Microbial Nutrition and Growth

Microbial nutrition and growth
Overview

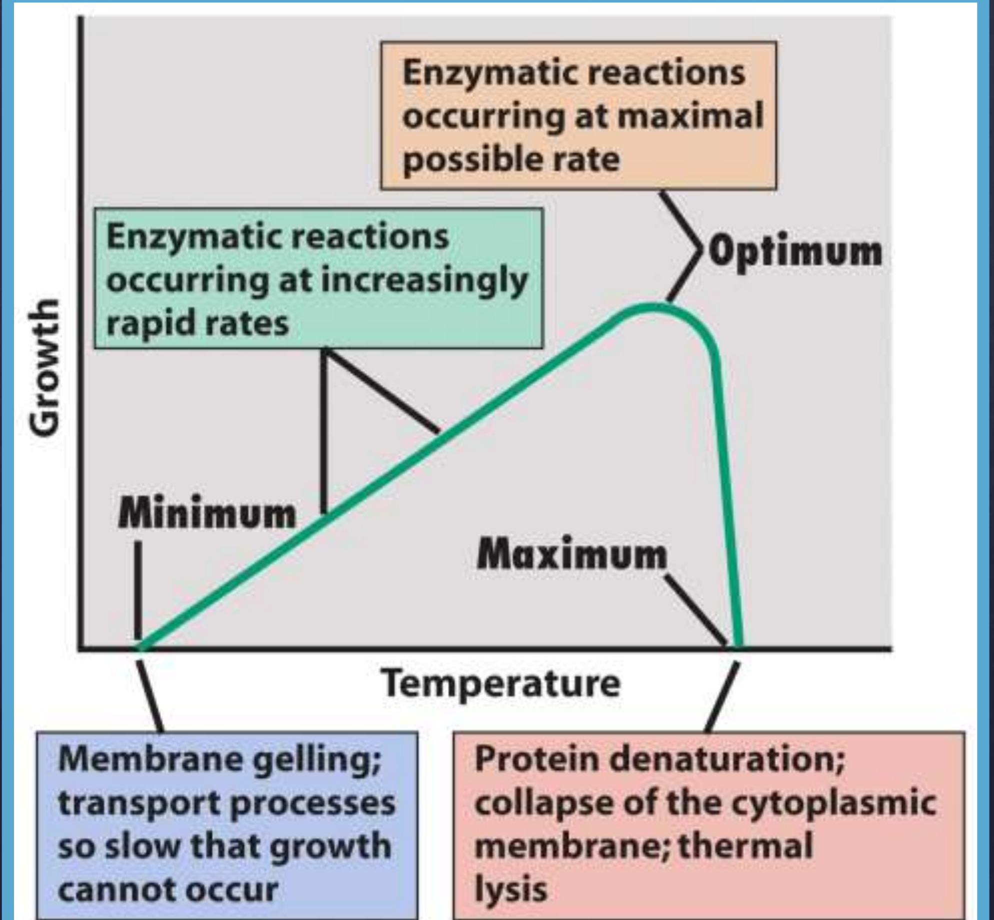
- Growth requirements and classification
- Physical parameters that effect growth and classification based on growth patterns
- Chemical parameters that effect growth and classification based on growth patterns
- Population growth -- growth curve
- Population growth -- Methods

Environmental Effects on Bacterial Growth

- Temperature
- pH
- Osmotic pressure
- Oxygen classes

Temperature and Microbial Growth

- **Cardinal temperatures**
  - minimum
  - optimum
  - maximum
- Temperature is a major environmental factor controlling microbial growth.



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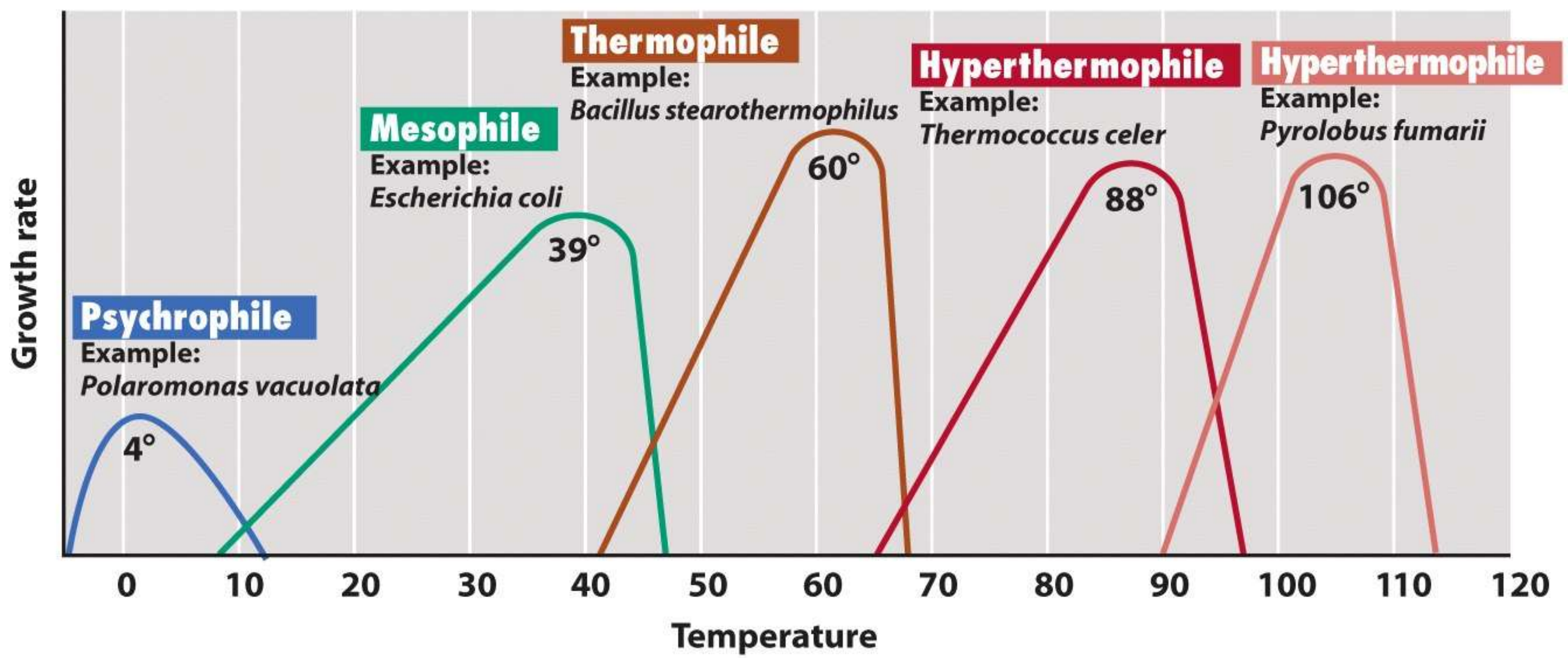
Temperature

- **Minimum Temperature**: Temperature below which growth ceases, or lowest temperature at which microbes will grow.
- **Optimum Temperature**: Temperature at which its growth rate is the fastest.
- **Maximum Temperature**: Temperature above which growth ceases, or highest temperature at which microbes will grow.

