Stress Physiology

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Influence of Environmental Factors on Growth

- The growth of microorganisms also is greatly affected by the chemical and physical nature of their surroundings.
- The ability of some microorganisms to adapt to extreme and inhospitable environments is truly remarkable.
- Microorganisms that grow in such harsh conditions are often called **extremophiles.**

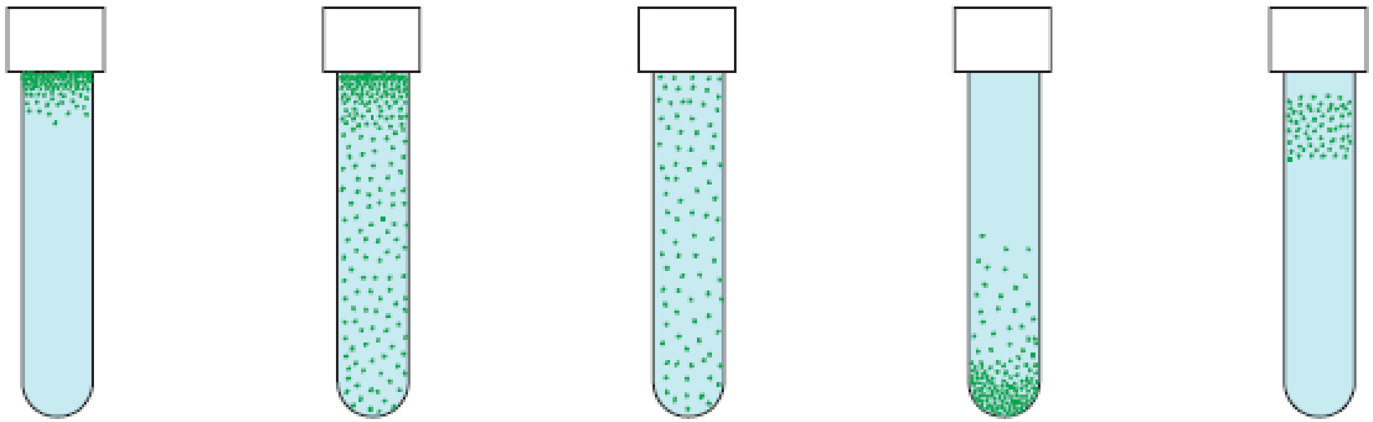
Table 6.3 Microbial Responses to Environmental Factors

Descriptive Term	Definition	Representative Microorganisms
Solute and Water Activity		
Osmotolerant	Able to grow over wide ranges of water activity or osmotic concentration	*Staphylococcus aureus, Saccharomyces rouxii*
Halophile	Requires high levels of sodium chloride, usually above about 0.2 M, to grow	*Halobacterium, Dunaliella, Ectothiorhodospira*
pH		
Acidophile	Growth optimum between pH 0 and 5.5	*Sulfolobus, Picrophilus, Ferroplasma, Acontium, Cyanidium caldarium*
Neutrophile	Growth optimum between pH 5.5 and 8.0	*Escherichia, Euglena, Paramecium*
Alkalophile	Growth optimum between pH 8.5 and 11.5	*Bacillus alcalophilus, Natronobacterium*
Temperature		
Psychrophile	Grows well at 0°C and has an optimum growth temperature of 15°C or lower	*Bacillus psychrophilus, Chlamydomonas nivalis*
Psychrotroph	Can grow at 0–7°C; has an optimum between 20 and 30°C and a maximum around 35°C	*Listeria monocytogenes, Pseudomonas fluorescens*
Mesophile	Has growth optimum around 20–45°C	*Escherichia coli, Neisseria gonorrhoeae, Trichomonas vaginalis*
Thermophile	Can grow at 55°C or higher; optimum often between 55 and 65°C	*Bacillus stearothermophilus, Thermus aquaticus, Cyanidium caldarium, Chaetomium thermophile*
Hyperthermophile	Has an optimum between 80 and about 113°C	*Sulfolobus, Pyrococcus, Pyrodictium*
Oxygen Concentration		
Obligate aerobe	Completely dependent on atmospheric O_2 for growth.	*Micrococcus luteus, Pseudomonas, Mycobacterium;* most algae, fungi, and protozoa
Facultative anaerobe	Does not require O_2 for growth, but grows better in its presence.	*Escherichia, Enterococcus, Saccharomyces cerevisiae*
Aerotolerant anaerobe	Grows equally well in presence or absence of O_2	*Streptococcus pyogenes*
Obligate anaerobe	Does not tolerate O_2 and dies in its presence.	*Clostridium, Bacteroides, Methanobacterium, Trepomonas agilis*
Microaerophile	Requires O_2 levels below 2–10% for growth and is damaged by atmospheric O_2 (20%).	*Campylobacter, Spirillum volutans, Treponema pallidum*
Pressure		
Barophilic	Growth more rapid at high hydrostatic pressures.	*Photobacterium profundum, Shewanella benthica, Methanococcus jannaschii*

