Chapter 6

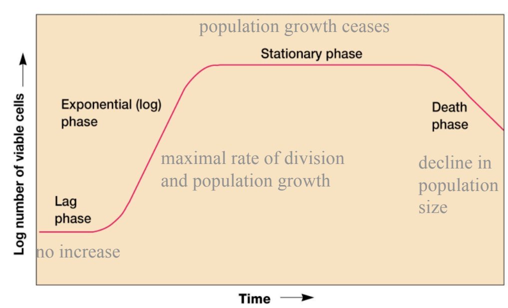
Microbial Growth

Growth

- ✓ increase in cellular constituents that may result in:
  - ✑increase in cell number
    - ⇨e.g., when microorganisms reproduce by budding or binary fission
  - ✑increase in cell size
    - ⇨e.g., coenocytic microorganisms have nuclear divisions that are not accompanied by cell divisions
- ✓ microbiologists usually study population growth rather than growth of individual cells

The Growth Curve

- ✓ microorganisms are cultivated in batch culture
  - ✑incubated in a closed vessel with a single batch of medium
- ✓ plotted as logarithm of cell number versus time
- ✓ four distinct phases



Lag Phase

- ✓ cell synthesizing new components
  - ✑e.g., to replenish spent materials
  - ✑e.g., to adapt to new medium or other conditions
  - ✑Synthesize enzymes
- ✓ varies in length
  - ✑can be very short or even absent

Exponential/Log Phase

- ✓ rate of growth is constant
- ✓ population is most uniform in terms of chemical and physical properties during this phase
- ✓ Balanced growth
  - ✑Shift up- poor medium to rich medium
  - ✑Shift down - rich medium to poor medium

Effect of nutrient concentration on growth

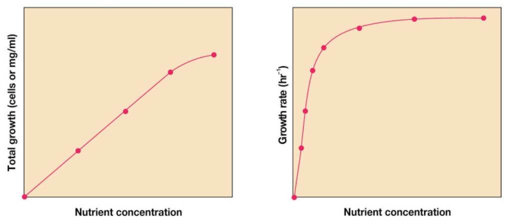


Table 6.1 An Example of Exponential Growth

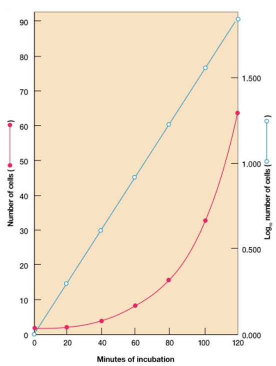
Time[a]	Division Number	2^n	Population ($N_0 \times 2^n$)	$\log_{10} N_t$
0	0	$2^0 = 1$	1	0.000
20	1	$2^1 = 2$	2	0.301
40	2	$2^2 = 4$	4	0.602
60	3	$2^3 = 8$	8	0.903
80	4	$2^4 = 16$	16	1.204
100	5	$2^5 = 32$	32	1.505
120	6	$2^6 = 64$	64	1.806

[a]The hypothetical culture begins with one cell having a 20-minute generation time.

cells are dividing and doubling in number at regular intervals

each individual cell divides at a slightly different time

curve rises smoothly rather than as discrete steps



Balanced growth

- ✓ log phase, cells exhibit balanced growth
  - cellular constituents manufactured at constant rates relative to each other

Unbalanced growth

- ✓ rates of synthesis of cell components vary relative to each other
- ✓ occurs under a variety of conditions
  - change in nutrient levels
    - shift-up (poor medium to rich medium)
    - shift-down (rich medium to poor medium)
  - change in environmental conditions

Stationary Phase

- ✓ total number of viable cells remains constant
  - 10^9 for bacteria or 10^6 for protozoa
  - may occur because metabolically active cells stop reproducing
  - may occur because reproductive rate is balanced by death rate

Possible reasons for entry into stationary phase

- ✓ nutrient limitation
- ✓ limited oxygen availability
- ✓ toxic waste accumulation
- ✓ critical population density reached

Starvation responses

- ✓ morphological changes
  - e.g., endospore formation
- ✓ decrease in size, protoplast shrinkage, and nucleoid condensation
- ✓ production of starvation proteins
- ✓ long term survival
- ✓ increased virulence