Classification of Microorganisms by Temperature Requirements



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Temperature Classes of Organisms

- **Mesophiles (20 – 45C)**
  - Midrange temperature optima
  - Found in warm-blooded animals and in terrestrial and aquatic environments in temperate and tropical latitudes
- **Psychrophiles (0-20C)**
  - Cold temperature optima
  - Most extreme representatives inhabit permanently cold environments
- **Thermophiles (50- 80C)**
  - Growth temperature optima between 45ºC and 80ºC
- **Hyperthermophiles**
  - Optima greater than 80°C
  - These organisms inhabit hot environments including boiling hot springs, as well as undersea hydrothermal vents that can have temperatures in excess of 100ºC

Table 6.5
Temperature Ranges for Microbial Growth

Microorganism	*Cardinal Temperatures (°C)* Minimum	Optimum	Maximum
Nonphotosynthetic Procaryotes			
Bacillus psychrophilus	−10	23–24	28–30
Micrococcus cryophilus	−4	10	24
Pseudomonas fluorescens	4	25–30	40
Staphylococcus aureus	6.5	30–37	46
Enterococcus faecalis	0	37	44
Escherichia coli	10	37	45
Neisseria gonorrhoeae	30	35–36	38
Thermoplasma acidophilum	45	59	62
Bacillus stearothermophilus	30	60–65	75
Thermus aquaticus	40	70–72	79
Sulfolobus acidocaldarius	60	80	85
Pyrococcus abyssi	67	96	102
Pyrodictium occultum	82	105	110
Pyrolobus fumarii	90	106	113
Photosynthetic Bacteria			
Rhodospirillum rubrum	ND[a]	30–35	ND
Anabaena variabilis	ND	35	ND
Oscillatoria tenuis	ND	ND	45–47
Synechococcus eximius	70	79	84

Table 6.1 Presently known upper temperature limits for growth of living organisms

Group	Upper temperature limits (°C)
Animals	
Fish and other aquatic vertebrates	38
Insects	45–50
Ostracods (crustaceans)	49–50
Plants	
Vascular plants	45
Mosses	50
Eukaryotic microorganisms	
Protozoa	56
Algae	55–60
Fungi	60–62
Prokaryotes	
Bacteria	
Cyanobacteria	70–74
Anoxygenic phototrophs	70–73
Chemoorganotrophic/chemolithotrophic *Bacteria*	95
Archaea	
Chemoorganotrophic/chemolithotrophic *Archaea*	113[a]

[a] The upper temperature limit for growth of the organism *Pyrolobus fumarii*. Related species of *Pyrodictium* may be able to grow up to as high as 121°C.

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pH and Microbial Growth

pH – measure of [H^+]

each organism has a pH range and a pH optimum

acidophiles – optimum in pH range 1-4
alkalophiles – optimum in pH range 8.5-11

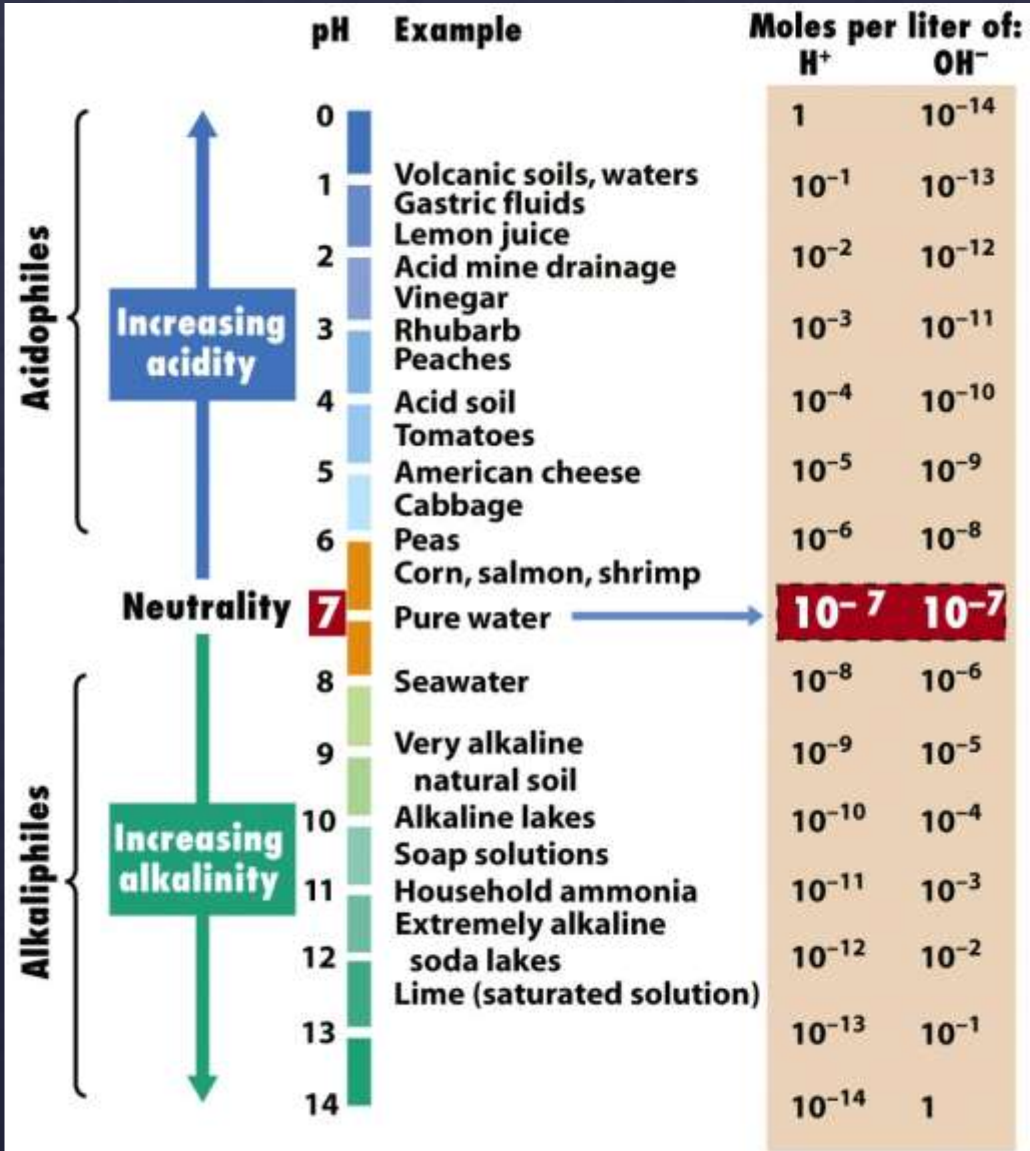
lactic acid bacteria – 4-7
***Thiobacillus thiooxidans* – 2.2-2.8**
fungi – 4-6