Adaptations to oxygen toxicity

- Almost all multicellular organisms are completely dependent on atmospheric O_2 for growth—that is, they are **obligate aerobes**.
- Oxygen serves as the terminal electron acceptor for the electron-transport chain in aerobic respiration.
- In addition, aerobic eucaryotes employ O_2 in the synthesis of sterols and unsaturated fatty acids.
- **Facultative anaerobes** do not require O_2 for growth but do grow better in its presence.
- In the presence of oxygen they will use aerobic respiration.
- **Aerotolerant anaerobes** such as *Enterococcus faecalis* simply ignore O_2 and grow equally well whether it is present or not.
- In contrast, **strict or obligate anaerobes** (e.g., *Bacteroides, Fusobacterium, Clostridium pasteurianum, Methanococcus)* do not tolerate O_2 at all and die in its presence.
- Aerotolerant and strict anaerobes cannot generate energy through respiration and must employ fermentation or anaerobic respiration pathways for this purpose.
- Finally, there are aerobes such as *Campylobacter,* called **microaerophiles**, that are damaged by the normal atmospheric level of O_2 (20%) and require O_2 levels below the range of 2 to 10% for growth.

Oxygen and Bacterial Growth



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

Oxidizing Agent

- These different relationships with O_2 appear due to several factors, including the inactivation of proteins and the effect of toxic O_2 derivatives.
- Enzymes can be inactivated when sensitive groups like sulfhydryls are oxidized.
- A notable example is the nitrogen-fixation enzyme nitrogenase which is very oxygen sensitive.
- Oxygen accepts electrons and is readily reduced because its two outer orbital electrons are unpaired.
- Flavoproteins, several other cell constituents, and radiation promote oxygen reduction.
- The result is usually some combination of the reduction products **superoxide radical, hydrogen peroxide, and hydroxyl radical.**

$$O_2 + e^- \rightarrow O_2^{-}\cdot \text{ (superoxide radical)}$$

$$O_2^{-}\cdot + e^- + 2H^+ \longrightarrow H_2O_2 \text{ (hydrogen peroxide)}$$

$$H_2O_2 + e^- + H^+ \longrightarrow H_2O + OH\cdot \text{ (hydroxyl radical)}$$

- These products of oxygen reduction are extremely toxic because they are powerful oxidizing agents and rapidly destroy cellular constituents.
- A microorganism must be able to protect itself against such oxygen products or it will be killed.
- Many microbes possess enzymes for protection against toxic O_2 products.
- Obligate aerobes and facultative anaerobes usually contain the enzymes superoxide dismutase (SOD) and catalase, which catalyze the destruction of superoxide radical and hydrogen peroxide, respectively.
- Peroxidase also can be used to destroy hydrogen peroxide.

$$2O_2^{\overline{\cdot}} + 2H^+ \xrightarrow{\text{superoxide dismutase}} O_2 + H_2O_2$$

$$2H_2O_2 \xrightarrow{\text{catalase}} 2H_2O + O_2$$

$$H_2O_2 + NADH + H^+ \xrightarrow{\text{peroxidase}} 2H_2O + NAD^+$$

- Aerotolerant microorganisms may lack catalase but almost always have superoxide dismutase.
- The aerotolerant *Lactobacillus plantarum* uses manganous ions instead of superoxide dismutase to destroy the superoxide radical.
- All strict anaerobes lack both enzymes or have them in very low concentrations and therefore cannot tolerate O_2.

