Chapter 5 & 6

Microbial Nutrition and Growth

The Common Nutrient Requirements

- ✓ macroelements (macronutrients)
  - ✒ C, O, H, N, S, P, K, Ca, Mg, and Fe
  - ✒ required in relatively large amounts
- ✓ micronutrients (trace elements)
  - ✒ Mn, Zn, Co, Mo, Ni, and Cu
  - ✒ required in trace amounts
  - ✒ often supplied in water or in media components

Requirements for Carbon, Hydrogen, and Oxygen

- ✓ often satisfied together
- ✓ autotrophs
  - ✒ use carbon dioxide as their sole or principal carbon source
- ✓ heterotrophs
  - ✒ use organic molecules as carbon sources

Nutritional Types of Microorganisms

Table 5.1 Sources of Carbon, Energy, and Electrons

Carbon Sources	
Autotrophs	CO_2 sole or principal biosynthetic carbon source (*pp. 207–8*)[a]
Heterotrophs	Reduced, preformed, organic molecules from other organisms (*chapters 9 and 10*)
Energy Sources	
Phototrophs	Light (*pp. 195–201*)
Chemotrophs	Oxidation of organic or inorganic compounds (*chapter 9*)
Electron Sources	
Lithotrophs	Reduced inorganic molecules (*pp. 193–94*)
Organotrophs	Organic molecules (*chapter 9*)

[a]For each category, the location of material describing the participating metabolic pathways is given within the parentheses.

Table 5.2 Major Nutritional Types of Microorganisms

Major Nutritional Types[a]	Sources of Energy, Hydrogen/Electrons, and Carbon	Representative Microorganisms
Photolithotrophic autotrophy (Photolithoautotrophy)	Light energy Inorganic hydrogen/electron (H/e^-) donor CO_2 carbon source	Algae Purple and green sulfur bacteria Cyanobacteria
Photoorganotrophic heterotrophy (Photoorganoheterotrophy)	Light energy Organic H/e^- donor Organic carbon source (CO_2 may also be used)	Purple nonsulfur bacteria Green nonsulfur bacteria
Chemolithotrophic autotrophy (Chemolithoautotrophy)	Chemical energy source (inorganic) Inorganic H/e^- donor CO_2 carbon source	Sulfur-oxidizing bacteria Hydrogen bacteria Nitrifying bacteria Iron-oxidizing bacteria
Chemoorganotrophic heterotrophy (Chemoorganoheterotrophy)	Chemical energy source (organic) Organic H/e^- donor Organic carbon source	Protozoa Fungi Most nonphotosynthetic bacteria (including most pathogens)

[a]Bacteria in other nutritional categories have been found. The categories are defined in terms of energy, electron, and carbon sources. Condensed versions of these names are given in parentheses.

Mixotrophy (i.e. Purple non-sulfur bacteria)

- •chemical energy source (inorganic)
- •inorganic H/e^- donor
- •organic carbon source

Requirements for Nitrogen, Phosphorus, and Sulfur

- ✓ needed for synthesis of important molecules (e.g., amino acids, nucleic acids)
- ✓ nitrogen supplied in numerous ways
- ✓ phosphorus usually supplied as inorganic phosphate
- ✓ sulfur usually supplied as sulfate via assimilatory sulfate reduction

Sources of nitrogen

- ✓organic molecules
- ✓ammonia
- ✓nitrate via assimilatory nitrate reduction
- ✓nitrogen gas via nitrogen fixation

Growth Factors

- ✓organic compounds
- ✓essential cell components (or their precursors) that the cell cannot synthesize
- ✓must be supplied by environment if cell is to survive and reproduce

Classes of growth factors

- ✓amino acids
  - ✒needed for protein synthesis
- ✓purines and pyrimidines
  - ✒needed for nucleic acid synthesis
- ✓vitamins
  - ✒function as enzyme cofactors