internal pH regulated by BUFFERS and near neutral adjusted with ion pumps

Human blood and tissues has pH 7.2$\pm$0.2

pH and Microbial Growth

- The acidity or alkalinity of an environment can greatly affect microbial growth.
- Most organisms grow best between pH 6 and 8, but some organisms have evolved to grow best at low or high pH. The internal pH of a cell must stay relatively close to neutral even though the external pH is highly acidic or basic.
  - **Acidophiles :** organisms that grow best at low pH (*Helicobacter pylori*, ***Thiobacillus thiooxidans***)
  - **Alkaliphiles** : organismsa that grow best at high pH (*Vibrio cholera*)
  - Most of pathogenic bacteria are neutrophiles



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Osmotic Effects on Microbial Growth

- Osmotic pressure depends on the surrounding solute concentration and water availability
- Water availability is generally expressed in physical terms such as water activity (a_w)
- Water activity is the ratio of the vapor pressure of the air in equilibrium with a substance or solution to the vapor pressure of pure water (aw 1.00).

$$a_w = \frac{P\ solu}{P\ water}$$

Environmental factors and growth

1. Osmotic Effect and water activity

organisms which thrive in high solute – osmophiles
organisms which tolerate high solute – osmotolerant

organisms which thrive in high salt – halophiles
organisms which tolerate high salt – halotolerant

organisms which thrive in high pressure – barophiles
organisms which tolerate high pressure – barotolerant

Table 6.2 Water activity of several substances

Water activity (a_w)	Material	Example organisms[a]
1.000	Pure water	*Caulobacter, Spirillum*
0.995	Human blood	*Streptococcus, Escherichia*
0.980	Seawater	*Pseudomonas, Vibrio*
0.950	Bread	Most gram-positive rods
0.900	Maple syrup, ham	Gram-positive cocci such as *Staphylococcus*
0.850	Salami	*Saccharomyces rouxii* (yeast)
0.800	Fruit cake, jams	*Saccharomyces bailii, Penicillium* (fungus)
0.750	Salt lakes, salted fish	*Halobacterium, Halococcus*
0.700	Cereals, candy, dried fruit	*Xeromyces bisporus* and other xerophilic fungi

[a] Selected examples of prokaryotes or fungi capable of growth in culture media adjusted to the stated water activity.

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Halophiles and Related Organisms

- In nature, osmotic effects are of interest mainly in habitats with high salt environments that have reduced water availability
- **Halophiles** : have evolved to grow best at reduced water potential, and some (**extreme halophiles e.g.** ***Halobacterium*****,** ***Dunaliella***) even require high levels of salts for growth.
- **Halotolerant** : can tolerate some reduction in the water activity of their environment but generally grow best in the absence of the added solute