Growth Conditions

- When large volumes of aerobic microorganisms are cultured, either the culture vessel is shaken to aerate the medium or sterile air must be pumped through the culture vessel.
- Precisely the opposite problem arises with anaerobes; all O_2 must be excluded.
- This can be accomplished in several ways.

(1) Special anaerobic media containing reducing agents such as thioglycollate or cysteine may be used.

- The medium is boiled during preparation to dissolve its components; boiling also drives off oxygen very effectively.
- The reducing agents will eliminate any dissolved O_2 remaining within the medium so that anaerobes can grow beneath its surface.

(2) Oxygen also may be eliminated from an anaerobic system by removing air with a vacuum pump and flushing out residual O_2 with nitrogen gas.

- Often CO_2 as well as nitrogen is added to the chamber since many anaerobes require a small amount of CO_2 for best growth.

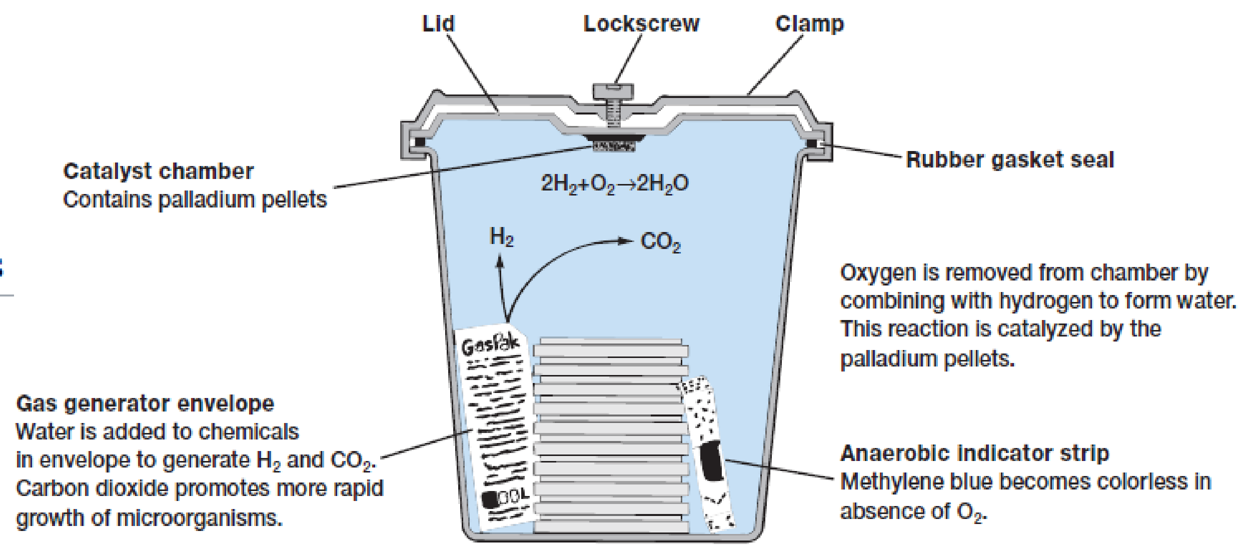
... Growth Conditions

(3) One of the most popular ways of culturing small numbers of anaerobes is by use of a Gas-Pak jar.

- In this procedure the environment is made anaerobic by using hydrogen and a palladium catalyst to remove O_2 through the formation of water.
- The reducing agents in anaerobic agar also remove oxygen, as mentioned previously.

(4) Plastic bags or pouches make convenient containers when only a few samples are to be incubated anaerobically.

- These have a catalyst and calcium carbonate to produce an anaerobic, carbon-dioxide rich atmosphere.
- A special solution is added to the pouch's reagent compartment; petri dishes or other containers are placed in the pouch; it then is clamped shut and placed in an incubator.