Table 5.3 Functions of Some Common Vitamins in Microorganisms

Vitamin	Functions	Examples of Microorganisms Requiring Vitamin*
Biotin	Carboxylation (CO_2fixation) One-carbon metabolism	*Leuconostoc mesenteroides* (B) *Saccharomyces cerevisiae* (F) *Ochromonas malhamensis* (A) *Acanthamoeba castellanii* (P)
Cyanocobalamin (B_{12})	Molecular rearrangements One-carbon metabolism—carries methyl groups	*Lactobacillus* spp. (B) *Euglena gracilis* (A) Diatoms and many other algae (A) *Acanthamoeba castellanii* (P)
Folic acid	One-carbon metabolism	*Enterococcus faecalis* (B) *Tetrahymena pyriformis* (P)
Lipoic acid	Transfer of acyl groups	*Lactobacillus casei* (B)
Pantothenic acid	Precursor of coenzyme A—carries acyl groups (pyruvate oxidation, fatty acid metabolism)	*Tetrahymena*spp. (P) *Proteus morganii* (B) *Hanseniaspora*spp. (F) *Paramecium*spp. (P)
Pyridoxine (B_6)	Amino acid metabolism (e.g., transamination)	*Lactobacillus* spp. (B)
Niacin (nicotinic acid)	Precursor of NAD and NADP—carry electrons and hydrogen atoms	*Tetrahymena pyriformis* (P) *Brucella abortus,Haemophilus influenzae* (B)
Riboflavin (B_{12})	Precursor of FAD and FMN—carry electrons or hydrogen atoms	*Blastocladia pringsheimii* (F) *Crithidia fasciculata* (P) *Caulobacter vibrioides* (B) *Dictyostelium*spp. (F) *Tetrahymena pyriformis* (P)
Thiamine (B_1)	Aldehyde group transfer (pyruvate decarboxylation, α-keto acid oxidation)	*Bacillus anthracis* (B) *Phycomyces blakesleeanus* (F) *Ochromonas malhamensis* (A) *Colpidium campylum* (P)

*The representative microorganisms are members of the following groups: bacteria (B), fungi (F), algae (A), and protozoa (P).

Practical importance of growth factors

- ✓development of quantitative growth-response assays for measuring concentrations of growth factors in a preparation
- ✓industrial production of growth factors by microorganisms

Uptake of Nutrients by the Cell

Most common mechanisms are:

- ✒facilitated diffusion
- ✒active transport
- ✒group translocation

Facilitated Diffusion

✓similar to passive diffusion
- ✒movement of molecules is not energy dependent
- ✒direction of movement is from high concentration to low concentration
- ✒size of concentration gradient impacts rate of uptake

Facilitated diffusion…

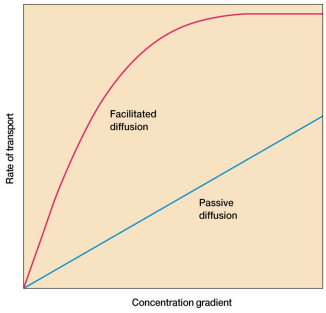
✓differs from passive diffusion
- ✒uses carrier molecules (permeases)
- ✒smaller concentration gradient is required for significant uptake of molecules
- ✒effectively transports glycerol, sugars, and amino acids

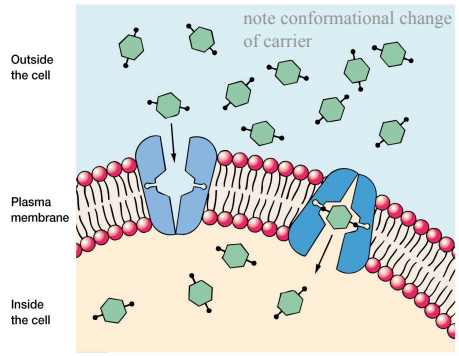
✓more prominent in eucaryotic cells than in procaryotic cells

•rate of facilitated diffusion increases more rapidly and at a lower concentration

