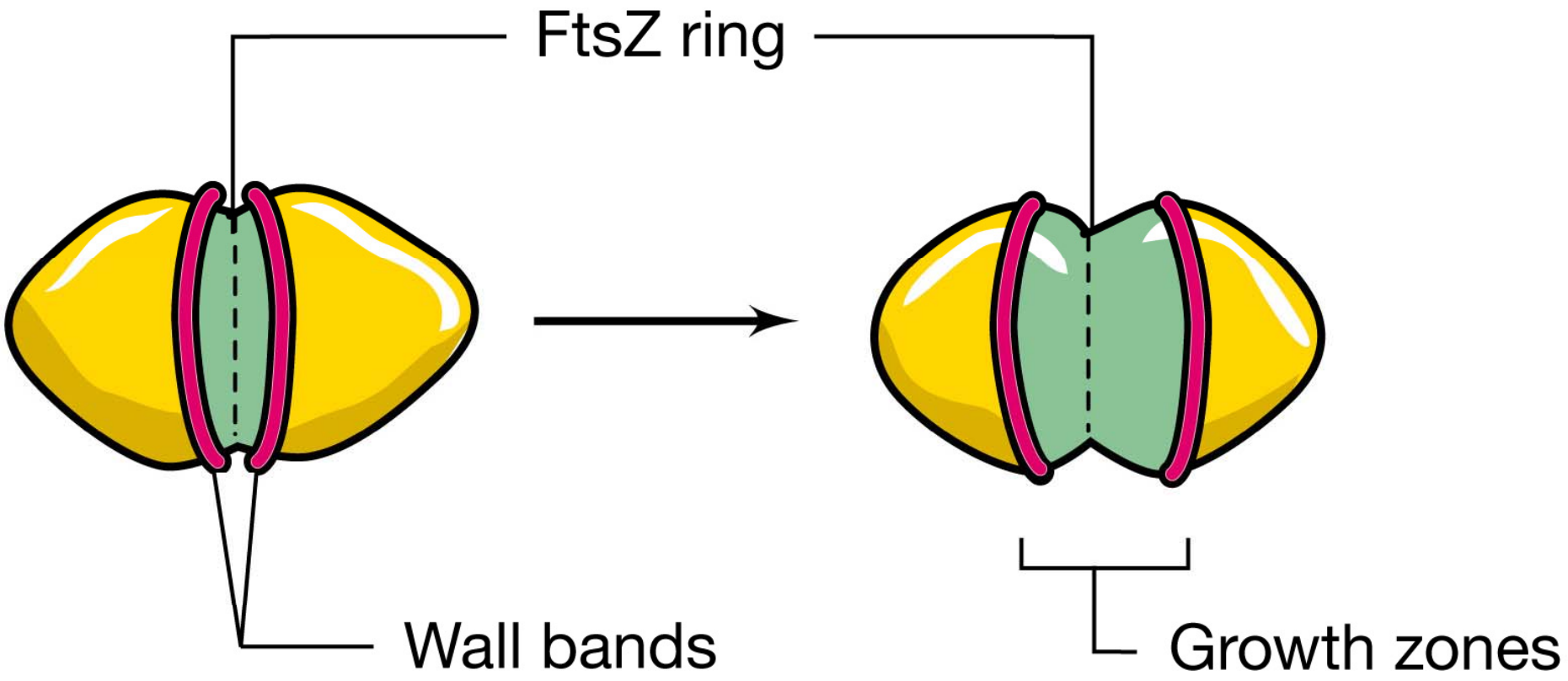
phase

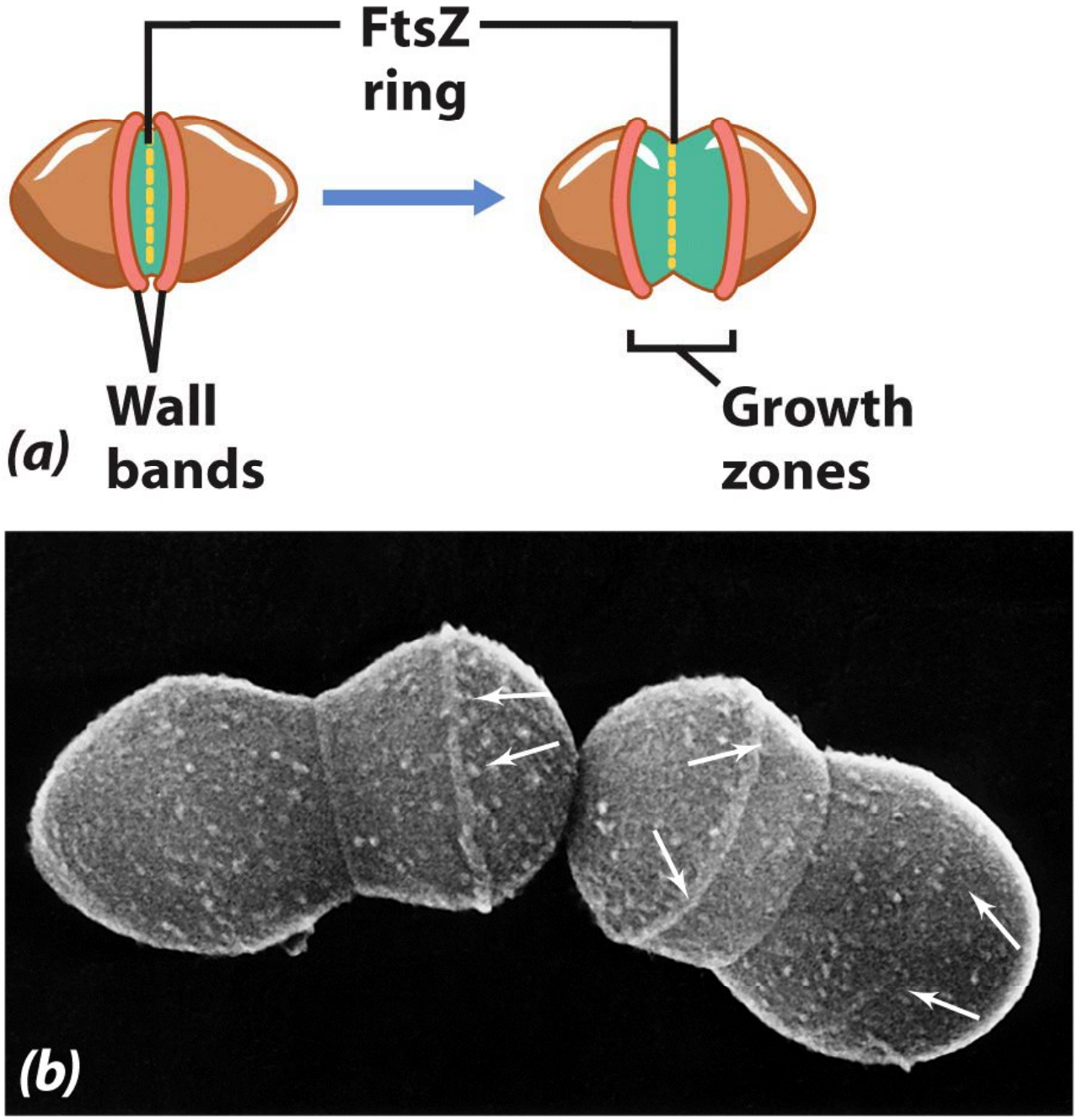
DNA stain

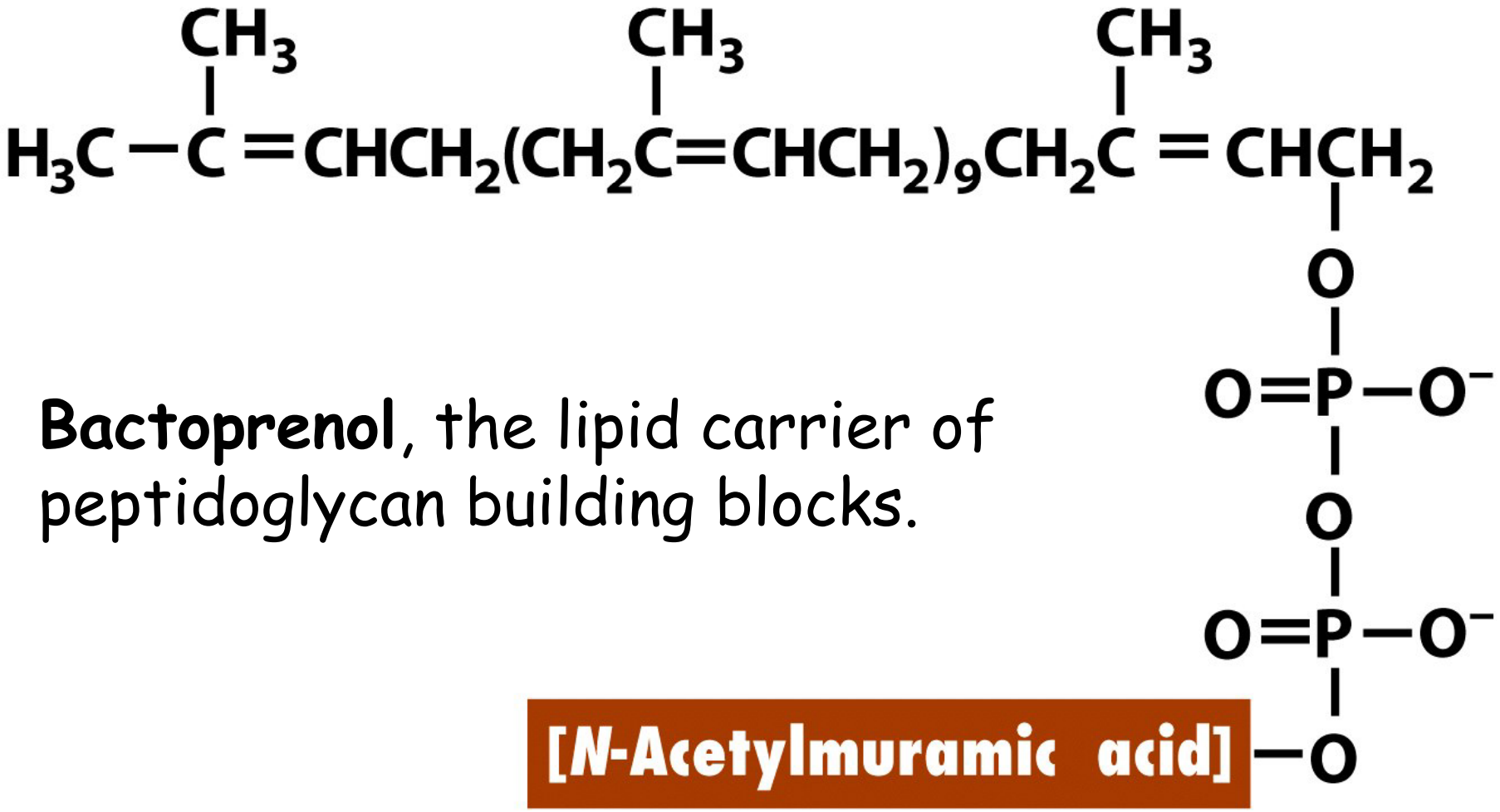
FtsZ stain

DNA & FtsZ

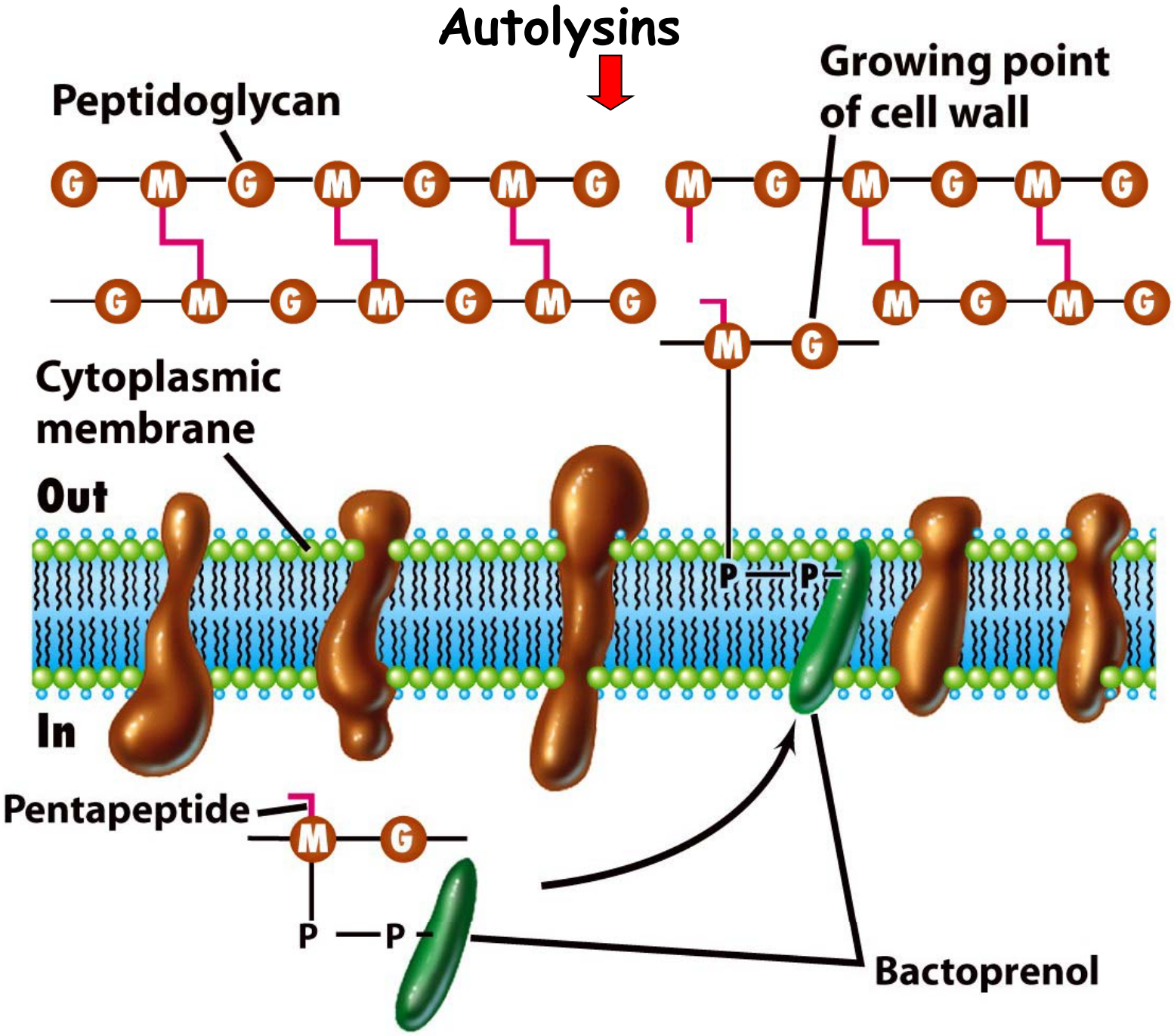
FtsZ similar to Tubulin
MreB similar to Actin