Death Phase

- ✓ cells dying at exponential rate
- ✓ death
  - irreversible loss of ability to reproduce
- ✓ in some cases, death rate slows due to accumulation of resistant cells

The Mathematics of Growth

- ✓ generation (doubling) time
  - time required for the population to double in size
- ✓ mean growth rate constant
  - number of generations per unit time
  - usually expressed as generations per hour

Mathamatics

- ✓ N_o = initial population number
- ✓ N_t = population at time t
- ✓ N = the number of generations in time t
- ✓ k = mean growth rate constant
- ✓ g = mean generation or doubling time

$$n = \frac{\log N_t - \log N_o}{\log 2}$$

$$k = \frac{n}{t} = \frac{\log N_t - \log N_o}{0.301t} \qquad g = \frac{1}{k}$$

- ✓ $N_o = 10^2$
- ✓ $N_t = 10^9$
- ✓ t = 10 hr

$$k = \frac{9-2}{0.301 * 10hr} = 2.3 generations/hr$$

$$g = \frac{1}{2.3 gen/hr} = .43 hr/gen = 26 min/gen$$

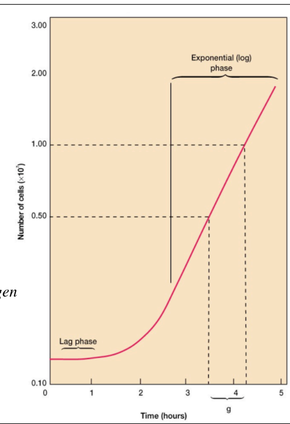


Table 6.2 Generation Times for Selected Microorganisms

Microorganism	Temperature (°C)	Generation Time (Hours)
Bacteria		
Beneckea natriegens	37	0.16
Escherichia coli	40	0.35
Bacillus subtilis	40	0.43
Staphylococcus aureus	37	0.47
Pseudomonas aeruginosa	37	0.58
Clostridium botulinum	37	0.58
Rhodospirillum rubrum	25	4.6–5.3
Anabaena cylindrica	25	10.6
Mycobacterium tuberculosis	37	≈12
Treponema pallidum	37	33
Algae		
Scenedesmus quadricauda	25	5.9
Chlorella pyrenoidosa	25	7.75
Asterionella formosa	20	9.6
Euglena gracilis	25	10.9
Ceratium tripos	20	82.8
Protozoa		
Tetrahymena geleii	24	2.2–4.2
Leishmania donovani	26	10–12
Paramecium caudatum	26	10.4
Acanthamoeba castellanii	30	11–12
Giardia lamblia	37	18
Fungi		
Saccharomyces cerevisiae	30	2
Monilinia fraa	25	30

Measurement of Microbial Growth

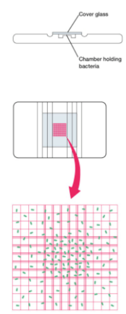
- ✓ can measure changes in number of cells in a population
- ✓ can measure changes in mass of population

Measurement of Cell Numbers

- ✓ Direct cell counts
  - ✑counting chambers
  - ✑electronic counters
  - ✑on membrane filters
- ✓ Viable cell counts
  - ✑plating methods
  - ✑membrane filtration methods

Counting chambers

- ✓ easy, inexpensive, and quick
- ✓ useful for counting both eucaryotes and procaryotes
- ✓ cannot distinguish living from dead cells



Electronic counters

- ✓ microbial suspension forced through small orifice
- ✓ movement of microbe through orifice impacts electric current that flows through orifice
- ✓ instances of disruption of current are counted

Electronic counters…

- ✓ cannot distinguish living from dead cells
- ✓ quick and easy to use
- ✓ useful for large microorganisms and blood cells, but not procaryotes