•diffusion rate reaches a plateau when carrier becomes saturated

carrier saturation effect





Active Transport

✓energy-dependent process
- ✒ATP or proton motive force used

✓moves molecules against the gradient

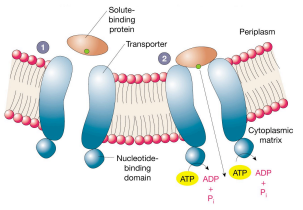
✓concentrates molecules inside cell

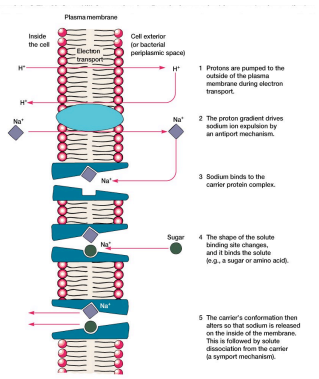
✓involves carrier proteins (permeases)
- ✒carrier saturation effect is observed

ABC transporters

✓ATP-binding cassette transporters

✓observed in bacteria, archaea, and eucaryotes



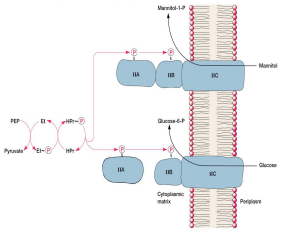


antiport

symport

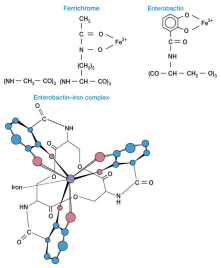
Group Translocation

- ✓ molecules are modified as they are transported across the membrane
- ✓ energy-dependent process



Iron Uptake

- ✓ ferric iron is very insoluble so uptake is difficult
- ✓ microorganisms use siderophores to aid uptake
- ✓ siderophore complexes with ferric ion
- ✓ complex is then transported into cell



Culture Media

- ✓ preparations devised to support the growth (reproduction) of microorganisms
- ✓ can be liquid or solid
  - ✒ solid media are usually solidified with agar
- ✓ important to study of microorganisms

Synthetic or Defined Media

- ✓ all components and their concentrations are known

Table 5.4 Examples of Defined Media

BG–11 Medium for Cyanobacteria	Amount (g/liter)
$NaNO_3$	1.5
$K_2HPO_4 \cdot 3H_2O$	0.04
$MgSO_4 \cdot 7H_2O$	0.075
$CaCl_2 \cdot 2H_2O$	0.036
Citric acid	0.006
Ferric ammonium citrate	0.006
EDTA (Na:Mg salt)	0.001
Na_2CO_3	0.02
Trace metal solution[a]	1.0 ml/liter
Final pH 7.4	

Medium for *Escherichia coli*	Amount (g/liter)
Glucose	1.0
Na_2HPO_4	16.4
KH_2PO_4	1.5
$(NH_4)_2SO_4$	2.0
$MgSO_4 \cdot 7H_2O$	200.0 mg
$CaCl_2$	10.0 mg
$FeSO_4 \cdot 7H_2O$	0.5 mg
Final pH 6.8–7.0	

Sources: Data from Rippka, et al. *Journal of General Microbiology*,111:1–61, 1979; and S.S. Cohen, and R. Arbogast, *Journal of Experimental Medicine*,91:619, 1950.
[a]The trace metal solution contains H_3BO_3, $MnCl_2 \cdot 4H_2O$, $ZnSO_4 \cdot 7H_2O$, $Na_2MoO_4 \cdot 2H_2O$, $CuSO_4 \cdot 5H_2O$, and $Co(NO_3)_2 \cdot 6H_2O$.

Complex Media

- ✓ contain some ingredients of unknown composition and/or concentration

Table 5.5 Some Common Complex Media

Nutrient Broth	Amount (g/liter)
Peptone (gelatin hydrolysate)	5
Beef extract	3
Tryptic Soy Broth	
Tryptone (pancreatic digest of casein)	17
Peptone (soybean digest)	3
Glucose	2.5
Sodium chloride	5
Dipotassium phosphate	2.5
MacConkey Agar	
Pancreatic digest of gelatin	17.0
Pancreatic digest of casein	1.5
Peptic digest of animal tissue	1.5
Lactose	10.0
Bile salts	1.5
Sodium chloride	5.0
Neutral red	0.03
Crystal violet	0.001
Agar	13.5

Some media components

- ✓ peptones
  - ✒ protein hydrolysates prepared by partial digestion of various protein sources
- ✓ extracts
  - ✒ aqueous extracts, usually of beef or yeast
- ✓ agar
  - ✒ sulfated polysaccharide used to solidify liquid media