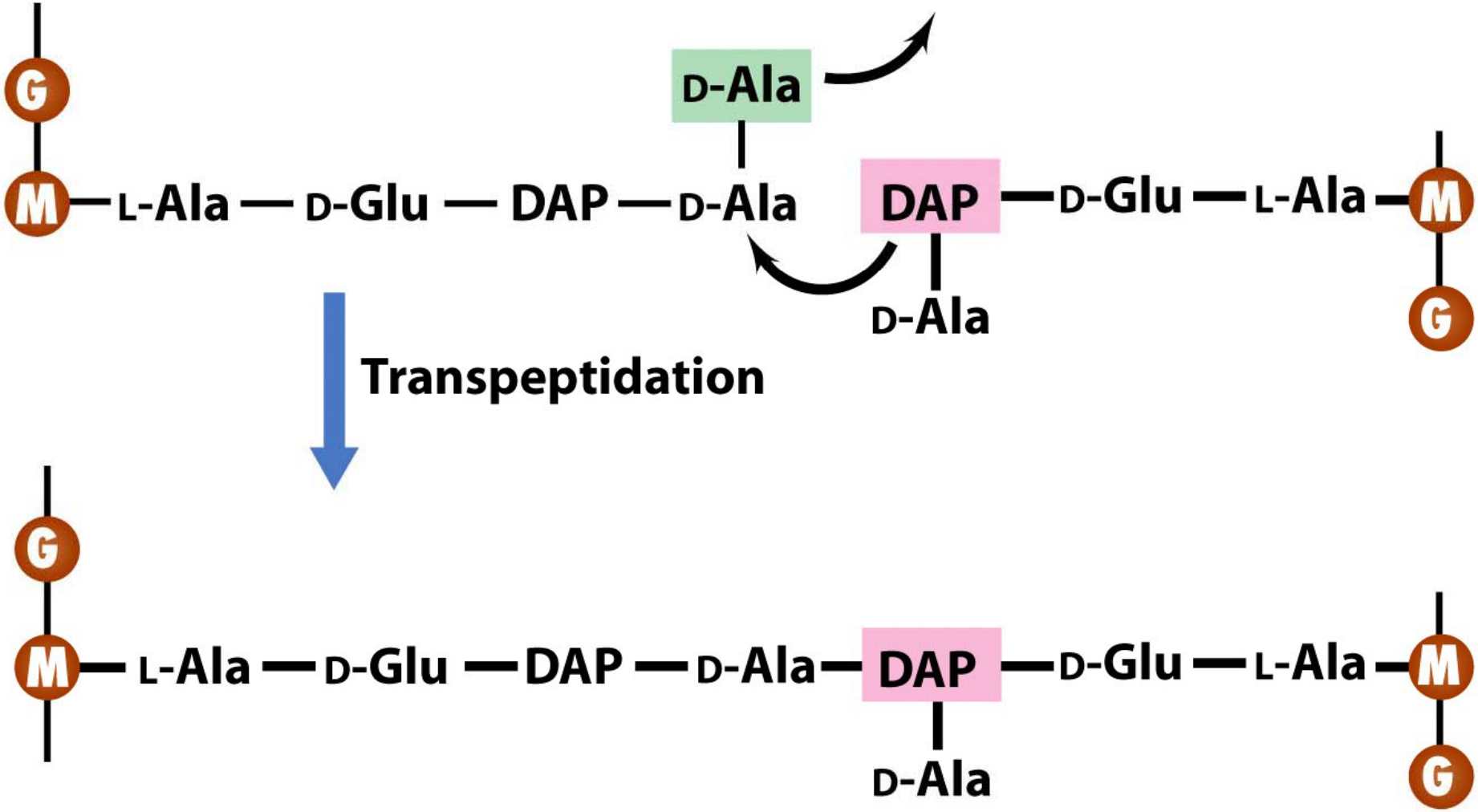

FtsZ
ring
Wall
bands
(a)
Growth
zones
(b)



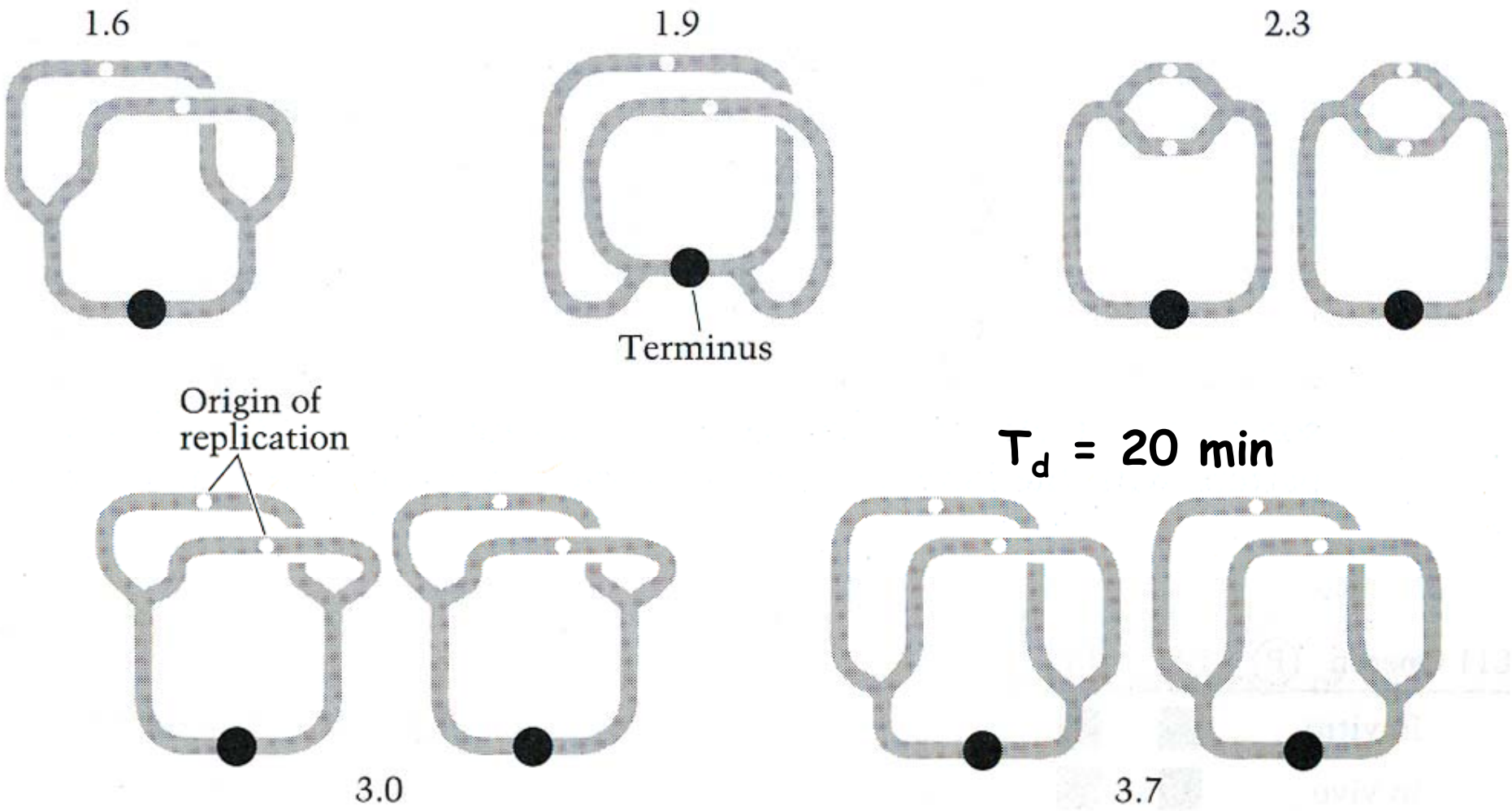
Bactoprenol, the lipid carrier of peptidoglycan building blocks.


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.)