Direct counts on membrane filters

- ✓ 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

Plating methods

- ✓ measure number of viable cells
- ✓ population size is expressed as colony forming units (CFU)

plate dilutions of population on suitable solid medium

↓

count number of colonies

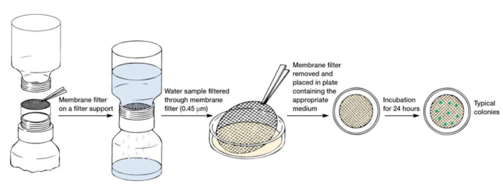
↓

calculate number of cells in population

Plating methods…

- ✓ simple and sensitive
- ✓ widely used for viable counts
  - ✑food, water, and soil
- ✓ inaccurate results
  - ✑cells clump together
  - ✑Dead cells

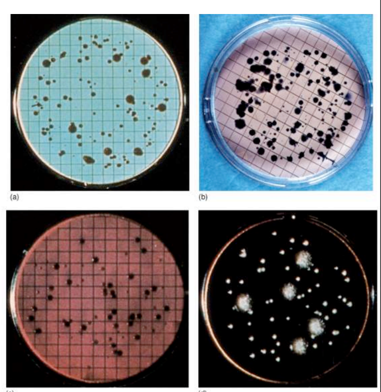
Membrane filtration methods



especially useful for analyzing aquatic samples

Colonies on membranes

a. Standard methods agar
b. Fecal coliform medium
c. m-endo medium
d. Wort agar



Measurement of Cell Mass

- ✓ dry weight
  - ✑time consuming and not very sensitive
- ✓ quantity of a particular cell constituent
  - ✑e.g., protein, DNA, ATP, or chlorophyll
  - ✑useful if amount of substance in each cell is constant
- ✓ turbidometric measures (light scattering)
  - ✑quick, easy, and sensitive

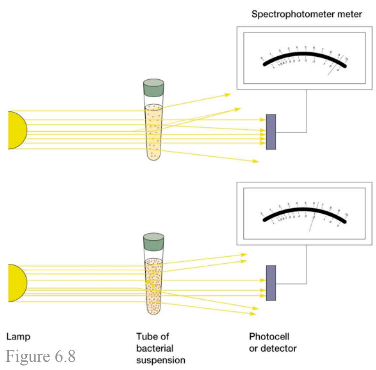


Figure 6.8

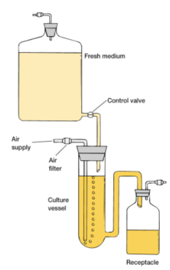
more cells
↓
more light scattered
↓
less light detected

The Continuous Culture of Microorganisms

- ✓ growth in an open system
  - continual provision of nutrients
  - continual removal of wastes
- ✓ maintains cells in log phase
  - constant biomass concentration for extended periods
- ✓ achieved using a continuous culture system

The Chemostat

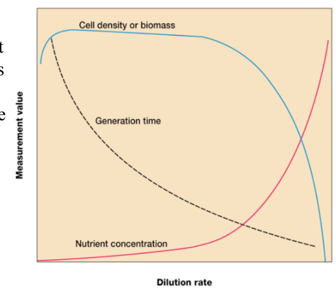
- ✓ rate of incoming medium = rate of removal of medium from vessel
- ✓ an essential nutrient is in limiting quantities



Dilution rate and microbial growth

dilution rate – rate at which medium flows through vessel relative to vessel size

note: cell density maintained at wide range of dilution rates and chemostat operates best at low dilution rate



The Turbidostat

- ✓ regulates the flow rate of media through vessel to maintain a predetermined turbidity or cell density
- ✓ dilution rate varies
- ✓ no limiting nutrient
- ✓ turbidostat operates best at high dilution rates

Importance of continuous culture methods

- ✓ constant supply of cells in exponential phase growing at a known rate
- ✓ study of microbial growth at very low nutrient concentrations, close to those present in natural environment
- ✓ study of interactions of microbes under conditions resembling those in aquatic environments
- ✓ food and industrial microbiology

The Influence of Environmental Factors on Growth

- ✓ most organisms grow in fairly moderate environmental conditions
- ✓ extremophiles
  - grow under harsh conditions that would kill most other organisms