Types of Media

- ✓ general purpose media
  - ✒ support the growth of many microorganisms
  - ✒ e.g., tryptic soy agar
- ✓ enriched media
  - ✒ general purpose media supplemented by blood or other special nutrients
  - ✒ e.g., blood agar

Types of media…

- ✓ selective media
  - ✒ favor the growth of some microorganisms and inhibit growth of others
  - ✒ e.g., MacConkey agar
    - ☞ selects for gram-negative bacteria

Types of media…

- ✓ differential media
  - ✒ distinguish between different groups of microorganisms based on their biological characteristics
  - ✒ e.g., blood agar
    - ☞ hemolytic versus nonhemolytic bacteria
  - ✒ e.g., MacConkey agar
    - ☞ lactose fermenters versus nonfermenters

Isolation of Pure Cultures

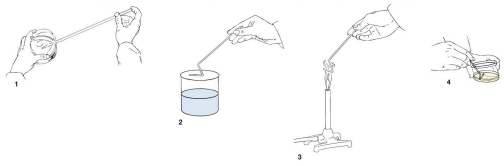
- ✓ pure culture
  - ✒ population of cells arising from a single cell
- ✓ spread plate, streak plate, and pour plate are techniques used to isolate pure cultures

The Spread Plate and Streak Plate

- ✓ involve spreading a mixture of cells on an agar surface so that individual cells are well separated from each other
- ✓ each cell can reproduce to form a separate colony (visible growth or cluster of microorganisms)

1. dispense cells onto medium in petri dish

4. spread cells across surface



2. - 3. sterilize spreader

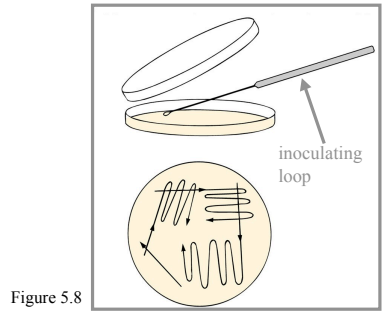


Figure 5.8

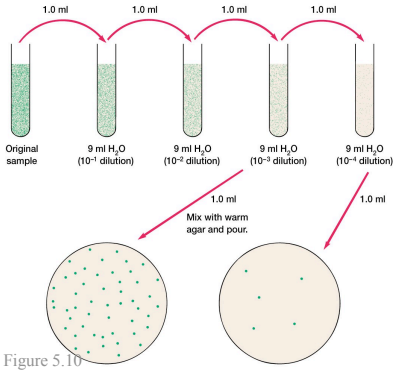
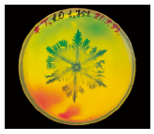
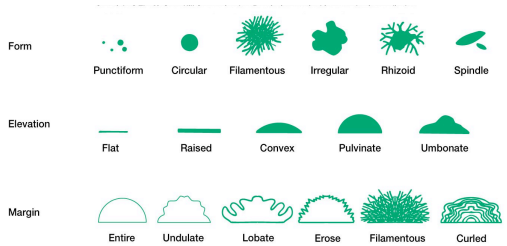


Figure 5.10

Colony Morphology and Growth

✓ individual species form characteristic colonies





Colony growth

✓ most rapid at edge of colony

- oxygen and nutrients are more available at edge

✓ slowest at center of colony

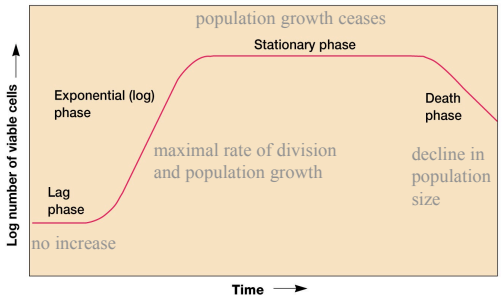
✓ in nature, many microorganisms form biofilms on surfaces

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

- ✓ observed when microorganisms are cultivated in batch culture
  - ✒ culture incubated in a closed vessel with a single batch of medium
- ✓ usually plotted as logarithm of cell number versus time
- ✓ usually has 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
  - ✒ in some cases 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