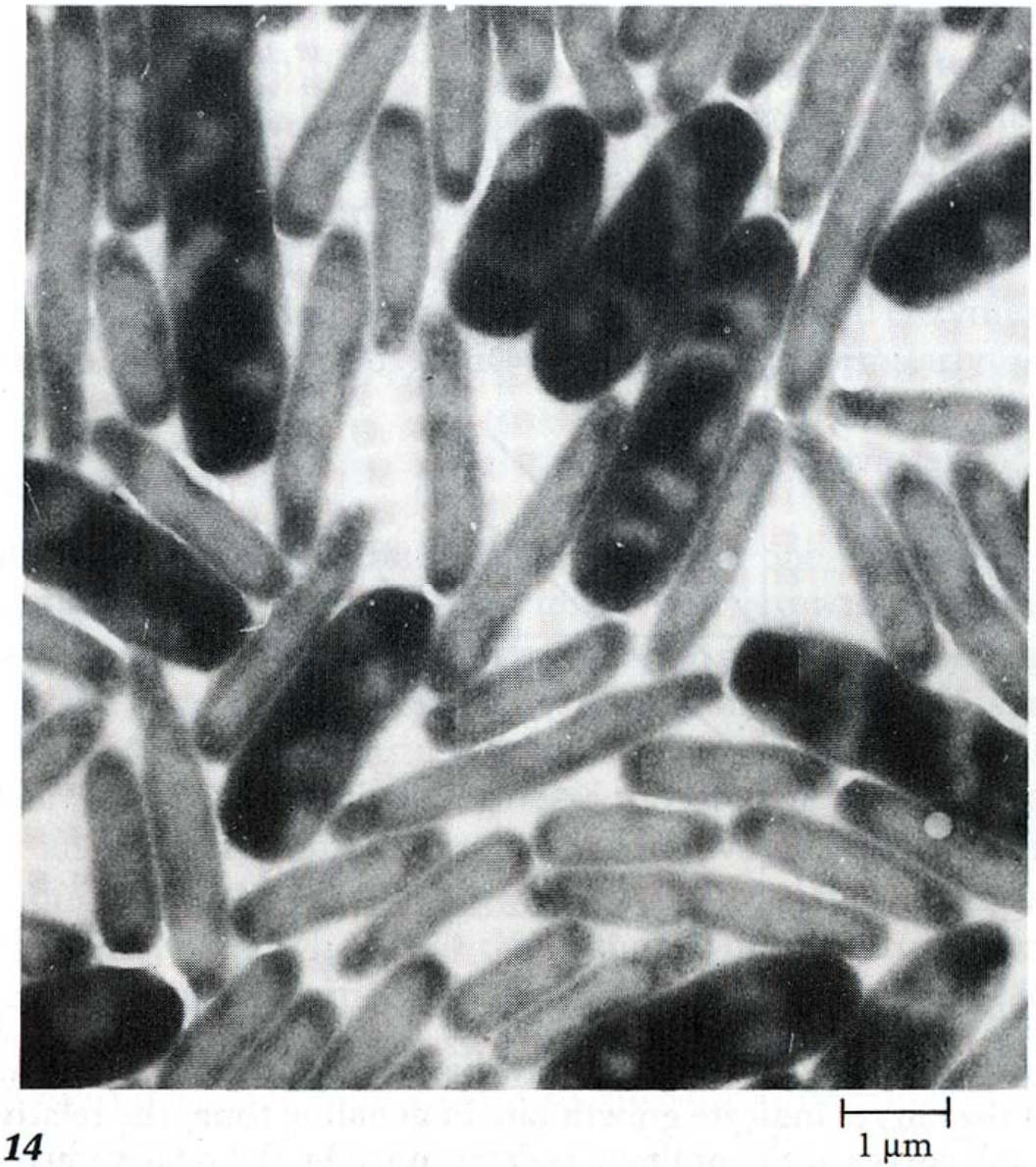


Figure 14

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

  $t_{\frac{1}{2}} = \ln 2/\mu = 0.693/\mu = g$

- Phases of Growth in Batch culture

  Lag, Log, Stationary, Death

- Measurement of Growth

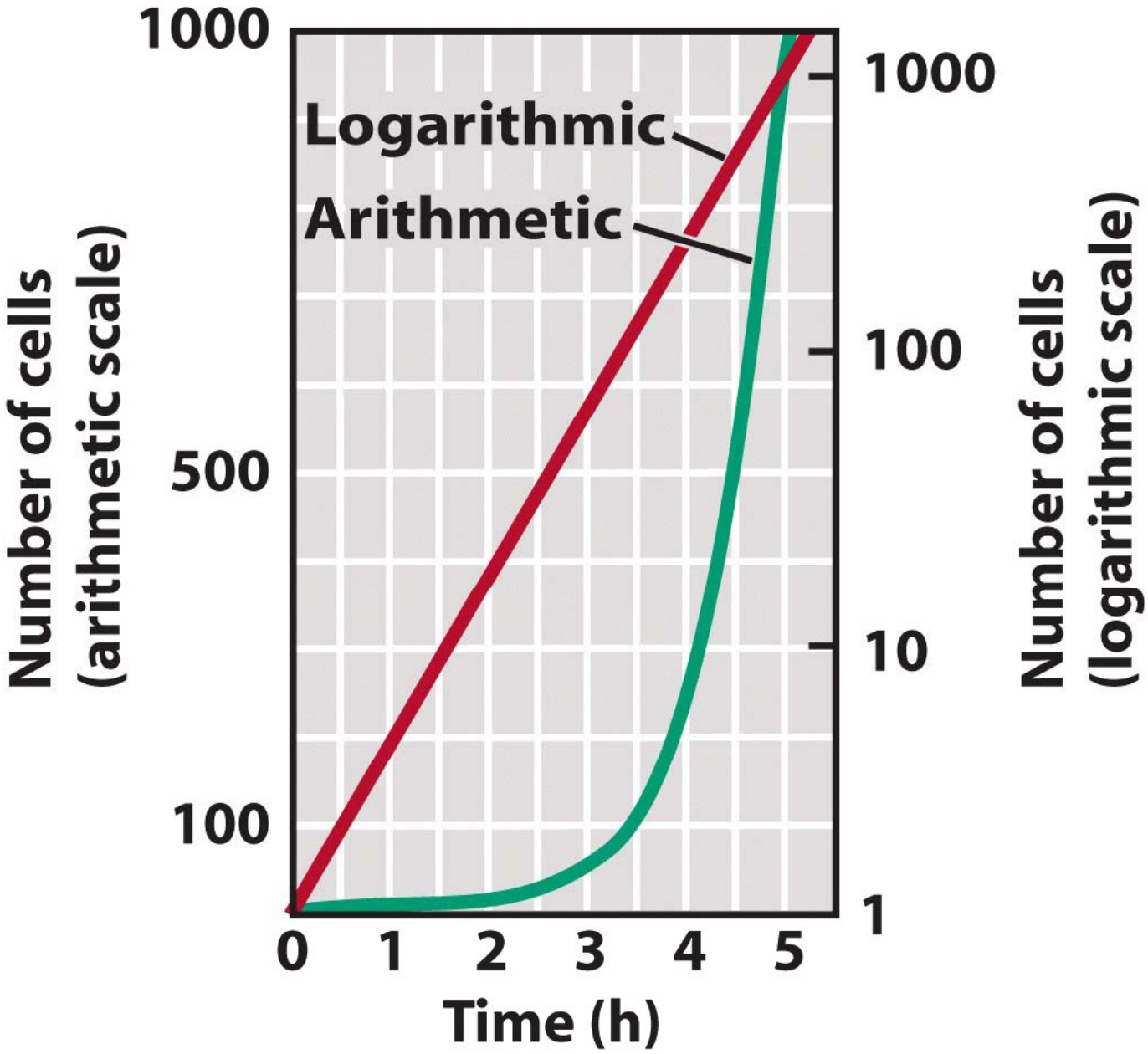
  Total cell counts

  Viable cell counts

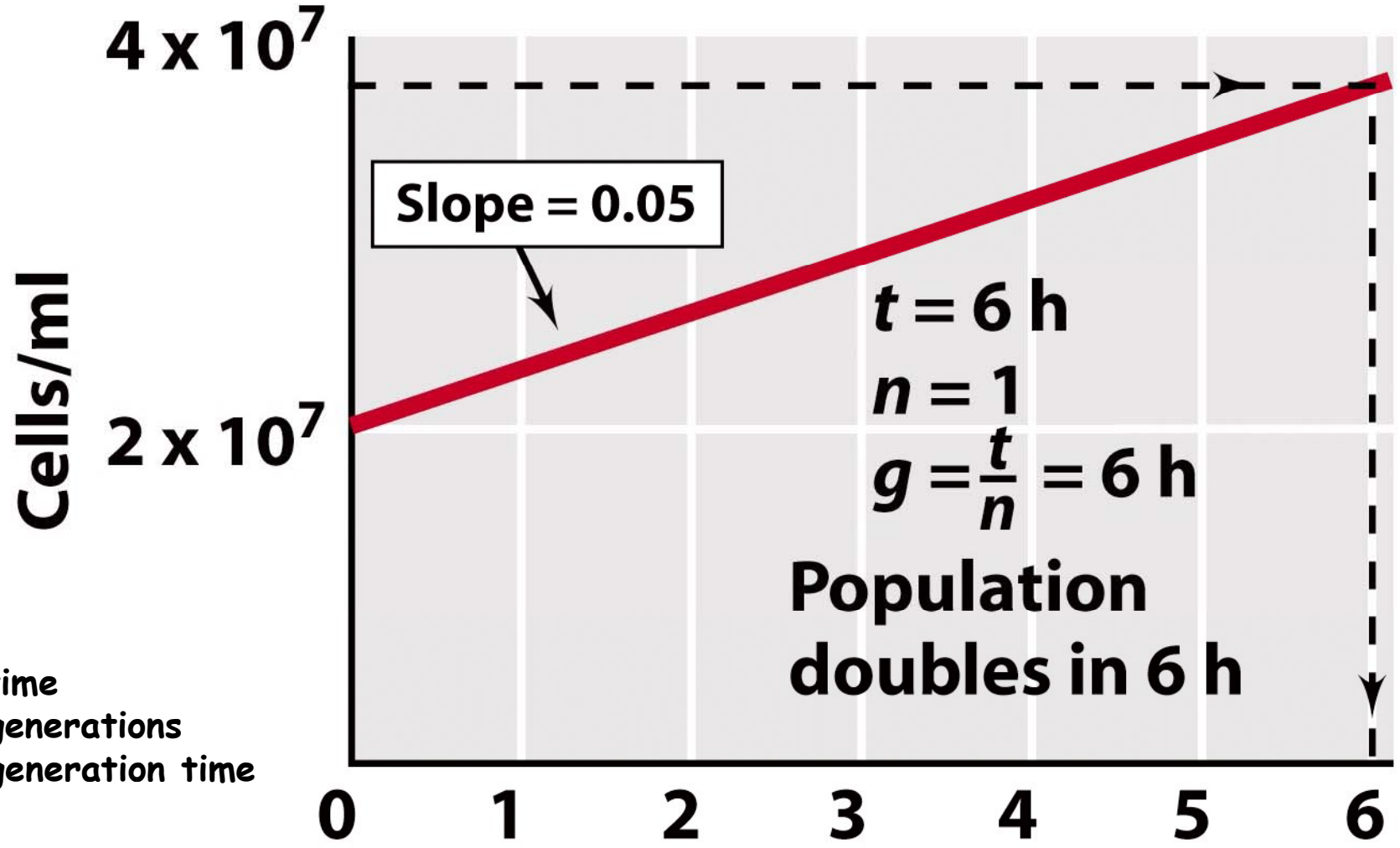
  Turbidity

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)

How to estimate the generation times of an exponential microbial culture using semi-log plots.