Solutes and Water Activity

- ✓ water activity (a_w)
  - amount of water available to organisms
  - reduced by interaction with solute molecules (osmotic effect)

    higher [solute] ⇒ lower a_w
  - reduced by adsorption to surfaces (matric effect)

$$a_w = \frac{P_{soln}}{P_{water}}$$

Table 6.4 Approximate Lower a_w Limits for Microbial Growth

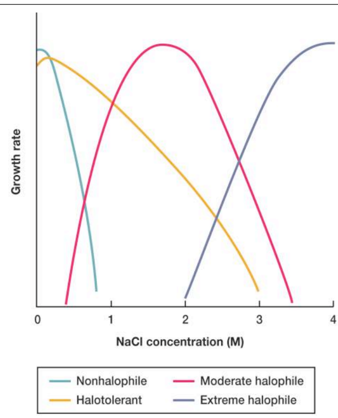
Water Activity	Environment	Bacteria	Fungi	Algae
1.00—Pure water	Blood Plant wilt Seawater	Most gram-negative nonhalophiles		
	Vegetables, meat, fruit			
0.95	Bread	Most gram-positive rods	*Basidiomycetes*	Most algae
0.90	Ham	Most cocci, *Bacillus*	*Fusarium* *Mucor, Rhizopus* Ascomycetous yeasts	
0.85	Salami	*Staphylococcus*	*Saccharomyces rouxii* (in salt)	
0.80	Preserves		*Penicillium*	
0.75	Salt lakes Salted fish	*Halobacterium* *Actinospora*	*Aspergillus*	*Dunaliella*
0.70			*Aspergillus*	
	Cereals,candy,dried fruit			
0.60			*Saccharomyces rouxii* (in sugars)	
	Chocolate Honey Dried milk		*Xeromyces bisporus*	
0.55—DNA disordered				

Adapted from A. D. Brown, "Microbial Water Stress," in *Bacteriological Reviews*,40(4):803–846 1976. Copyright ©1976 by the American Society for Microbiology. Reprinted by permission.

Osmotolerant organisms

- ✓ grow over wide ranges of water activity
- ✓ many use compatible solutes to increase their internal osmotic concentration
  - solutes that are compatible with metabolism and growth
- ✓ some have proteins and membranes that require high solute concentrations for stability and activity
- ✓ halophiles
  - require high levels of NaCl to grow

Salt on growth



pH

- ✓ negative logarithm of the hydrogen ion concentration

$$pH = -\log[H+]$$

pH	$[H^+]$ (Molarity)		Environmental examples	Microbial examples
0	10^{-0} (1.0)	Increasing acidity	Concentrated nitric acid	*Ferroplasma* *Picrophilus oshimae*
1	10^{-1}		Gastric contents, acid thermal springs	*Dunaliella acidophila*
2	10^{-2}		Lemon juice Acid mine drainage	*Cyanidium caldarium* *Thiobacillus thiooxidans* *Sulfolobus acidocaldarius*
3	10^{-3}		Vinegar, ginger ale Pineapple	
4	10^{-4}		Tomatoes, orange juice Very acid soil	
5	10^{-5}		Cheese, cabbage Bread	*Physarum polycephalum* *Acanthamoeba castellanii*
6	10^{-6}		Beef, chicken Rain water Milk Saliva	*Lactobacillus acidophilus* *E. coli, Pseudomonas aeruginosa,* *Euglena gracilis, Paramecium bursaria*
7	10^{-7}	Neutrality	Pure water Blood	*Staphyloccus aureus*
8	10^{-8}		Seawater	*Nitrosomonas* spp.
9	10^{-9}		Strongly alkaline soil Alkaline lakes	
10	10^{-10}		Soap	*Microcystis aeruginosa* *Bacillus alcalophilus*
11	10^{-11}		Household ammonia	
12	10^{-12}		Saturated calcium hydroxide solution	
13	10^{-13}		Bleach Drain opener	
14	10^{-14}	Increasing alkalinity		

pH

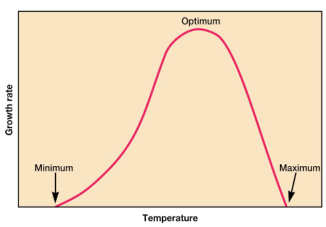
- ✓ acidophiles
  - growth optimum between pH 0 and pH 5.5
- ✓ neutrophiles
  - growth optimum between pH 5.5 and pH 7
- ✓ alkalophiles
  - growth optimum between pH 8.5 and pH 11.5