- **Xerophiles** : are able to grow in very dry environments

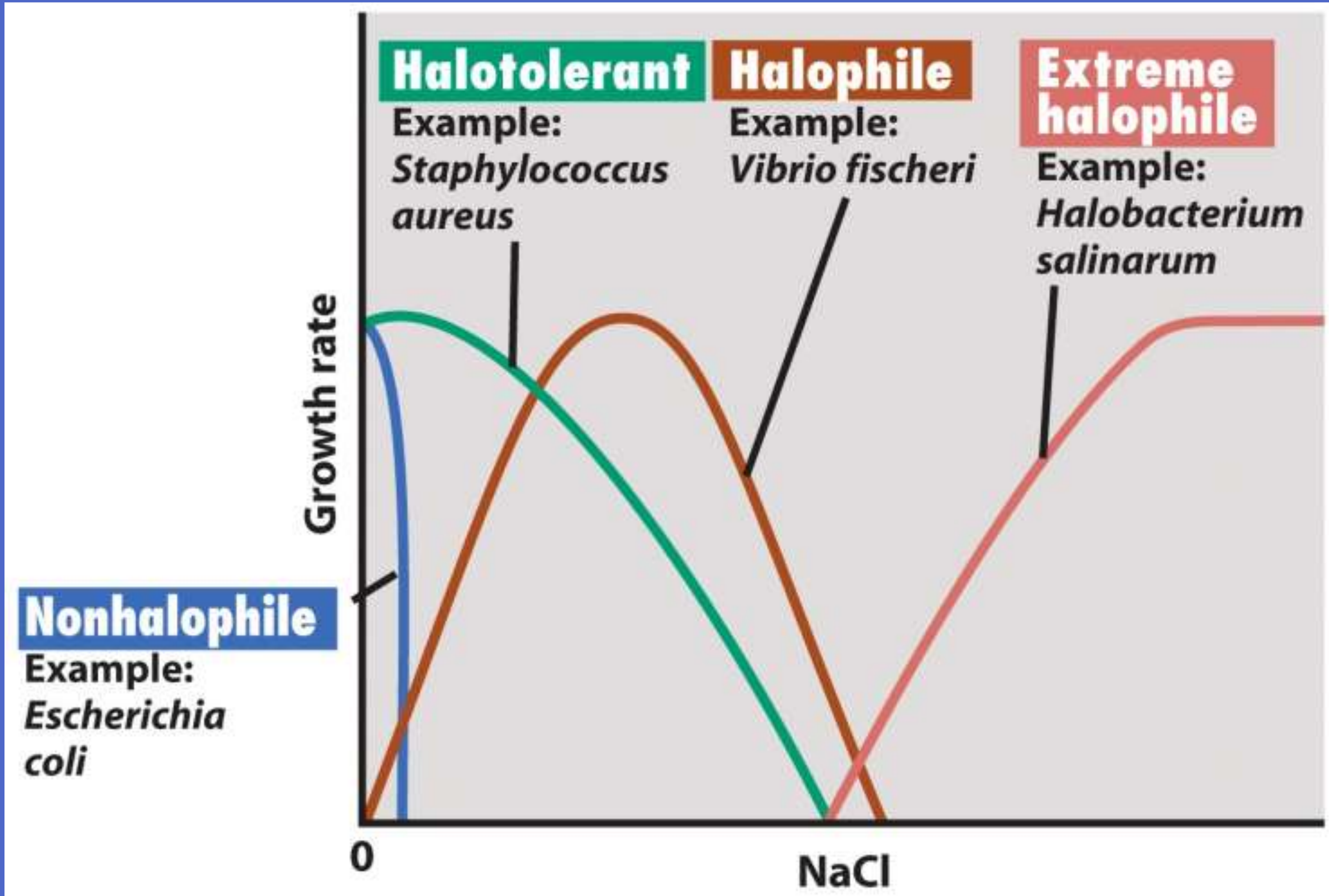


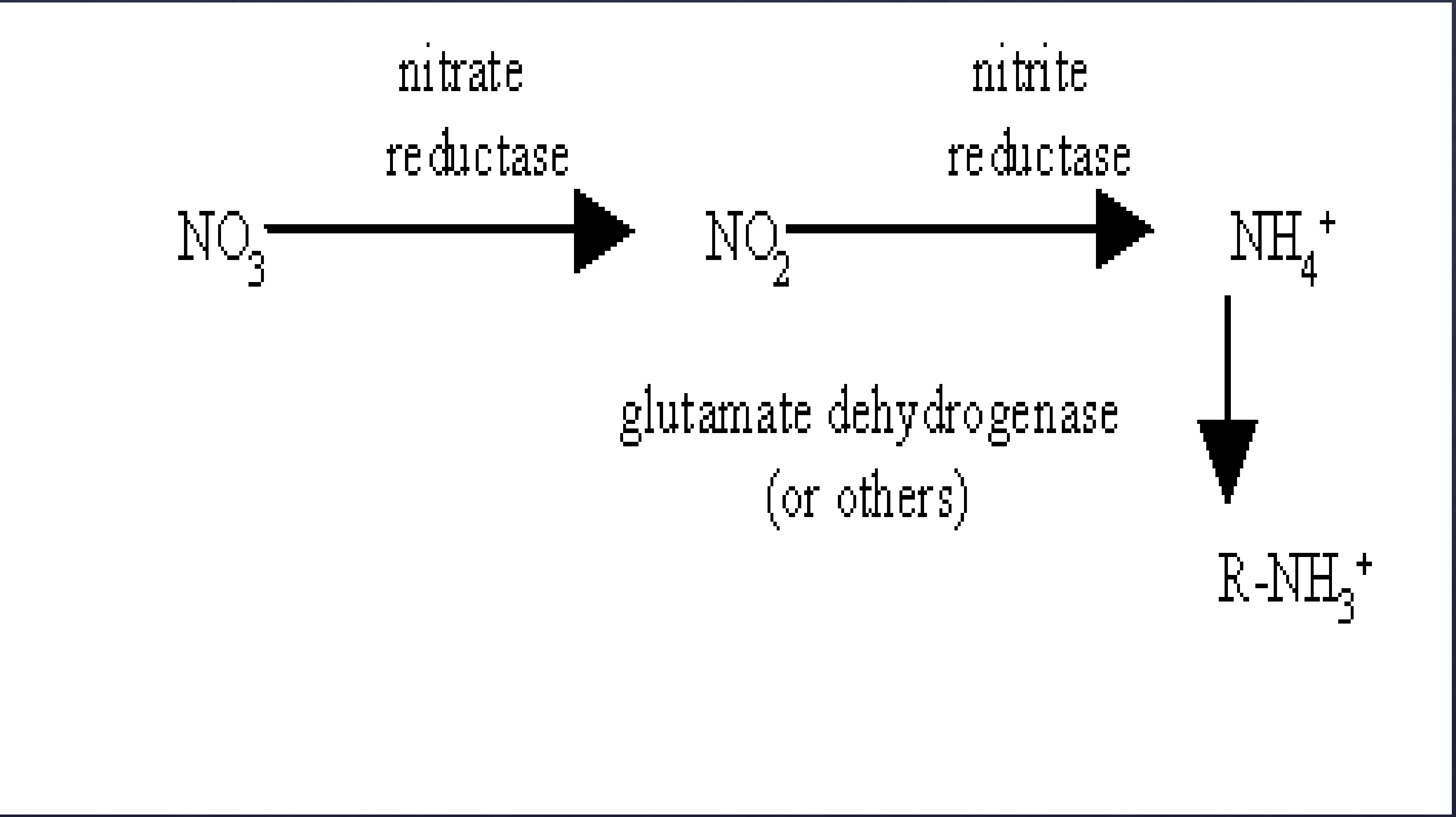
Figure 6-23 Brock Biology of Microorganisms 11/e

Microbial Nutrition

- Why is nutrition important?
  - The hundreds of chemical compounds present inside a living cell are formed from nutrients.
- **Macronutrients** : elements required in fairly large amounts
- **Micronutrients** : metals and organic compounds needed in very small amounts

Main Macronutrients

- Carbon (C, 50% of dry weight) and nitrogen (N, 12% of dry weight)
- **Autotrophs** are able to build all of their cellular organic molecules from carbon dioxide
- Nitrogen mainly incorporated in proteins, nucleic acids
- Most Bacteria can use Ammonia -NH_3 and many can also use NO_3^-
- Nitrogen fixers can utilize atmospheric nitrogen (N_2)


nitrate reductase
NO_3
NO_2
nitrite reductase
NH_4^+
glutamate dehydrogenase (or others)
$R\text{-}NH_3^+$

Microbial growth requirements

- Source of carbon for basic structures
- Source of cellular energy (ATP or related compounds) to drive metabolic reactions
- Source of high energy electrons/H, reducing power, typically in form of NADH/NADPH

Classification of organisms based on sources of C and energy used

Carbon Source	Energy Source: Light *(photo-)*	Energy Source: Chemical compounds *(chemo-)*
Carbon dioxide *(auto-)*	***Photoautotrophs*** • Plants, algae, and cyanobacteria use H_2O to reduce CO_2, producing O_2 as a byproduct • Photosynthetic green sulfur and purple sulfur bacteria do not use H_2O nor produce O_2	***Chemoautotrophs*** • Hydrogen, sulfur, and nitrifying bacteria
Organic compounds *(hetero-)*	***Photoheterotrophs*** • Green nonsulfur and purple nonsulfur bacteria	***Chemoheterotrophs*** • Aerobic respiration: most animals, fungi, and protozoa, and many bacteria • Anaerobic respiration: some animals, protozoa, and bacteria • Fermentation: some bacteria and yeasts

Nitrogen requirements

- Although many biological components within living organisms contain N, and N_2 is the most abundant component of air, very few organisms can "fix" or utilize N_2 by converting it to NH_3
- N is often growth limiting as organisms must find source as NH_4^+ for biosynthesis
- Photosynthetic organisms and many microbes can reduce NO_3^- to NH_4^+

Other Macronutrients

- Phosphate (P), sulfur (S), potassium (K), magnesium (Mg), calcium (Ca), sodium (Na), iron (Fe)
- Iron plays a major role in cellular respiration, being a key component of cytochromes and iron-sulfur proteins involved in electron transport.
- **Siderophores** : Iron-binding agents that cells produce to obtain iron from various insoluble minerals.