t = time
n = generations
g = generation time

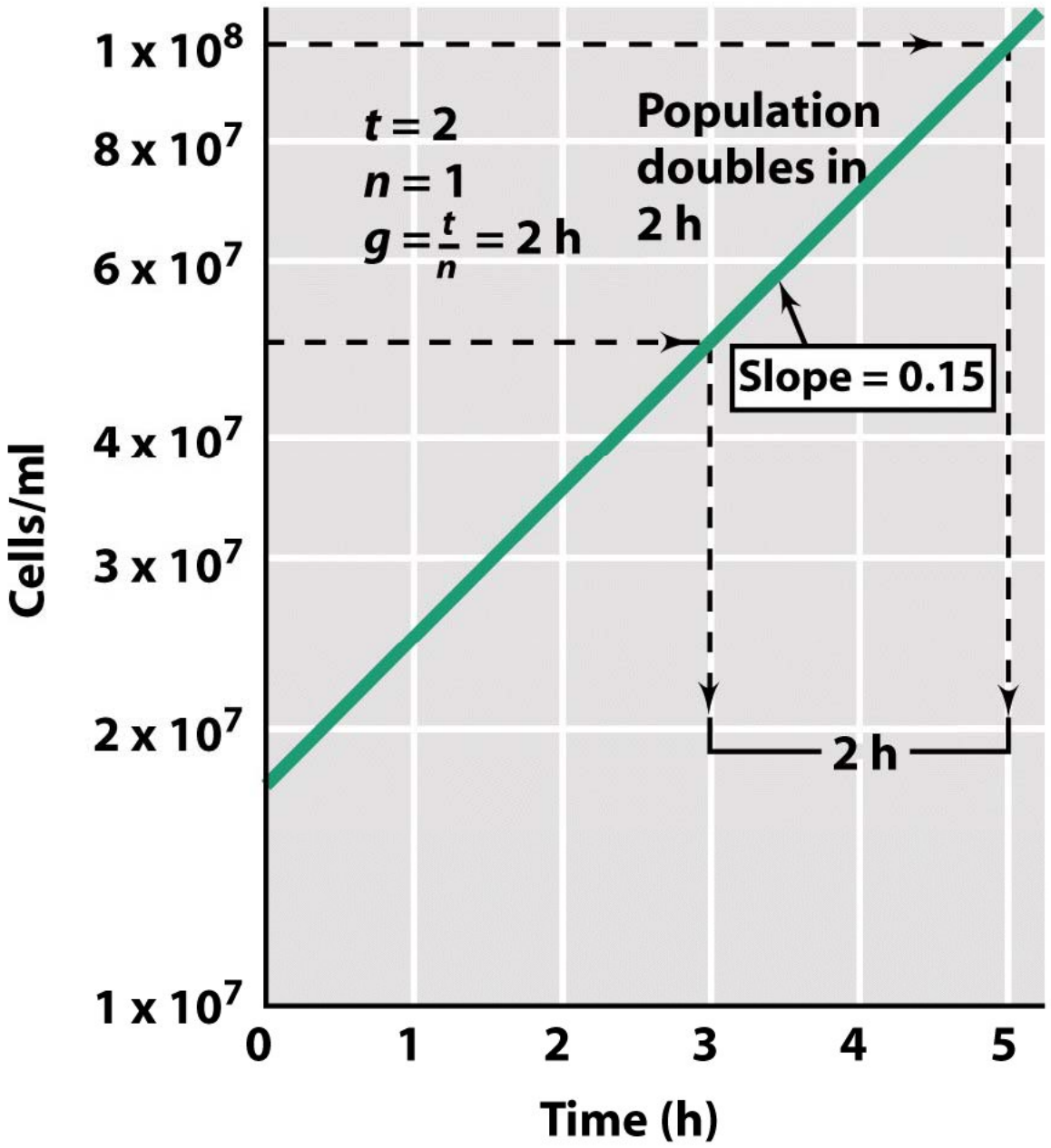

1 x 10^8
8 x 10^7
6 x 10^7
4 x 10^7
3 x 10^7
2 x 10^7
1 x 10^7
Cells/ml
t = 2
n = 1
g = t/n = 2 h
Population doubles in 2 h
Slope = 0.15
2 h
0
1
2
3
4
5
Time (h)

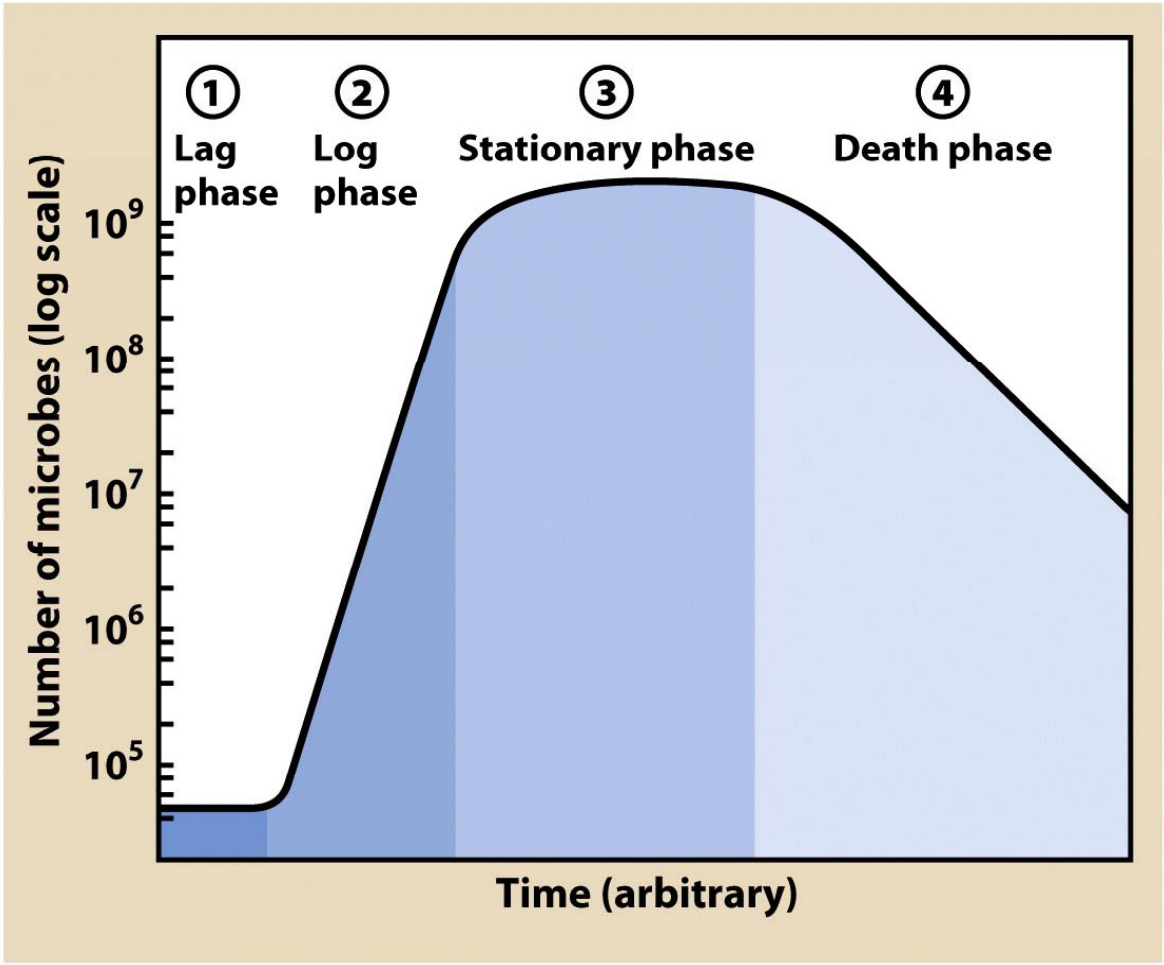
Table 6.1 **Approximate generation times for several organisms growing in media optimal for growth**

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

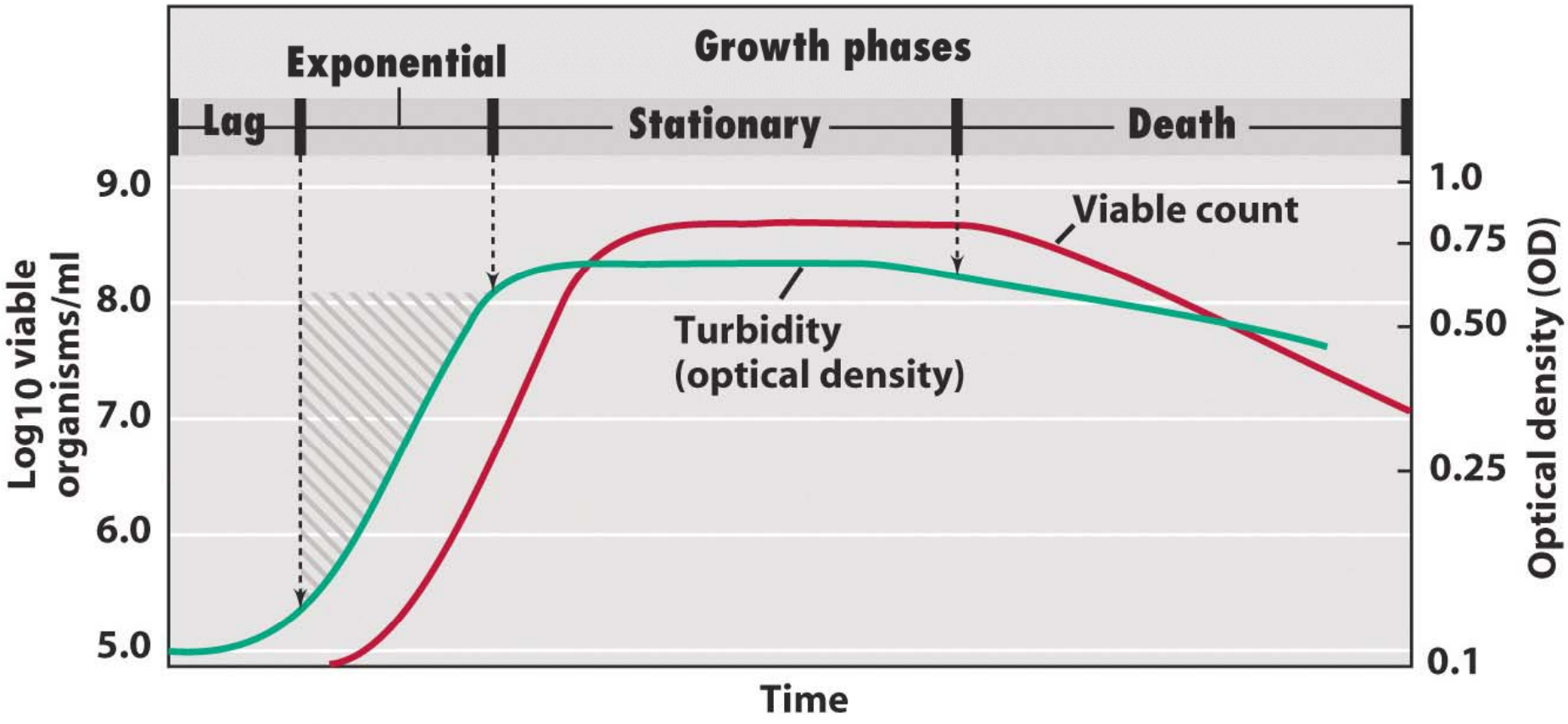
- Lag phase
  - Cells synthesizing materials, not dividing
- Log phase = exponential growth
  - $1 \rightarrow 2 \rightarrow 4 \rightarrow 8 \rightarrow 16 \ldots$
    - 10 doublings increases density by ~1000
    - $\log_{10}(N)$ increases linearly
- Stationary phase
  - Cells no longer growing
- Death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