Constituents of gas-pak sachets

1. Sodium borohydride - $NaBH_4$
2. Sodium bicarbonate - $NaHCO_3$
3. Citric acid - $C_3H_5O(COOH)_3$
4. Cobalt chloride - $CoCl_2$ (catalyst)

- $NaBH_4 + 2\ H_2O = NaBO_2 + 4\ H_2\uparrow$
- $C_3H_5O(COOH)_3 + 3\ NaHCO_3 + [CoCl_2] = C_3H_5O(COONa)_3 + 3\ CO_2 + 3\ H_2 + [CoCl_2]$
- $2\ H_2 + O_2 + [Catalyst] = 2\ H_2O + [Catalyst]$

Adaptations to pH

- pH is a measure of the hydrogen ion activity of a solution and is defined as the negative logarithm of the hydrogen ion concentration (expressed in terms of molarity).

$$pH = -\log[H^+] = \log(1/[H^+])$$

- The pH scale extends from pH 0.0 (1.0 M H^+) to pH 14.0 (1.0×10^{-14} M H^+), and each pH unit represents a tenfold change in hydrogen ion concentration.
- Habitats in which microorganisms grow vary widely—from pH 1 to 2 at the acid end to alkaline lakes and soil that may have pH values between 9 and 10.
- Each species has a definite pH growth range and pH growth optimum.
- **Acidophiles** have their growth optimum between pH 0 and 5.5; **neutrophiles**, between pH 5.5 and 8.0; and **alkalophiles** prefer the pH range of 8.5 to 11.5.
- **Extreme alkalophiles** have growth optima at pH 10 or higher.
- Most bacteria and protozoa are neutrophiles.
- Most fungi prefer slightly acid surroundings, about pH 4 to 6; algae also seem to favor slight acidity.
- There are many exceptions to these generalizations.
- For example, the alga *Cyanidium caldarium* and the archaeon *Sulfolobus acidocaldarius* are common inhabitants of acidic hot springs; both grow well around pH 1 to 3 and at high temperatures.

Effect of Drastic pH Variations

- Although microorganisms will often grow over wide ranges of pH and far from their optima, there are limits to their tolerance.
- Drastic variations in cytoplasmic pH can harm microorganisms by disrupting the plasma membrane or inhibiting the activity of enzymes and membrane transport proteins.
- Changes in the external pH also might alter the ionization of nutrient molecules and thus reduce their availability to the organism.

Mechanism of Survival in extreme pH Conditions

- Microorganisms must adapt to environmental pH changes to survive.
- Several proposed mechanisms for the maintenance of a neutral cytoplasmic pH are:
- In bacteria, potassium/proton and sodium/proton antiport systems probably correct small variations in pH.
- Extreme alkalophiles like *Bacillus alcalophilus* maintain their internal pH closer to neutrality by exchanging internal sodium ions for external protons.
- If the pH becomes too acidic, other mechanisms come into play.
- When the pH drops below about 5.5 to 6.0, *Salmonella typhimurium* and *E. coli* synthesize an array of new proteins as part of what has been called their acidic tolerance response.
- A proton-translocating ATPase contributes to this protective response, either by making more ATP or by pumping protons out of the cell.

... **Mechanism of Survival in extreme pH Conditions**

- If the external pH decreases to 4.5 or lower, chaperones such as acid shock proteins and heat shock proteins are synthesized.
- Presumably these prevent the acid denaturation of proteins and aid in the refolding of denatured proteins.
- Microorganisms frequently change the pH of their own habitat by producing acidic or basic metabolic waste products.
- Fermentative microorganisms form organic acids from carbohydrates, whereas chemolithotrophs like *Thiobacillus oxidize* reduced sulfur components to sulfuric acid.
- Other microorganisms make their environment more alkaline by generating ammonia through amino acid degradation.

Growth Conditions

- Buffers often are included in media to prevent growth inhibition by large pH changes.
- Phosphate is a commonly used buffer and a good example of buffering by a weak acid (H_2PO_4) and its conjugate base (HPO_4^{2-}).

$$H^+ + HPO_4^{2-} \longrightarrow H_2PO_4^-$$

$$OH^- + H_2PO_4^- \longrightarrow HPO_4^{2-} + HOH$$

- If protons are added to the mixture, they combine with the salt form to yield a weak acid.
- An increase in alkalinity is resisted because the weak acid will neutralize hydroxyl ions through proton donation to give water.
- Peptides and amino acids in complex media also have a strong buffering effect.

Adaptations to Osmotic Pressure

- Because a selectively permeable plasma membrane separates microorganisms from their environment, they can be affected by changes in the osmotic concentration of their surroundings.
- If a microorganism is placed in a hypotonic solution (one with a lower osmotic concentration), water will enter the cell and cause it to burst unless something is done to prevent the influx.
- The osmotic concentration of the cytoplasm can be reduced by use of inclusion bodies.
- Procaryotes also can contain pressure-sensitive channels that open to allow solute escape when the osmolarity of the environment becomes much lower than that of the cytoplasm.