Table 6.1 An Example of Exponential Growth

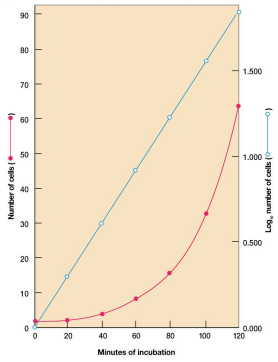
Time[a]	Division Number	2^n	Population ($N_0 \times 2^n$)	$\log_{10} N_t$
0	0	$2^0 = 1$	1	0.000
20	1	$2^1 = 2$	2	0.301
40	2	$2^2 = 4$	4	0.602
60	3	$2^3 = 8$	8	0.903
80	4	$2^4 = 16$	16	1.204
100	5	$2^5 = 32$	32	1.505
120	6	$2^6 = 64$	64	1.806

[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

✓during 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

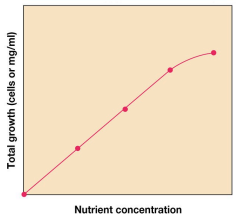
✒change in nutrient levels

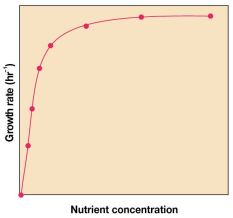
☞shift-up (poor medium to rich medium)

☞shift-down (rich medium to poor medium)

✒change in environmental conditions

Effect of nutrient concentration on growth





Stationary Phase

✓total number of viable cells remains constant

✒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

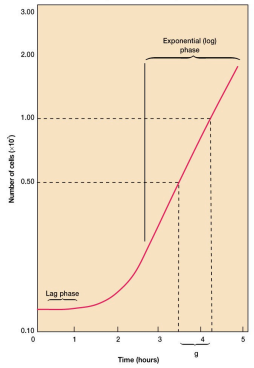


Table 6.2 Generation Times for Selected Microorganisms

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

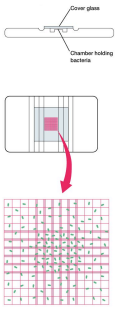
- ✓ can measure changes in number of cells in a population
- ✓ can measure changes in mass of population

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

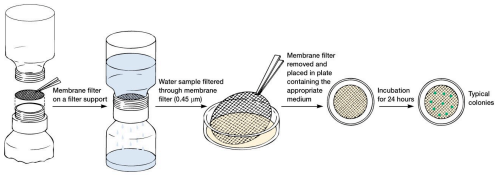
↓

calculate number of cells in population

Plating methods…

- ✓ simple and sensitive
- ✓ widely used for viable counts of microorganisms in food, water, and soil
- ✓ inaccurate results obtained if cells clump together

Membrane filtration methods



especially useful for analyzing aquatic samples

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

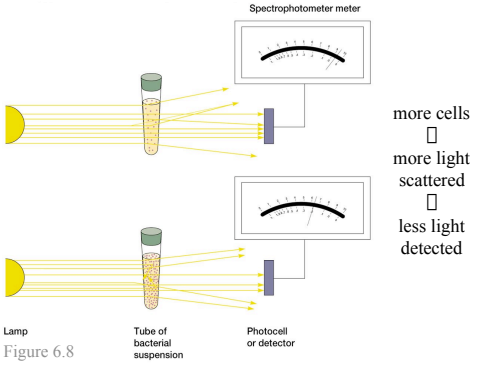


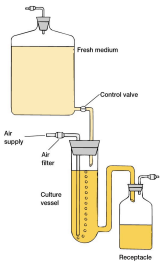
Figure 6.8

The Continuous Culture of Microorganisms

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

The Chemostat

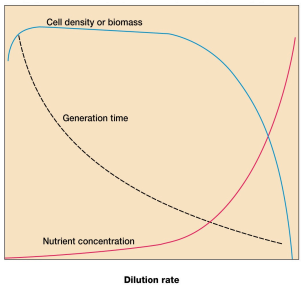
- ✓ rate of incoming medium = rate of removal of medium from vessel
- ✓ an essential nutrient is in limiting quantities



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)

Table 6.4 Approximate Lower a_w Limits for Microbial Growth

Water Activity	Environment	Bacteria	Fungi	Algae
1.00—Pure water	Blood Plant wilt { Vegetables, meat,fruit Seawater	Most gram-negative nonhalophiles		
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	Cereals,candy,dried fruit		*Aspergillus*	
0.60	Chocolate Honey Dried milk		*Saccharomyces rouxii* (in sugars) *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.