The Growth Cycle



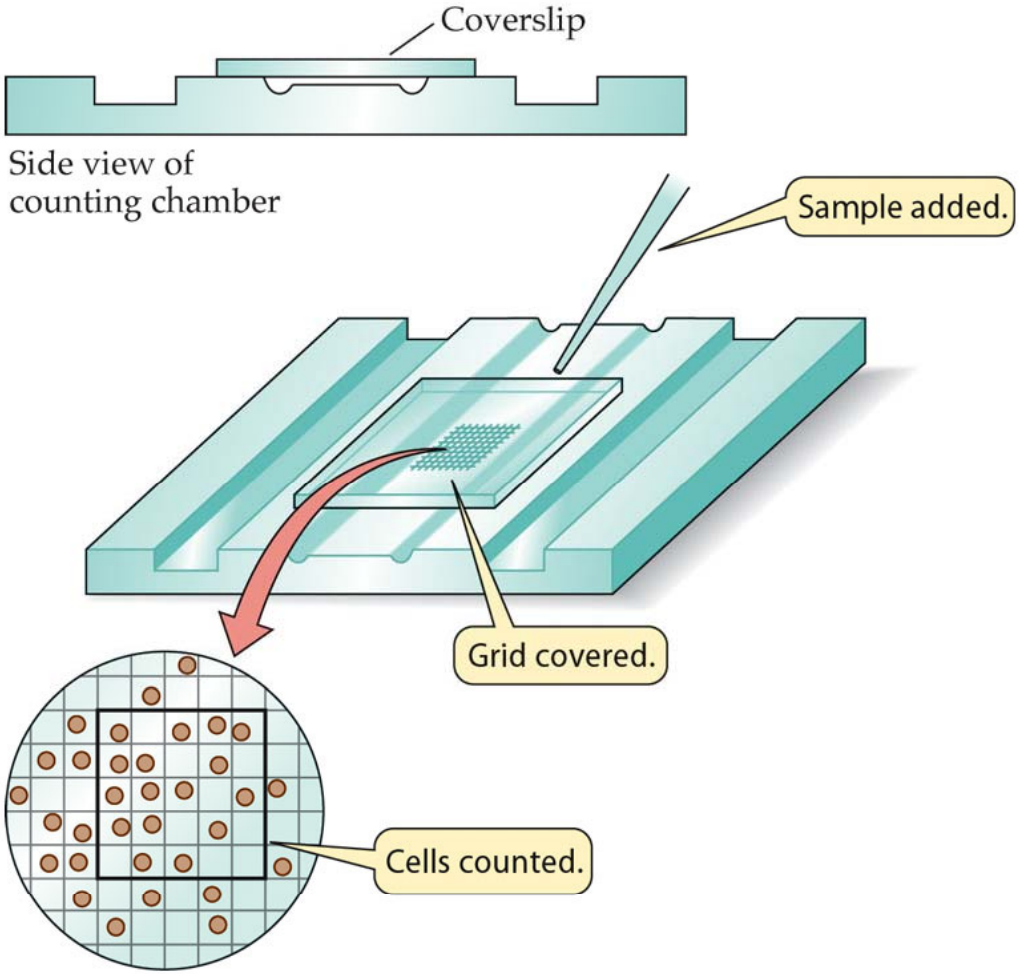
Log scale necessary to show wide range of concentrations

Cryptic Growth

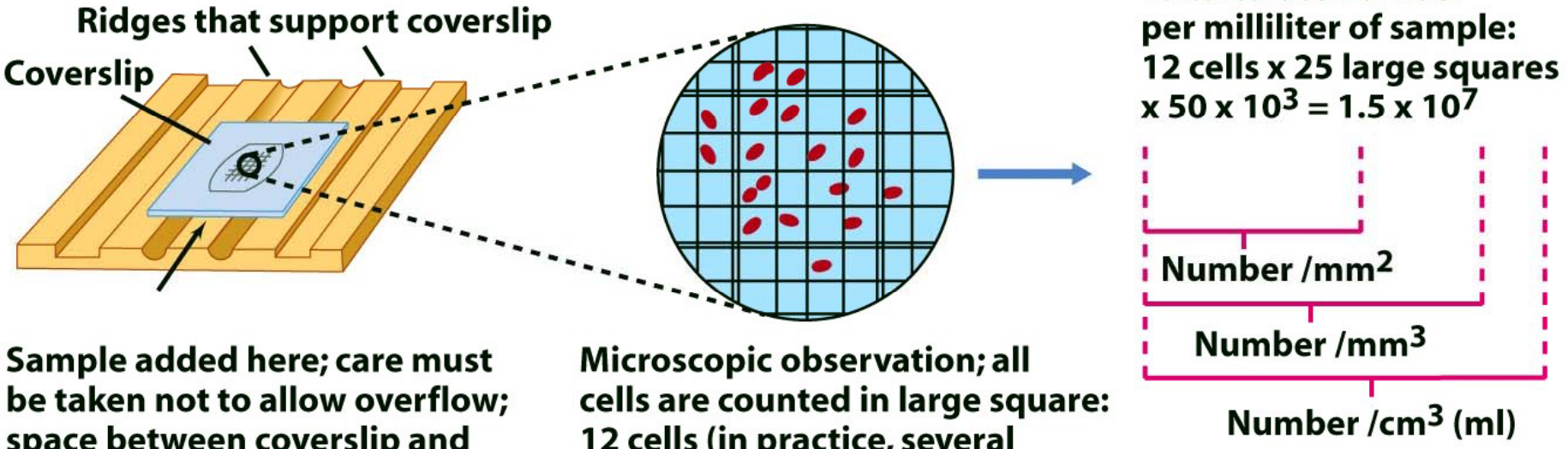


Typical growth curve for a bacterial population

Total Cell counts using the Petroff-Hausser Counter



Total Cell counts using the Petroff-Hausser Counter

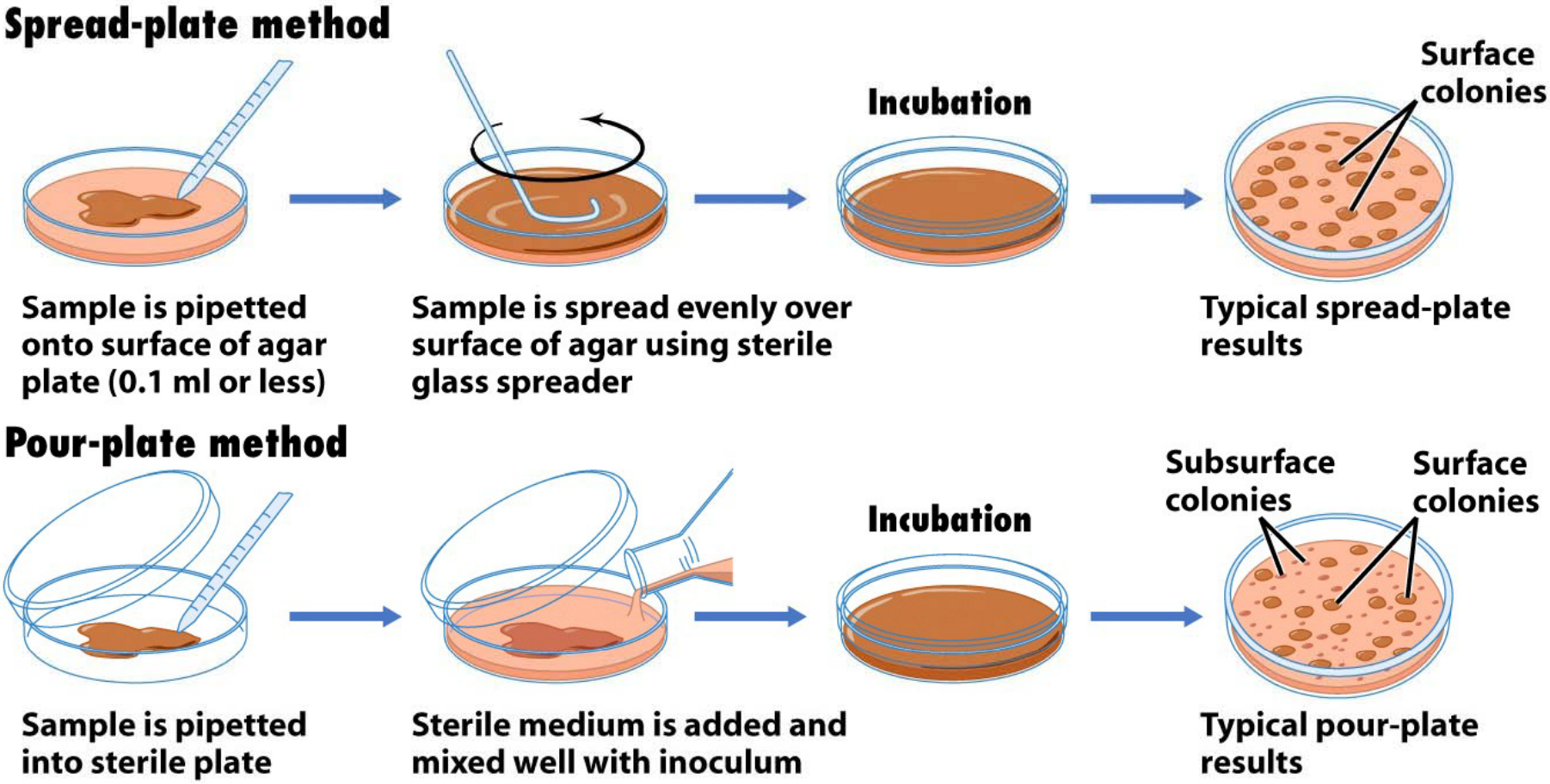


Sample added here; care must be taken not to allow overflow; space between coverslip and slide is 0.02 mm ($\frac{1}{50}$ mm). Whole grid has 25 large squares, a total area of 1 mm^2 and a total volume of 0.02 mm^3.

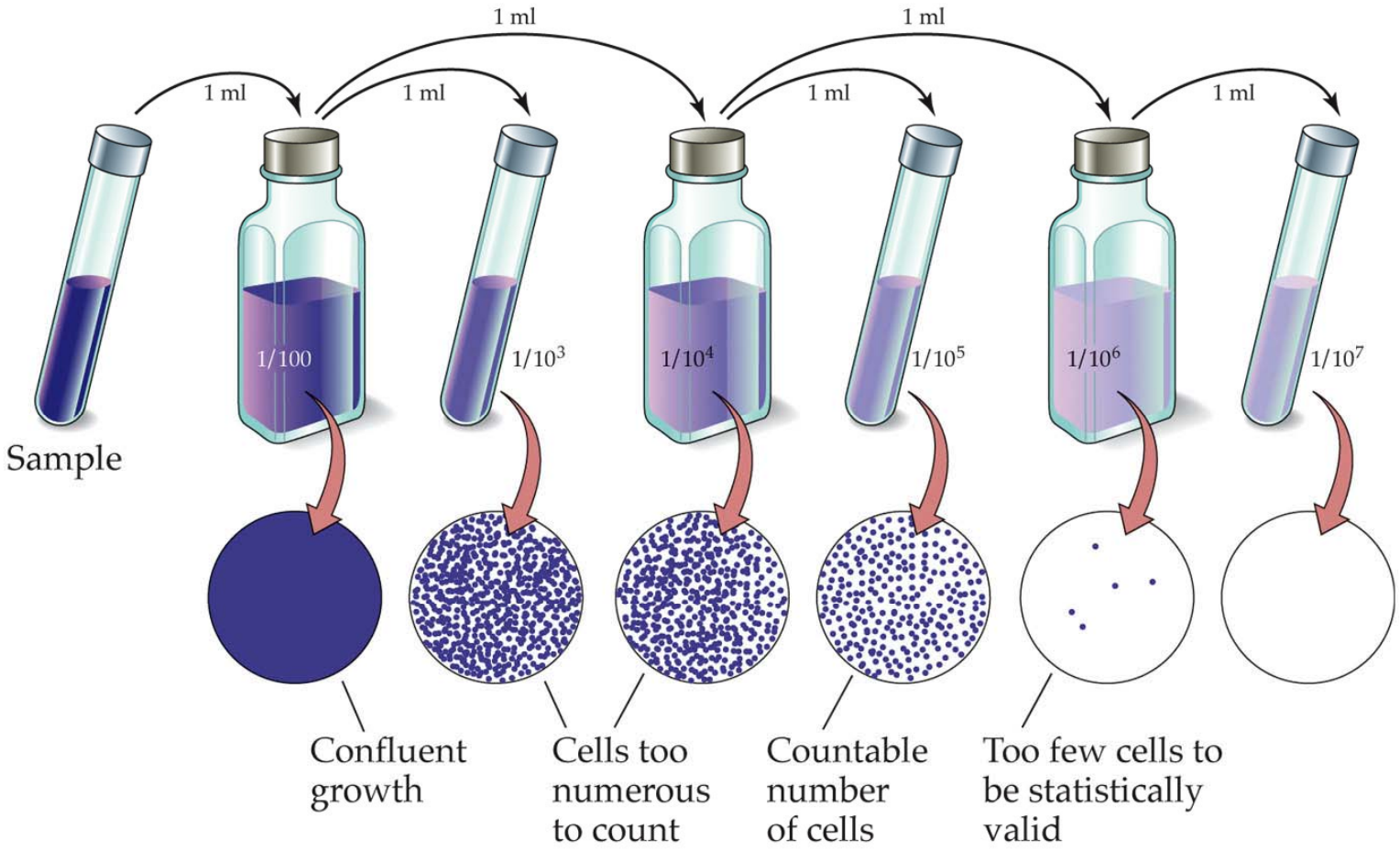
Microscopic observation; all cells are counted in large square: 12 cells (in practice, several squares are counted and the numbers averaged.)