Plasmolysis

- Most bacteria, algae, and fungi have rigid cell walls that maintain the shape and integrity of the cell.
- When microorganisms with rigid cell walls are placed in a hypertonic environment, water leaves and the plasma membrane shrinks away from the wall, a process known as plasmolysis.
- This dehydrates the cell and may damage the plasma membrane; the cell usually becomes metabolically inactive and ceases to grow.
- Many microorganisms keep the osmotic concentration of their protoplasm somewhat above that of the habitat by the use of compatible solutes, so that the plasma membrane is always pressed firmly against their cell wall.
- **Compatible solutes are** solutes that are compatible with metabolism and growth when at high intracellular concentrations.
- Most procaryotes increase their internal osmotic concentration in a hypertonic environment through the synthesis or uptake of choline, betaine, proline, glutamic acid, and other amino acids; elevated levels of potassium ions are also involved to some extent.

... **Plasmolysis**

- Algae and fungi employ sucrose and polyols—for example, arabitol, glycerol, and mannitol—for the same purpose.
- Polyols and amino acids are ideal solutes for this function because they normally do not disrupt enzyme structure and function.
- A few procaryotes like *Halobacterium salinarium* raise their osmotic concentration with potassium ions (sodium ions are also elevated but not as much as potassium).
- *Halobacterium's* enzymes have been altered so that they actually require high salt concentrations for normal activity.
- Since protozoa do not have a cell wall, they must use contractile vacuoles to eliminate excess water when living in hypotonic environments.

Water Activity & Osmotolerence

- The amount of water available to microorganisms can be reduced by interaction with solute molecules (the osmotic effect) or by adsorption to the surfaces of solids (the matric effect).
- Water availability can be expressed as:
  - Water activity (a_w)
  - water potential, which is related to a_w
- The water activity of a solution is 1/100 the relative humidity of the solution (when expressed as a percent).
- It is also equivalent to the ratio of the solution's vapor pressure (P_{soln}) *to that of pure water* (P_{water}).

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

- If, the relative humidity is 95%, then the sample's water activity, 0.95.
- Water activity is inversely related to osmotic pressure; if a solution has high osmotic pressure, its a_w is low.

... **Water Activity & Osmotolerence**

- Microorganisms differ greatly in their ability to adapt to habitats with low water activity **.**
- **A microorganism** must expend extra effort to grow in a habitat with a low a_w value because it must maintain a high internal solute concentration to retain water.
- Some microorganisms can do this and are **osmotolerant;** they will grow over wide ranges of water activity or osmotic concentration.
- For example, *Staphylococcus aureus* can be cultured in media containing any sodium chloride concentration up to about 3 M. It is well adapted for growth on the skin.
- The yeast *Saccharomyces rouxii* will grow in sugar solutions with a_w values as low as 0.6.
- Although a few microorganisms are truly osmotolerant, most only grow well at water activities around 0.98 (the approximate a_w for seawater) or higher.
- This is why drying food or adding large quantities of salt and sugar is so effective in preventing food spoilage.

Halophiles Osmotic Tolerence Ability

- Halophiles have adapted so completely to hypertonic, saline conditions that they require high levels of sodium chloride to grow, concentrations between about 2.8 M and saturation (about 6.2 M) for extreme halophilic bacteria.
- The archaeon *Halobacterium* can be isolated from the Dead Sea, the Great Salt Lake in Utah, and other aquatic habitats with salt concentrations approaching saturation.
- *Halobacterium* and other extremely halophilic bacteria have significantly modified the structure of their proteins and membranes rather than simply increasing the intracellular concentrations of solutes, the approach used by most osmotolerant microorganisms.
- These extreme halophiles accumulate enormous quantities of potassium in order to remain hypertonic to their environment; the internal potassium concentration may reach 4 to 7 M.
- The enzymes, ribosomes, and transport proteins of these bacteria require high levels of potassium for stability and activity.
- In addition, the plasma membrane and cell wall of *Halobacterium* are stabilized by high concentrations of sodium ion.
- If the sodium concentration decreases too much, the wall and plasma membrane literally disintegrate.
- Extreme halophilic bacteria have successfully adapted to environmental conditions that would destroy most organisms.
- In the process they have become so specialized that they have lost ecological flexibility and can prosper only in a few extreme habitats.