Representative Siderophore

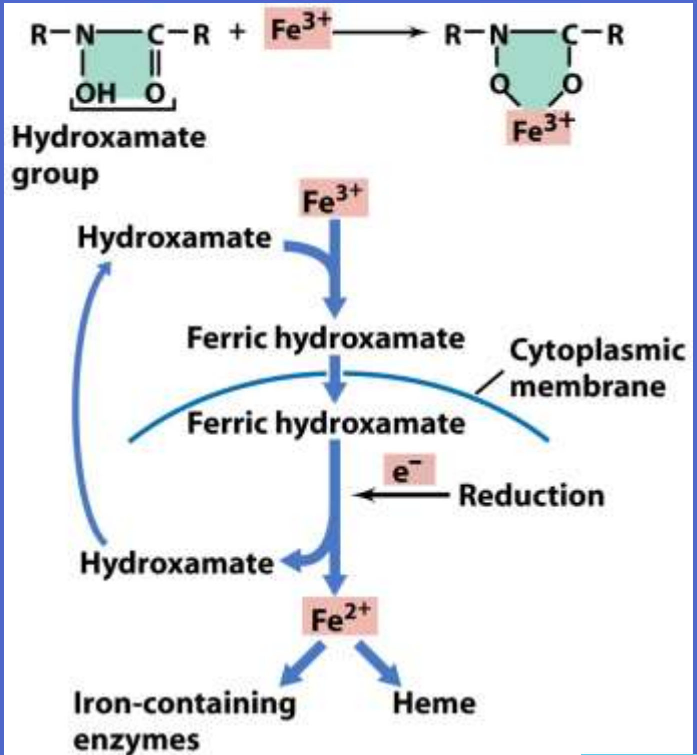


Figure 5-1a Brock Biology of Microorganisms 11/e

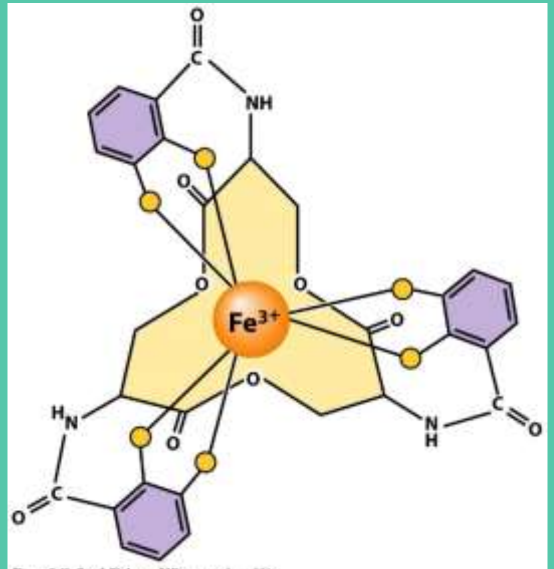


Figure 5-1b Brock Biology of Microorganisms 11/e

Aquachelin

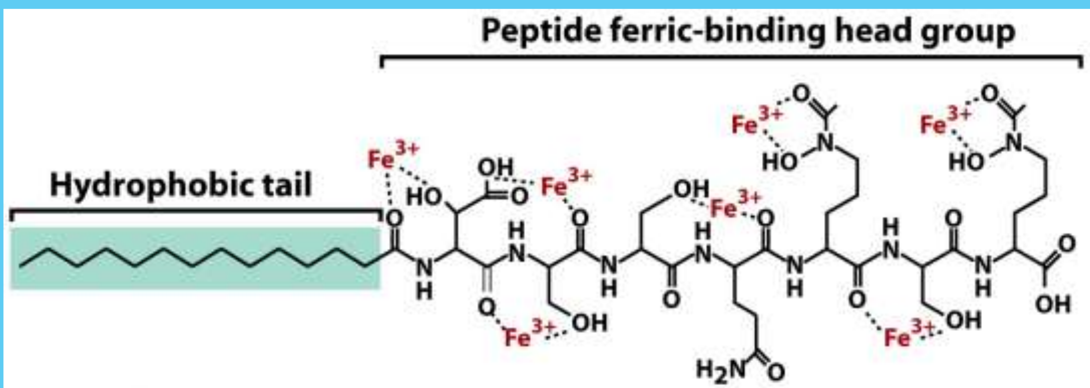


Figure 5-1c Brock Biology of Microorganisms 11/e

Table 5.1 Macronutrients in nature and in culture media

Element	Usual form of nutrient found in the environment	Chemical form supplied in culture media
Carbon (C)	CO_2, organic compounds	Glucose, malate, acetate, pyruvate, amino acids, hundreds of other compounds, or complex mixtures (yeast extract, peptone, and so on)
Hydrogen (H)	H_2O, organic compounds	H_2O, organic compounds
Oxygen (O)	H_2O, O_2, organic compounds	H_2O, O_2, organic compounds
Nitrogen (N)	NH_3, NO_3^-, N_2, organic nitrogen compounds	*Inorganic:* NH_4Cl, $(NH_4)_2SO_4$, KNO_3, N_2 *Organic:* Amino acids, nitrogen bases of nucleotides, many other N-containing organic compounds
Phosphorus (P)	PO_4^{3-}	KH_2PO_4, Na_2HPO_4
Sulfur (S)	H_2S, SO_4^{2-}, organic S compounds, metal sulfides (FeS, CuS, ZnS, NiS, and so on)	Na_2SO_4, $Na_2S_2O_3$, Na_2S, cysteine, or other organic sulfur compounds
Potassium (K)	K^+ in solution or as various K salts	KCl, KH_2PO_4
Magnesium (Mg)	Mg^{2+} in solution or as various Mg salts	$MgCl_2$, $MgSO_4$
Sodium (Na)	Na^+ in solution or as NaCl or other Na salts	NaCl
Calcium (Ca)	Ca^{2+} in solution or as $CaSO_4$ or other Ca salts	$CaCl_2$
Iron (Fe)	Fe^{2+} or Fe^{3+} in solution or as FeS, $Fe(OH)_3$, or many other Fe salts	$FeCl_3$, $FeSO_4$, various chelated iron solutions (Fe^{3+} EDTA, Fe^{3+} citrate, and so on)