Viable cell count methods

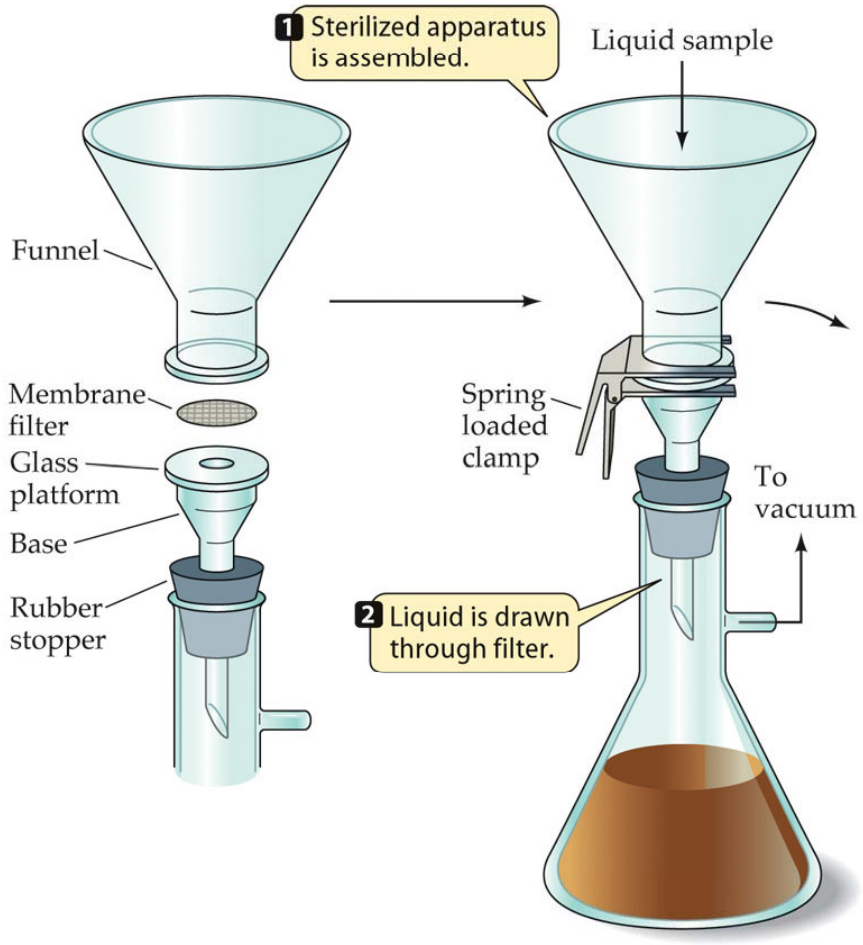
30-300 on standard Petri Dish



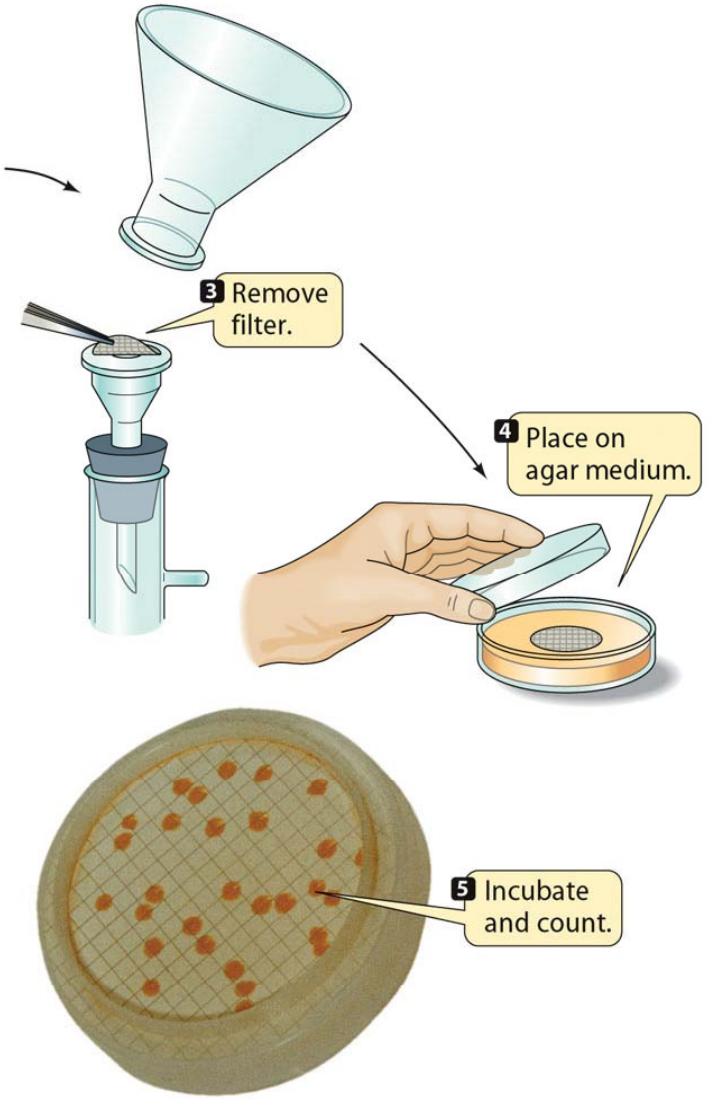
Counting the number of viable cells by serial dilution and plate count



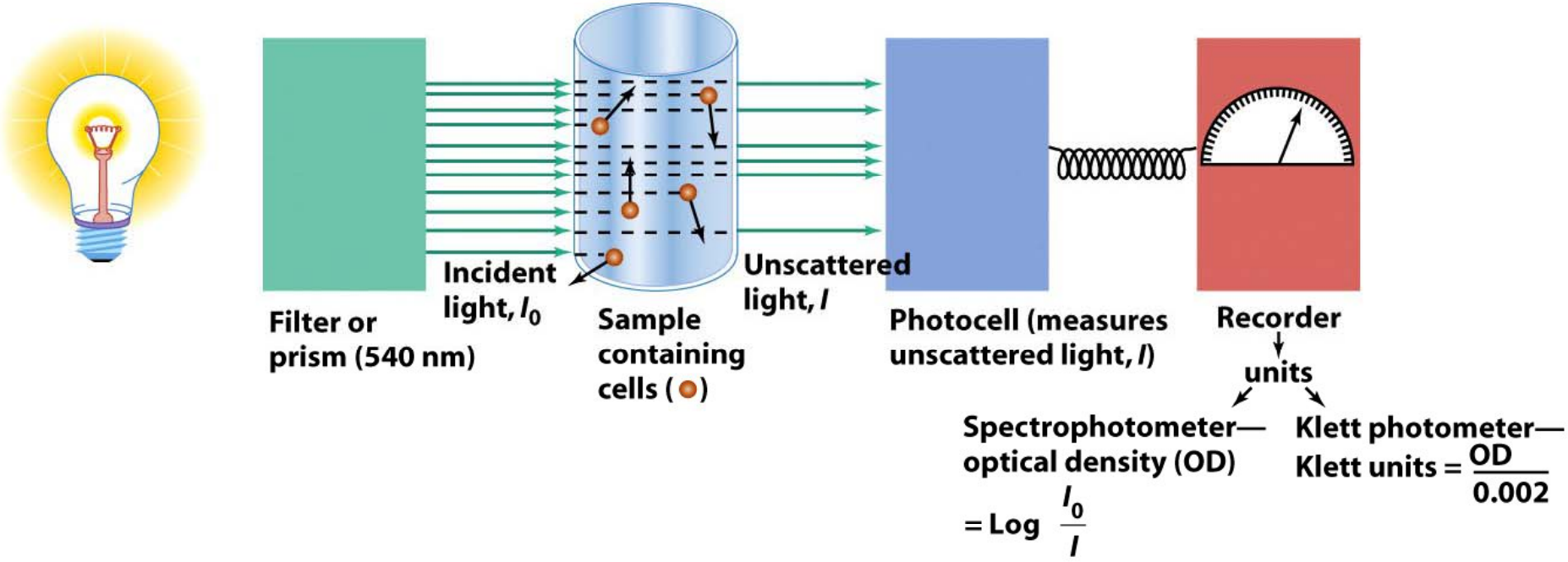
(Part 1) Concentration of cells by membrane filtration



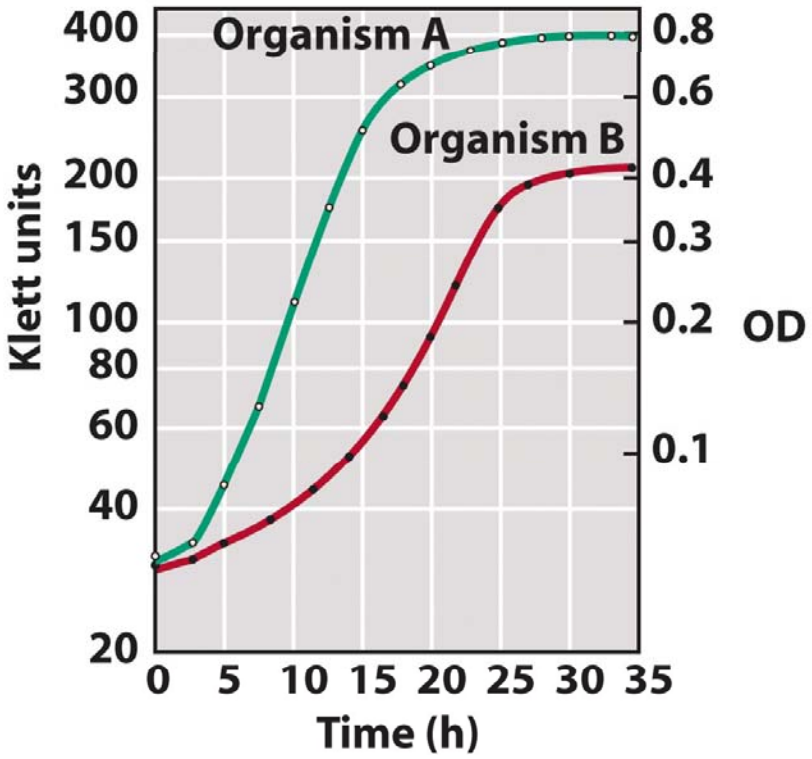
(Part 2) Concentration of cells by membrane filtration