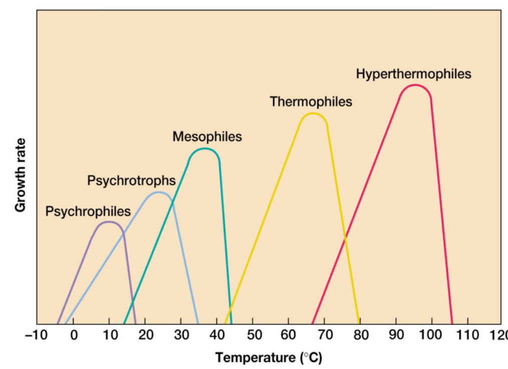
pH

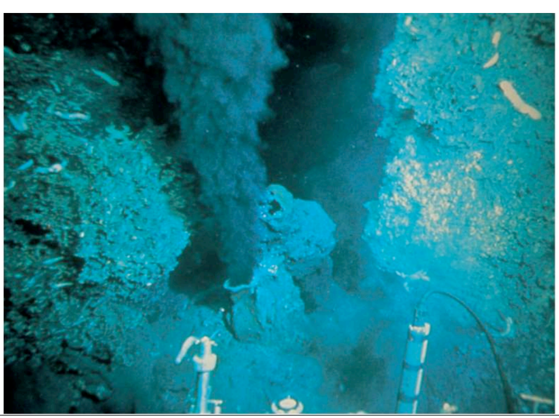
- ✓ most acidophiles and alkalophiles maintain an internal pH near neutrality
  - some use proton/ion exchange mechanisms to do so
- ✓ some synthesize proteins that provide protection
  - e.g., acid-shock proteins
- ✓ many microorganisms change pH of their habitat by producing acidic or basic waste products
  - most media contain buffers to prevent growth inhibition

Temperature

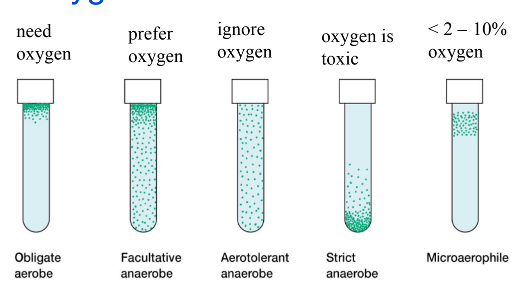
- ✓ organisms exhibit distinct cardinal growth temperatures
  - minimal
  - maximal
  - optimal





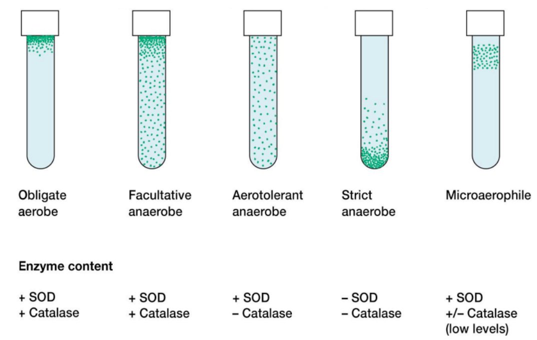


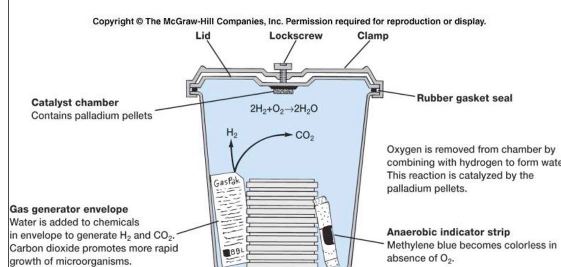
Oxygen Concentration



Basis of different oxygen sensitivities

- ✓ oxygen easily reduced to toxic products
  - ✑superoxide radical
  - ✑hydrogen peroxide
  - ✑hydroxyl radical
- ✓ aerobes produce protective enzymes
  - ✑superoxide dismutase (SOD)
  - ✑catalase