Table 5-1 Brock Biology of Microorganisms 11/e

Micronutrients

Table 5.2 Micronutrients (trace elements) needed by living organisms[a]

Element	Cellular function
Boron (B)	Present in an autoinducer for quorum sensing in bacteria; also found in some polyketide antibiotics
Chromium (Cr)	Required by mammals for glucose metabolism; no known microbial requirement
Cobalt (Co)	Vitamin B_{12}; transcarboxylase (propionic acid bacteria)
Copper (Cu)	Respiration, cytochrome *c* oxidase; photosynthesis, plastocyanin, some superoxide dismutases
Iron (Fe)[b]	Cytochromes; catalases; peroxidases; iron-sulfur proteins; oxygenases; all nitrogenases
Manganese (Mn)	Activator of many enzymes; present in certain superoxide dismutases and in the water-splitting enzyme in oxygenic phototrophs (Photosystem II)
Molybdenum (Mo)	Certain flavin-containing enzymes; some nitrogenases, nitrate reductases, sulfite oxidases, DMSO-TMAO reductases; some formate dehydrogenases
Nickel (Ni)	Most hydrogenases; coenzyme F_{430} of methanogens; carbon monoxide dehydrogenase; urease
Selenium (Se)	Formate dehydrogenase; some hydrogenases; the amino acid selenocysteine
Tungsten (W)	Some formate dehydrogenases; oxotransferases of hyperthermophiles
Vanadium (V)	Vanadium nitrogenase; bromoperoxidase
Zinc (Zn)	Carbonic anhydrase; alcohol dehydrogenase; RNA and DNA polymerases; and many DNA-binding proteins

[a] Not every micronutrient listed is required by all cells; some metals listed are found in enzymes present in only specific microorganisms.
[b] Needed in greater amounts than other trace metals.

Table 5-2 part 2 Brock Biology of Microorganisms 11/e

Need very little amount but critical to cell function. Often used as enzyme cofactors

Growth factors

Organic compounds, required in very small amount and then only by some cells

Table 5.3 Growth factors: Vitamins and their functions

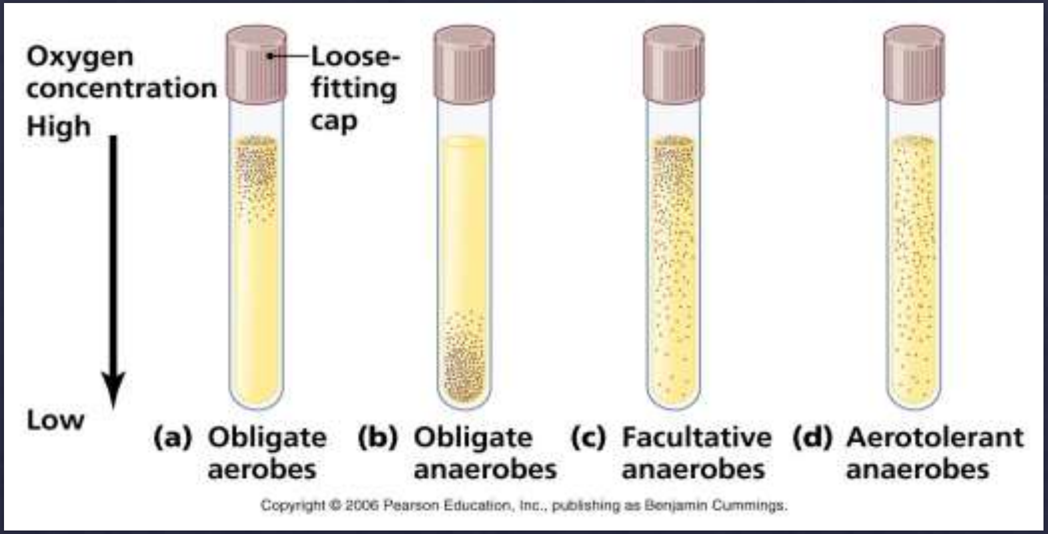
Vitamin	Function
p-Aminobenzoic acid	Precursor of folic acid
Folic acid	One-carbon metabolism; methyl group transfer
Biotin	Fatty acid biosynthesis; β-decarboxylations; some CO_2 fixation reactions
Cobalamin (B_{12})	Reduction of and transfer of single carbon fragments; synthesis of deoxyribose
Lipoic acid	Transfer of acyl groups in decarboxylation of pyruvate and α-ketoglutarate
Nicotinic acid (niacin)	Precursor of NAD^+ (see Figure 5.10); electron transfer in oxidation-reduction reactions
Pantothenic acid	Precursor of coenzyme A; activation of acetyl and other acyl derivatives
Riboflavin	Precursor of FMN (see Figure 5.15), FAD in flavoproteins involved in electron transport
Thiamine (B_1)	α-Decarboxylations; transketolase
Vitamins B_6 (pyridoxal-pyridoxamine group)	Amino acid and keto acid transformations
Vitamin K group; quinones	Electron transport; synthesis of sphingolipids
Hydroxamates	Iron-binding compounds; solubilization of iron and transport into cell

Table 5-3 Brock Biology of Microorganisms 11/e

Classification of organisms based on O_2 utilization

- Utilization of O_2 during metabolism yields toxic by-products including O_2^-, singlet oxygen ($^1O_2$) and/or H_2O_2.
- Toxic O_2 products can be converted to harmless substances if the organism has catalase (or peroxidase) and superoxide dismutase (SOD)
- SOD converts O_2^- into H_2O_2 and O_2
- Catalase breaks down H_2O_2 into H_2O and O_2
- Any organism that can live in or requires O_2 has SOD and catalase (peroxidase)

Classification of organisms based on O_2 utilization



- Obligate (strict) aerobes require O_2 in order to grow
- Obligate (strict) anaerobes cannot survive in O_2
- Facultative anaerobes grow better in O_2
- Aerotolerant organisms don't care about O_2
- Microaerophiles require low levels of O_2

Oxygen and Microbial Growth

- **Aerobes :**
  - **Obligate** : require oxygen to grow
  - **Facultative** : can live with or without oxygen but grow better with oxygen
  - **Microaerphiles :** require reduced level of oxygen

- **Anaerobes :**
  - **Aerotolerant anaerobes :** can tolerate oxygen but grow better without oxygen.
  - **Obligate :** do not require oxygen. Obligate anaerobes are killed by oxygen