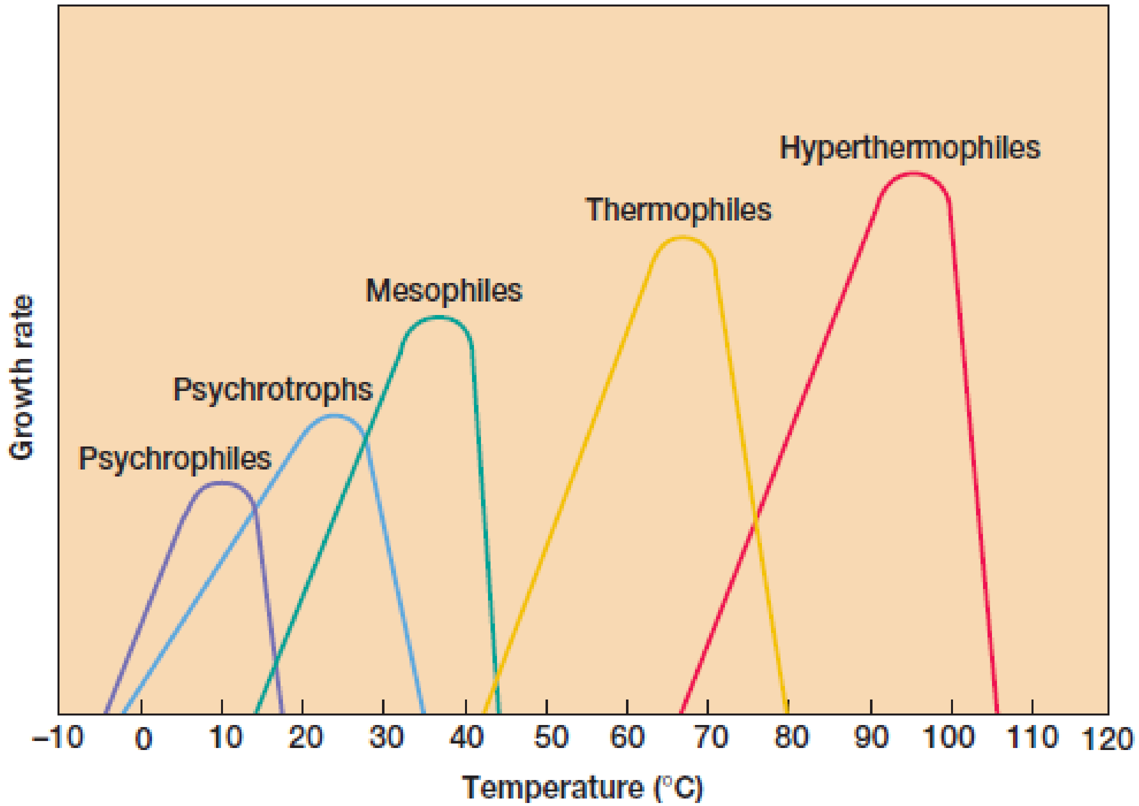
Adaptations to Temperature

- Microorganisms are particularly susceptible because they are usually unicellular and their temperature varies with that of the external environment.
- A most important factor influencing the effect of temperature on growth is the temperature sensitivity of enzyme catalyzed reactions.
- At low temperatures a temperature rise increases the growth rate because the velocity of an enzyme-catalyzed reaction, like that of any chemical reaction, will roughly double for every 10°C rise in temperature.
- Because the rate of each reaction increases, metabolism as a whole is more active at higher temperatures, and the microorganism grows faster.
- Beyond a certain point further increases actually slow growth, and sufficiently high temperatures are lethal.
- High temperatures damage microorganisms by denaturing enzymes, transport carriers, and other proteins.
- Microbial membranes are also disrupted by temperature extremes; the lipid bilayer simply melts and disintegrates.
- Thus, although functional enzymes operate more rapidly at higher temperatures, the microorganism may be damaged to such an extent that growth is inhibited because the damage cannot be repaired.