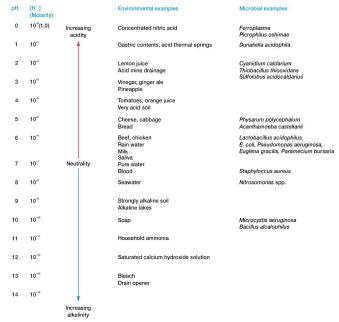
Osmotolerant organisms

- ✓ 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

pH

- ✓ negative logarithm of the hydrogen ion concentration

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



pH

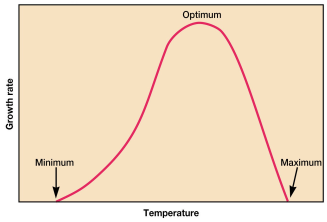
- ✓ acidophiles
  - ✒ growth optimum between pH 0 and pH 5.5
- ✓ neutrophiles
  - ✒ growth optimum between pH 5.5 and pH 7
- ✓ alkalophiles
  - ✒ growth optimum between pH 8.5 and pH 11.5

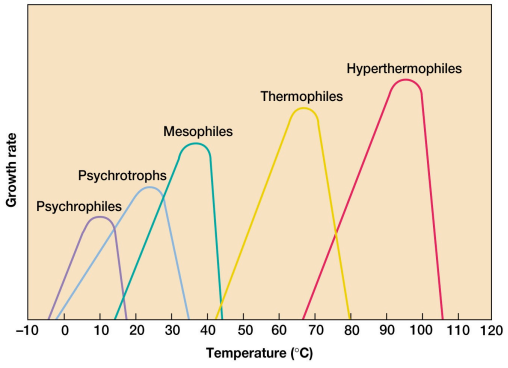
pH

- ✓ 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

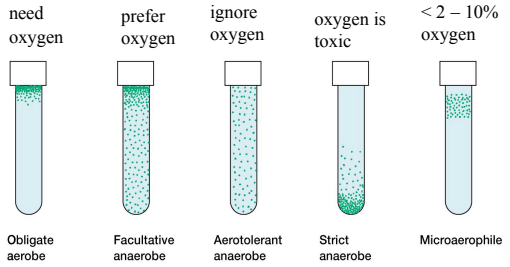
Temperature

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



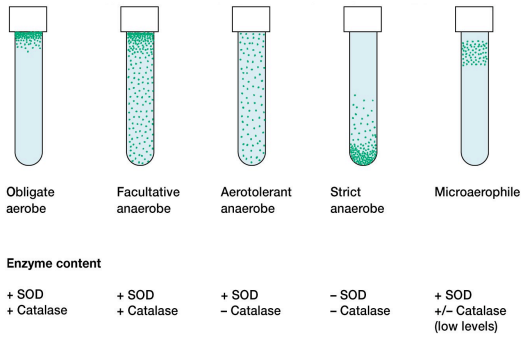


Oxygen Concentration



Basis of different oxygen sensitivities

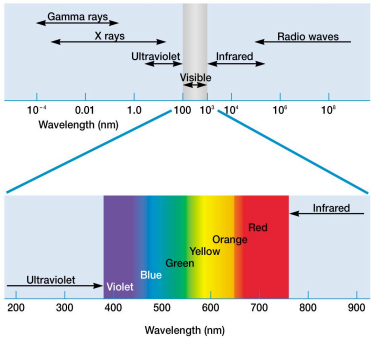
- ✓oxygen easily reduced to toxic products
  - ✒superoxide radical
  - ✒hydrogen peroxide
  - ✒hydroxyl radical
- ✓aerobes produce protective enzymes
  - ✒superoxide dismutase (SOD)
  - ✒catalase



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 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

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

Quorum Sensing and Microbial Populations

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