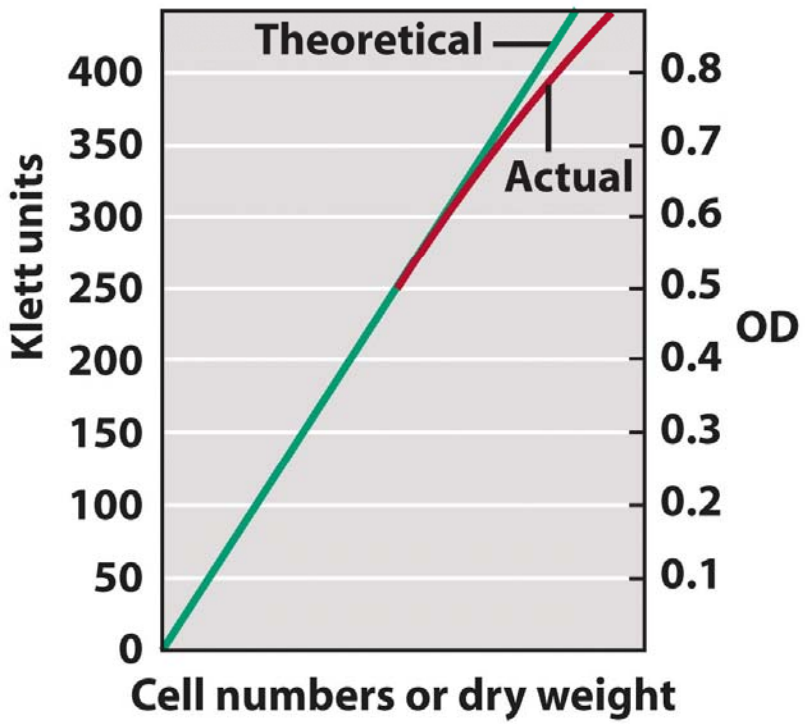


Turbidity measurements of microbial growth



Turbidity measurements of microbial growth



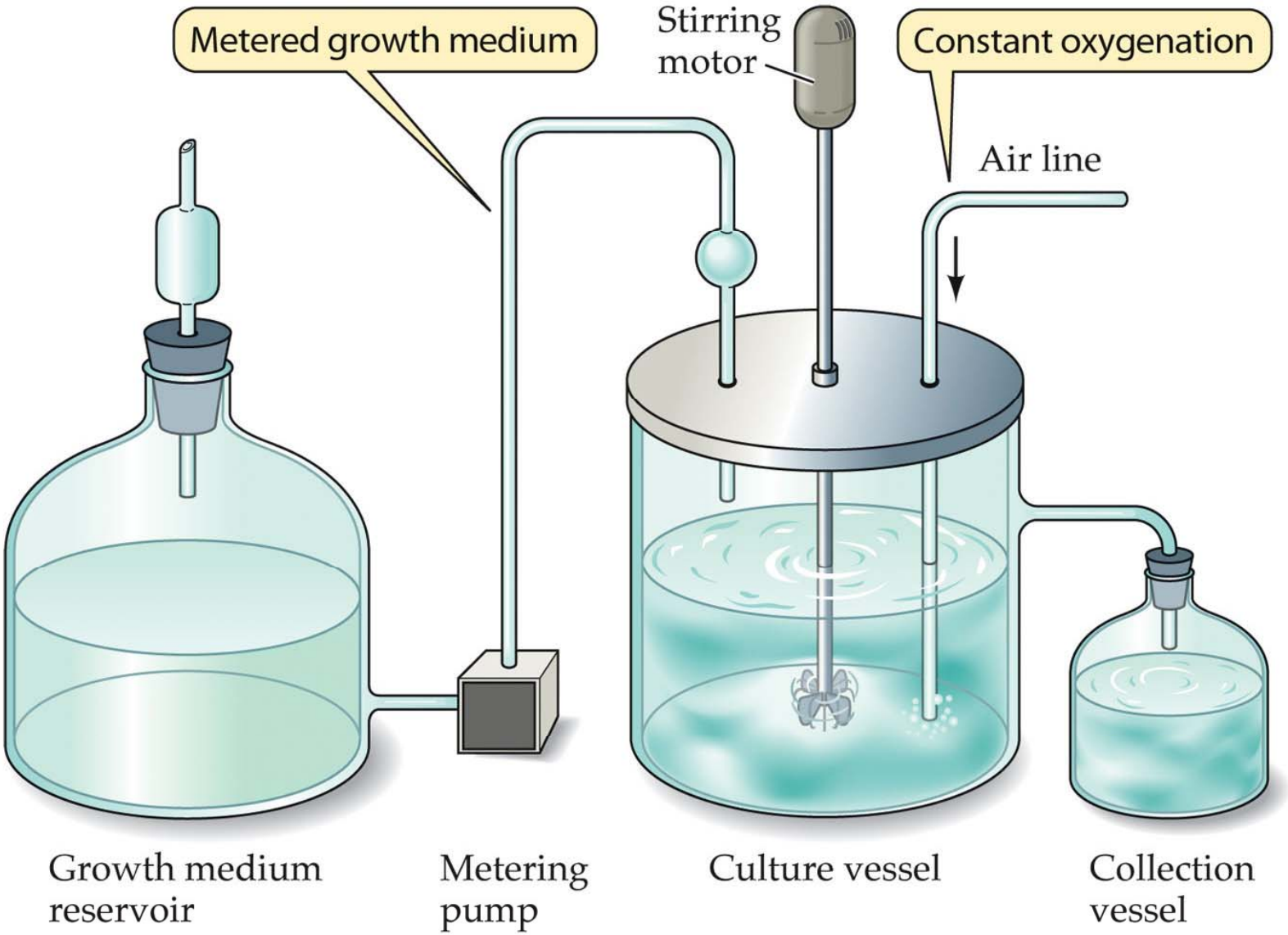


The Process of Growth

- Continuous Culture: The wonders of the **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:

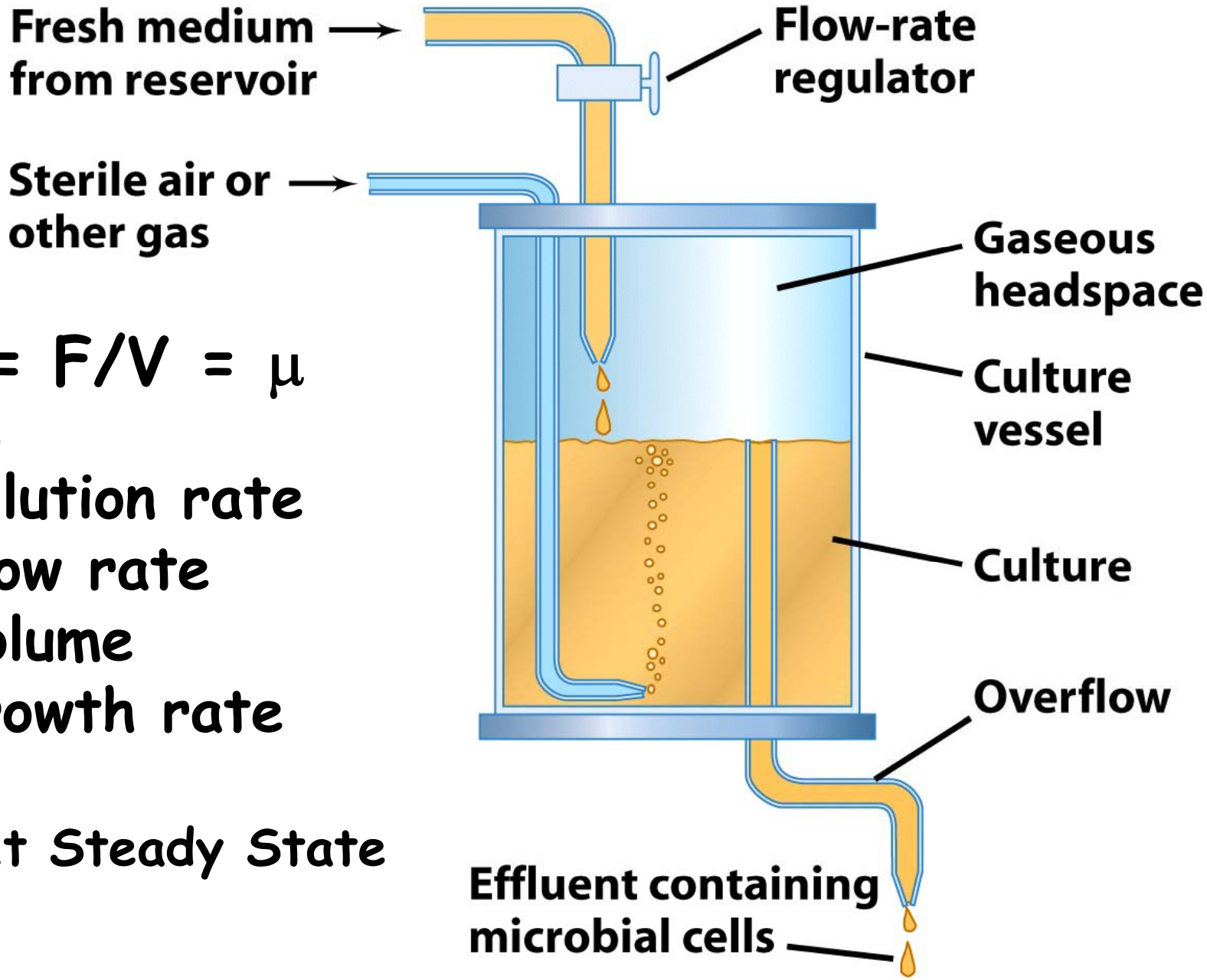
D = dilution rate

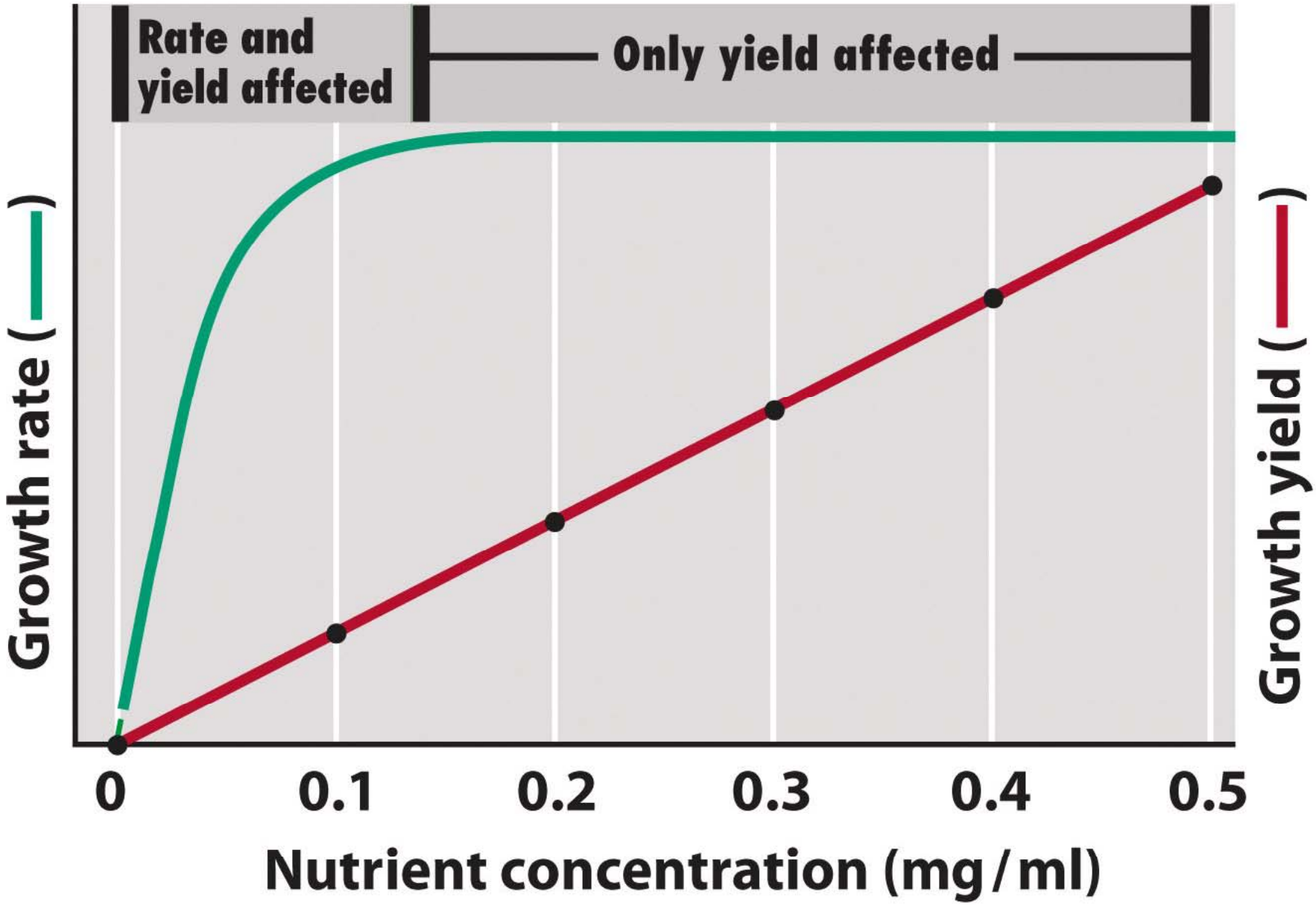
F = flow rate

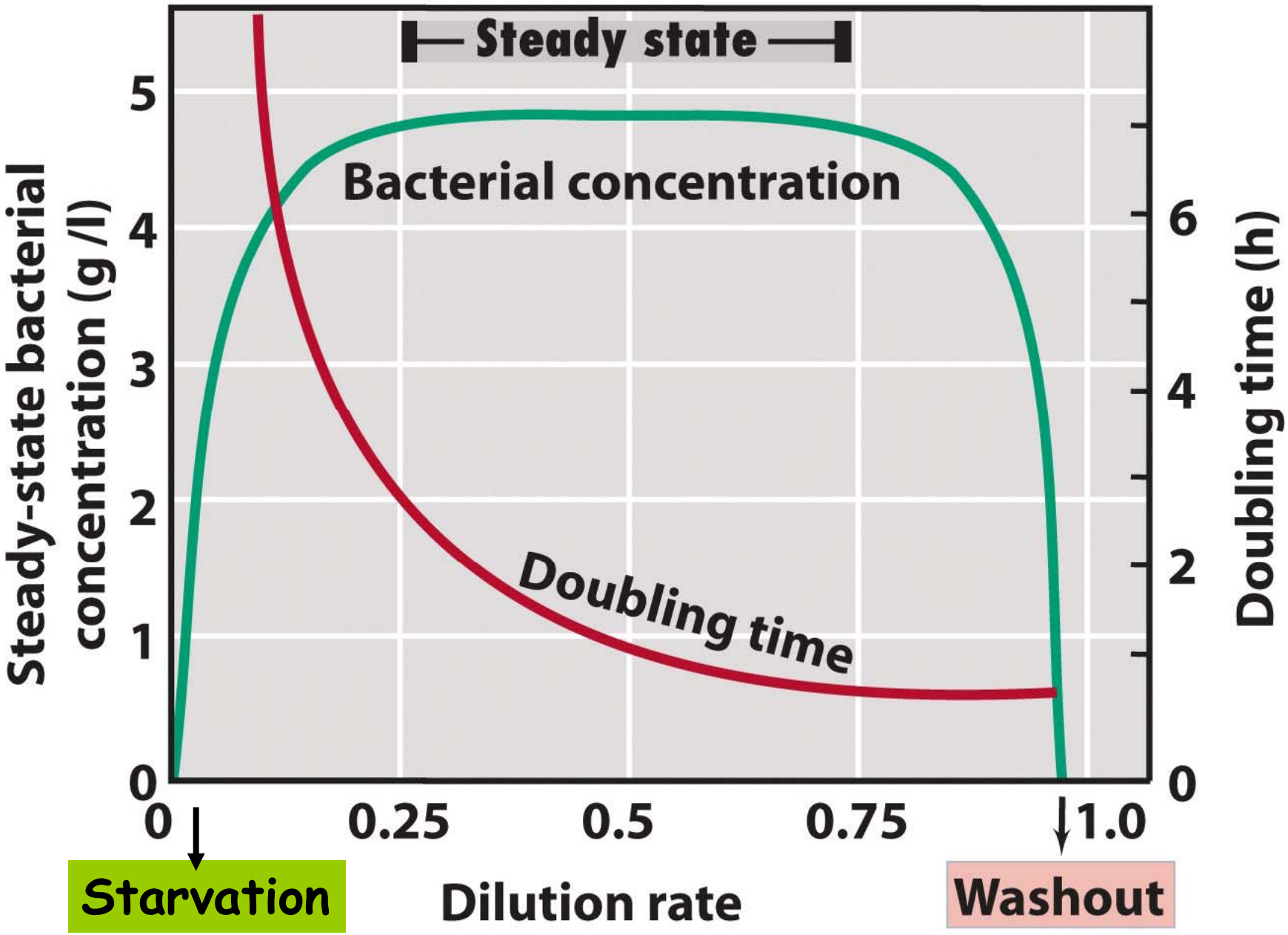
V = volume

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

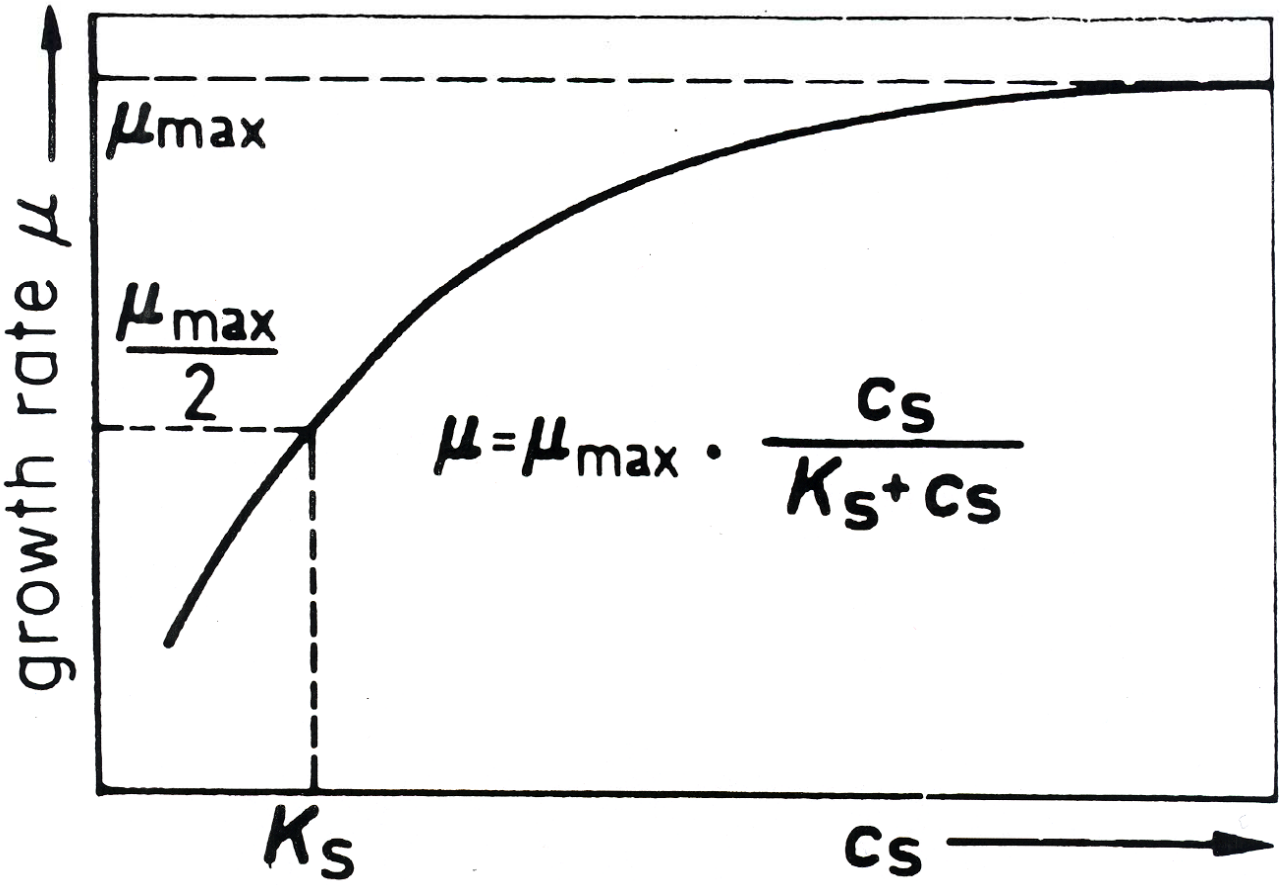


Fig. 6.10 *Dependence of growth rate* μ *on the substrate concentration* c_S.

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

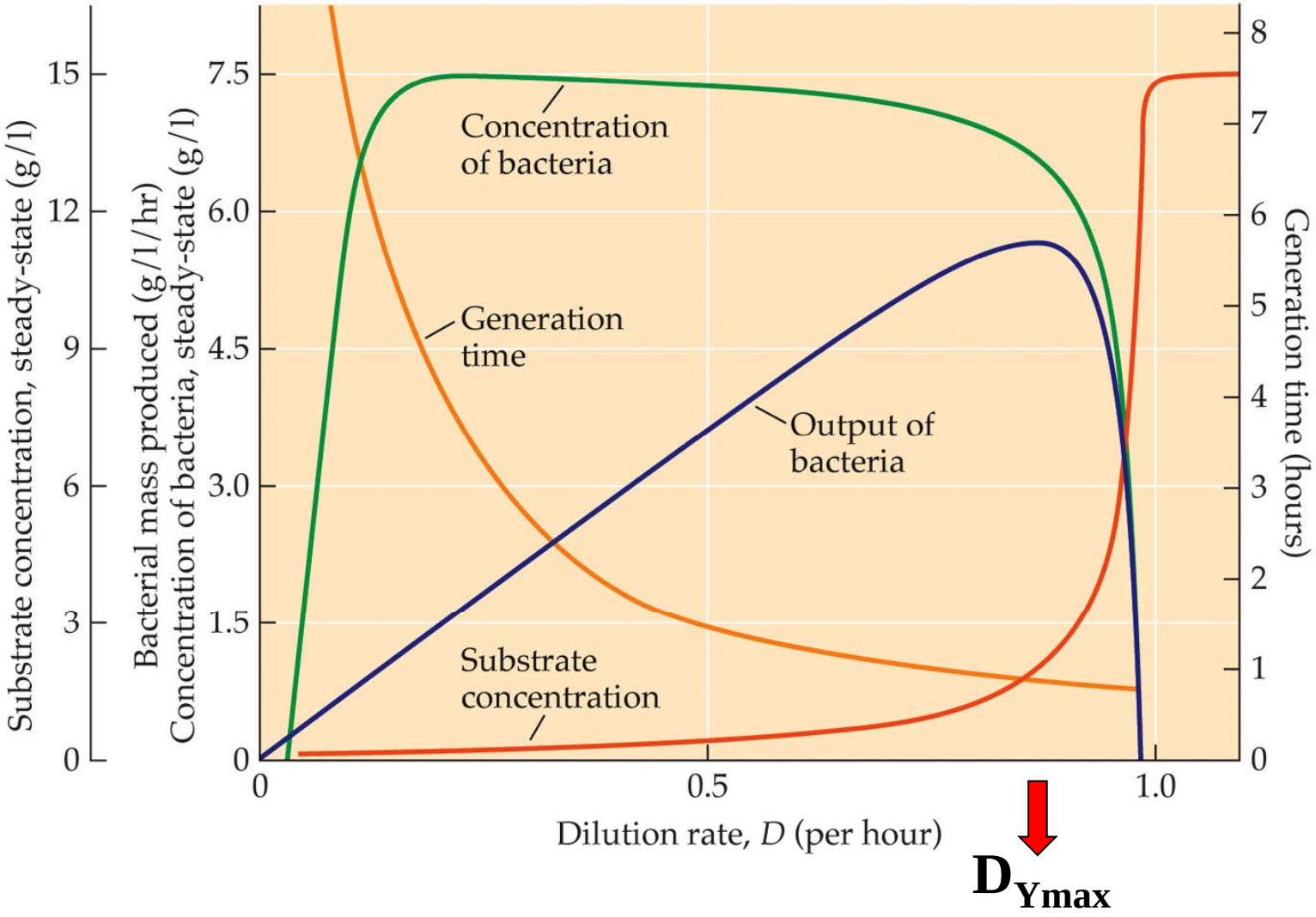


Table 6.2 Growth yields of anaerobic bacteria utilizing glucose as the energy source

	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).