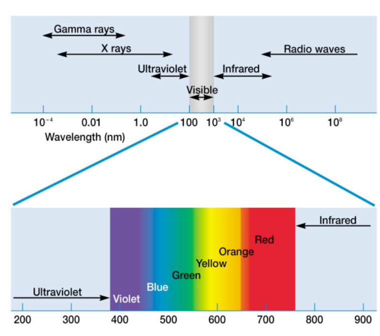




Pressure

- ✓ barotolerant organisms
  - ✑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

Radiation



Radiation damage

- ✓ ionizing radiation
  - x rays and gamma rays
  - Mutations → death
    - Disrupts chemical structure of many molecules, including DNA
    - Damage my be repaired by DNA repair mechanisms

Radiation damage…

- ✓ ultraviolet (UV) radiation
  - Mutation → death
  - Causes formation of thymine dimers in DNA
  - DNA damage can be repaired by two mechanisms
    - Photoreactivation - dimers split in presence of light
    - Dark reactivation - dimers excised and replaced in absence of light

Radiation damage…

- ✓ visible light
  - at high intensities generates singlet oxygen ($^1O_2$)
    - powerful oxidizing agent
  - carotenoid pigments
    - protect many light-exposed microorganisms from photooxidation

Microbial Growth in Natural Environments

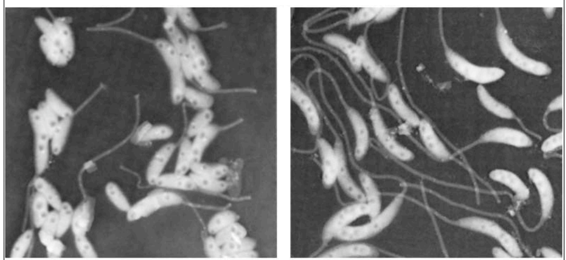
- ✓ microbial environments are complex, constantly changing, and may expose a microorganism to overlapping gradients of nutrients and environmental factors

Growth Limitation by Environmental Factors

- ✓ Leibig's law of the minimum
  - total biomass of organism determined by the nutrient present at lowest concentration
- ✓ Shelford's law of tolerance
  - above or below certain environmental limits, a microorganism will not grow, regardless of the nutrient supply

Responses to low nutrient levels

- ✓ oligotrophic environments
- ✓ morphological changes
  - increase surface area and ability to absorb nutrients
- ✓ mechanisms to sequester certain nutrients



(a) (b)

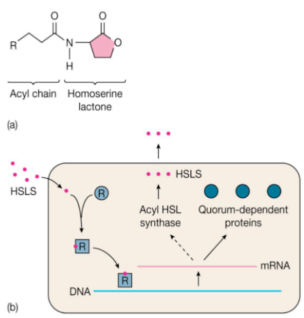
Caulobacter short stalks under nitrogen limiting conditions and long under phosphorus limiting conditions.

Counting Viable but Nonculturable Vegetative Procaryotes

- ✓ stressed microorganisms can temporarily lose ability to grow using normal cultivation methods
- ✓ microscopic and isotopic methods for counting viable but nonculturable cells have been developed
- ✓ Co-culture of cells

Quorum Sensing and Microbial Populations

- ✓ quorum sensing
  - ✑microbial communication and cooperation
  - ✑involves secretion and detection of chemical signals



Processes sensitive to quorum sensing: gram-negative bacteria

- ✓ bioluminescence (*Vibrio fischeri*)
- ✓ synthesis and release of virulence factors (*Pseudomonas aeruginosa*)
- ✓ conjugation (*Agrobacterium tumefaciens*)
- ✓ antibiotic production (*Erwinia carotovora, Pseudomonas aureofaciens*)
- ✓ biofilm production (*P. aeruginosa*)

Quorum sensing: gram-positive bacteria

- ✓ mediated by oligopeptide pheromone
- ✓ processes impacted by quorum sensing:
  - ✑mating (*Enterococcus faecalis*)
  - ✑transformation competence (*Streptococcus pneumoniae*)
  - ✑sporulation (*Bacillus subtilis*)
  - ✑production of virulence factors (*Staphylococcus aureus*)
  - ✑development of aerial mycelia (*Streptomyces griseus*)
  - ✑antibiotic production (*S. griseus*)