Temperature

- At very low temperatures, membranes solidify and enzymes don’t work rapidly.
- In summary, when organisms are above the optimum temperature, both function and cell structure are affected.
- If temperatures are very low, function is affected but not necessarily cell chemical composition and structure.
- Although the shape of the temperature dependence curve can vary, the temperature optimum is always closer to the maximum than to the minimum.
- The cardinal temperatures for a particular species are not rigidly fixed but often depend to some extent on other environmental factors such as pH and the available nutrients.
- Optima normally range from 0°C to as high as 75°C, whereas microbial growth occurs at temperatures extending from 20°C to over 100°C.
- The major factor determining this growth range seems to be water.
- Even at the most extreme temperatures, microorganisms need liquid water to grow.

- Microorganisms **can be placed in** one of five classes based on their temperature ranges for growth:



Psychrophiles

- Psychrophiles grow well at 0°C and have an optimum growth temperature of 15°C or lower; the maximum is around 20°C.
- They are readily isolated from Arctic and Antarctic habitats; because 90% of the ocean is 5°C or colder.
- The psychrophilic alga *Chlamydomonas nivalis* can actually turn a snowfield or glacier pink with its bright red spores.
- Psychrophiles are widespread among bacterial taxa and found in such genera as *Pseudomonas, Vibrio, Alcaligenes, Bacillus, Arthrobacter, Moritella, Photobacterium,* and *Shewanella.*
- Psychrophilic microorganisms have adapted to their environment in several ways.
- Their enzymes, transport systems, and protein synthetic mechanisms function well at low temperatures.
- The cell membranes of psychrophilic microorganisms have high levels of unsaturated fatty acids and remain semifluid when cold.
- Indeed, many psychrophiles begin to leak cellular constituents at temperatures higher than 20°C because of cell membrane disruption.

Psychrotrophs and Mesophiles

Psychrotrophs

- Many species can grow at 0 to 7°C even though they have optima between 20 and 30°C, and maxima at about 35°C.
- These are called psychrotrophs or facultative psychrophiles.
- Psychrotrophic bacteria and fungi are major factors in the spoilage of refrigerated foods.

Mesophiles

- Mesophiles are microorganisms with growth optima around 20 to 45°C; they often have a temperature minimum of 15 to 20°C.
- Their maximum is about 45°C or lower.
- Most microorganisms probably fall within this category.
- Almost all human pathogens are mesophiles, as might be expected since their environment is a fairly constant 37°C.

Thermophiles and Hyperthermophiles

Thermophiles

- Some microorganisms **can grow** at temperatures of 55°C or higher.
- Their growth minimum is usually around 45°C and optima between 55 and 65°C.
- The vast majority are procaryotes although a few algae and fungi are thermophilic.
- These organisms flourish in many habitats including composts, self-heating hay stacks, hot water lines, and hot springs.
- Thermophiles differ from mesophiles in having much more heat-stable enzymes and protein synthesis systems able to function at high temperatures.
- Their membrane lipids are also more saturated than those of mesophiles and have higher melting points; therefore thermophile membranes remain intact at higher temperatures.

Hyperthermophiles

- A few thermophiles can grow at 90°C or above and some have maxima above 100°C.
- Procaryotes that have growth optima between 80°C and about 113°C are called hyperthermophiles.
- They usually do not grow well below 55°C.
- *Pyrococcus abyssi* and *Pyrodictium occultum* are examples of marine hyperthermophiles found in hot areas of the seafloor.

Questions

- Define pH, acidophile, neutrophile, and alkalophile. How can microorganisms change the pH of their environment, and how does the microbiologist minimize this effect?
- Why does the growth rate rise with increasing temperature and then fall again at higher temperatures?
- Define psychrophile, psychrotroph, mesophile, thermophile, and hyperthermophile.
- Describe the five types of O_2 relationships seen in microorganisms.
- For what do aerobes use O_2? Why is O_2 toxic to many microorganisms and how do they protect themselves?
- Describe four ways in which anaerobes may be cultured.
- How do microorganisms adapt to hypotonic and hypertonic environments?
- What is plasmolysis?
- Define water activity and briefly describe how it can be determined.
- Why is it difficult for microorganisms to grow at low a_w values?
- What are halophiles and why does *Halobacterium* require sodium and potassium ions?