Table 6.4 Oxygen relationships of microorganisms

Group	Relationship to O_2	Type of metabolism	Example[a]	Habitat[b]
Aerobes				
Obligate	Required	Aerobic respiration	*Micrococcus luteus* (B)	Skin, dust
Facultative	Not required, but growth better with O_2	Aerobic respiration, anaerobic respiration, fermentation	*Escherichia coli* (B)	Mammalian large intestine
Microaerophilic	Required but at levels lower than atmospheric	Aerobic respiration	*Spirillum volutans* (B)	Lake water
Anaerobes				
Aerotolerant	Not required, and growth no better when O_2 present	Fermentation	*Streptococcus pyogenes* (B)	Upper respiratory tract
Obligate	Harmful or lethal	Fermentation or anaerobic respiration	*Methanobacterium formicicum* (A)	Sewage sludge digestors, anoxic lake sediments

[a] Letters in parentheses indicate phylogenetic status (B, *Bacteria*; A, *Archaea*). Representatives of either domain of prokaryotes are known in each category. Most eukaryotes are obligate aerobes, but facultative aerobes (for example, yeast) and obligate anaerobes (for example, certain protozoa and fungi) are known.

[b] Listed are typical habitats of the example organism.

Table 6-4 Brock Biology of Microorganisms 11/e

Test for Oxygen Requirements of Microorganisms

Thioglycolate broth : contains a reducing agent and provides aerobic and anaerobic conditions

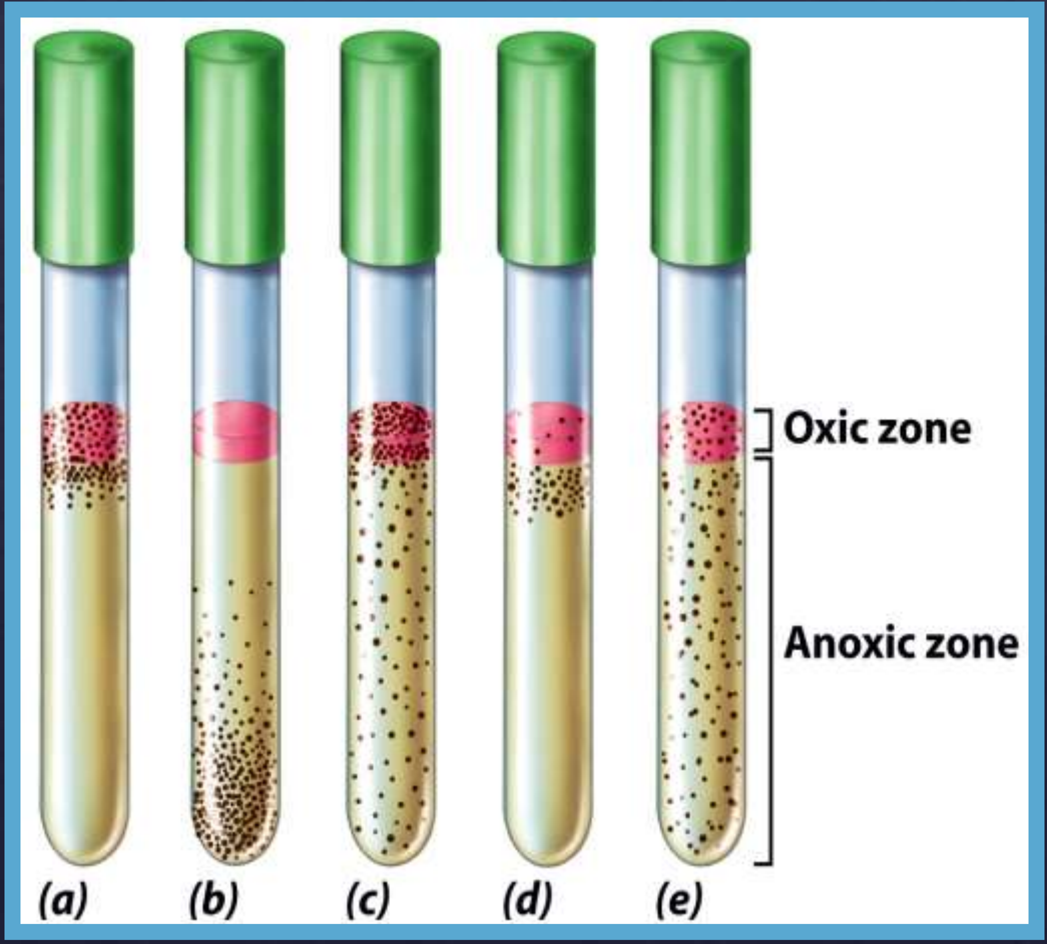
a) Aerobic

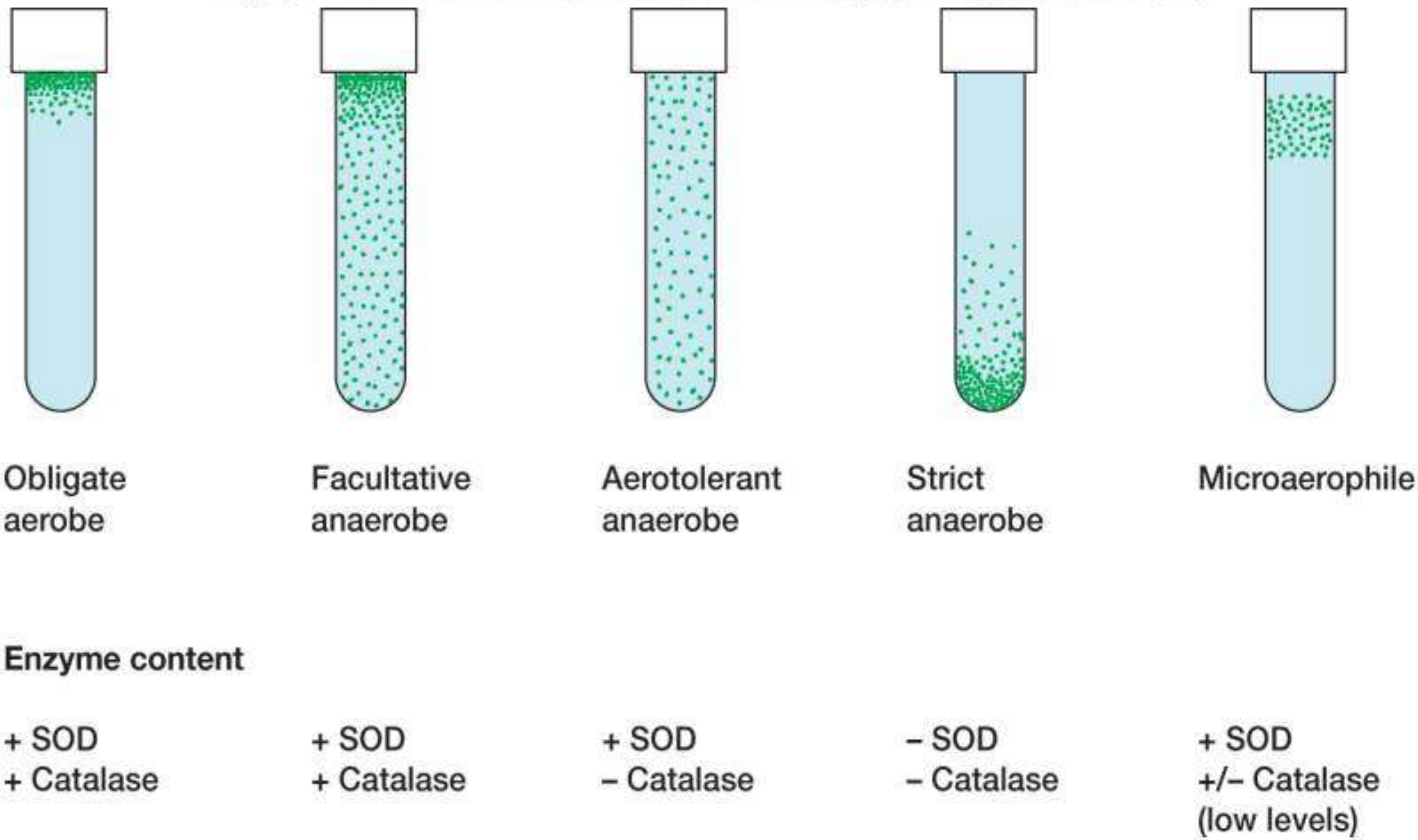
b) Anaerobic

c) Facultative

d) Microaerophil

e) Aerotolerant




Obligate aerobe
Facultative anaerobe
Aerotolerant anaerobe
Strict anaerobe
Microaerophile
Enzyme content
+ SOD
+ Catalase
+ SOD
+ Catalase
+ SOD
– Catalase
– SOD
– Catalase
+ SOD
+/– Catalase
(low levels)

Environmental factors and growth

4. Oxygen

anaerobes lack superoxide dismutase and/or catalase

anaerobes need high -, something to remove O_2

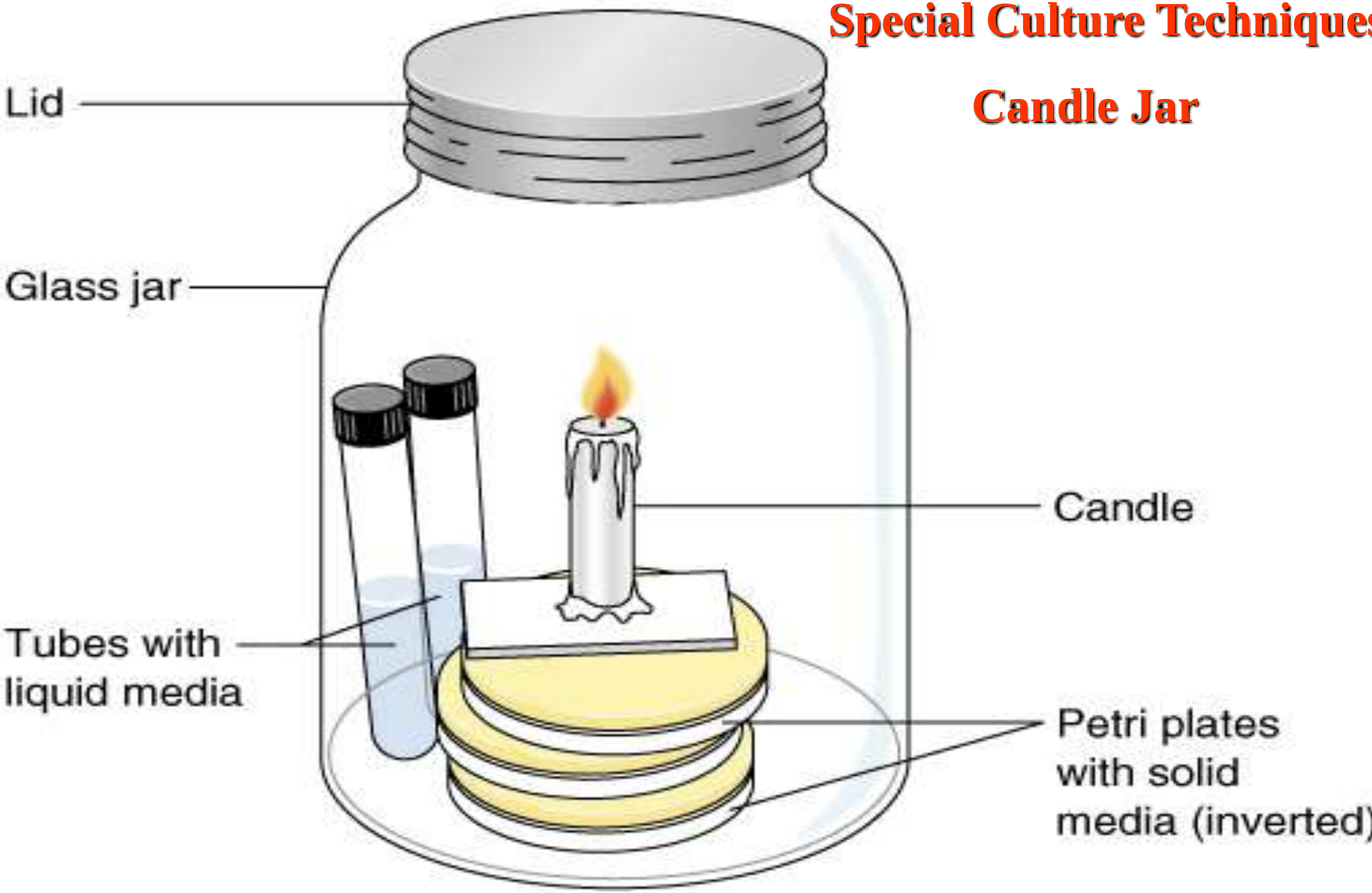
chemical: thioglycollate; pyrogallol + NaOH

H_2 generator + catalyst

physical: removal/replacement

Special Culture Techniques

Candle Jar



(a) Candle jar

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Special Culture Techniques

Gas Pack Jar Is Used for Anaerobic Growth