<u>Lecture 5</u> Environmental Factors affecting microbial growth

The kinds of organisms found in a given environment and the rates at which they grow can be influenced by a variety of factors, both physical and biochemical. **Physical factors** include pH, temperature, oxygen concentration, moisture, hydrostatic pressure, osmotic pressure, and radiation. **Nutritional** (*biochemical*) **factors** include availability of carbon, nitrogen, sulfur, phosphorus, trace elements, and, in some cases, vitamins.

<u>Temperature</u>

Microorganisms as a group are able to grow over a wide range of temperatures, from around freezing to above boiling point. The **<u>minimum temperature</u>** is the lowest temperature that permits a microbe's continued growth and metabolism; below this temperature, its activities are inhibited. Growth is slower at low temperatures because enzymes work less efficiently and also because lipids tend to harden and there is a loss of membrane fluidity. The **<u>maximum temperature</u>** is the highest temperature at which growth and metabolism can proceed. If the temperature rises slightly above maximum, growth will stop, but if it continues to rise beyond that point, the enzymes and nucleic acids will eventually become permanently inactivated (otherwise known as denaturation) and the cell will die. This is why heat works so well as an agent in microbial control. The

optimum temperature covers a small range, intermediate between the minimum and maximum, which promotes the fastest rate of growth and metabolism (rarely is the optimum a single point). Growth rates increase with temperature until the *optimum* temperature is reached, then the rate falls again.

Temperature is important not only in providing conditions for microbial growth, but also in preventing such growth. The refrigeration of food, usually at 4°C, reduces the growth of psychrophiles and prevents the growth of most other bacteria. Bacteria are more apt to survive extremes of cold than extremes of heat; enzymes are not denatured by chilling but can be permanently denatured by heat.

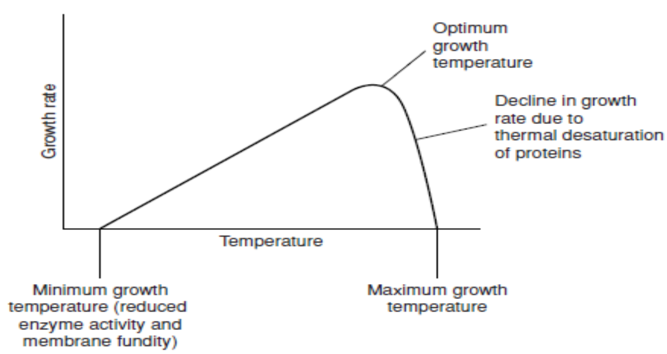
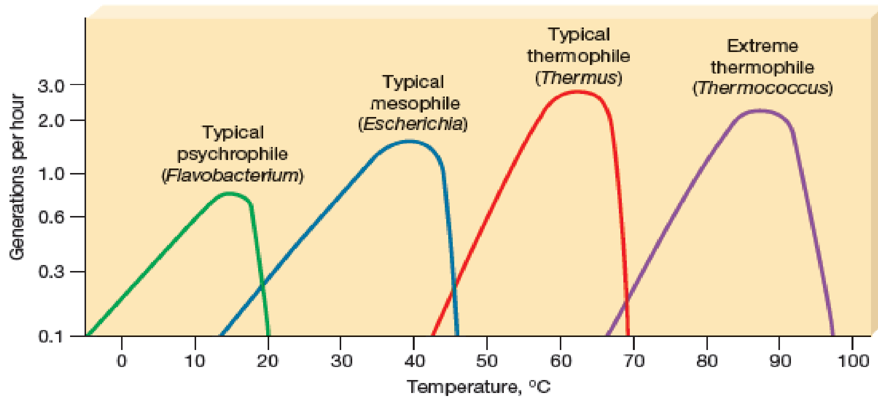


Figure 5.4 Effect of temperature on microbial growth rate. The factors governing the minimum, optimum and maximum temperatures for a particular organism are indicated. The curve is asymmetrical, with the optimum temperature being closer to the maximum than the minimum



According to their growth temperature range, bacteria can be classified as:

- Psychrophiles,

- Mesophiles, or

- Thermophiles.

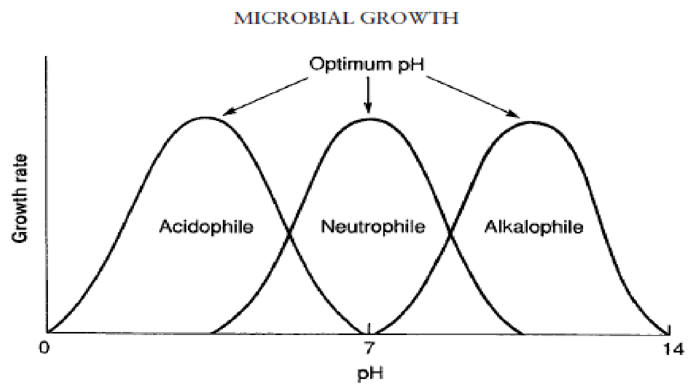
Most bacteria, however, do not tolerate the whole temperature range of a category, and some tolerate a range that overlaps categories. Within these groups, bacteria are further classified as obligate or facultative. **Obligate** means that the organism *must* have the specified environmental condition. **Facultative** means that the organism is *able* to adjust to and tolerate the environmental condition, but it can also live in other conditions. **Psychrophiles** or cold-loving organisms, grow best at temperatures of 15 °C to 20°C, although some live quite well at 0°C. Psychrophiles live mostly in cold water and soil. *Achromobacter*, *Flavobacterium*, *Pseudomonas*, and *Micrococcus are* the most examples of psychrophilic bacteria.

Bacteria such as *Staphylococcus aureus* and *Listeria monocytogenes* are a concern because they can grow in refrigerated food and cause foodborne illness.

Mesophiles which include most bacteria, grow best at temperatures between 25°C and 40°C. Organisms in this group inhabit animals and plants as well as soil and water in temperate, subtropical, and tropical regions. Most human pathogens have optima somewhere between 30°C and 40°C (human body temperature is 37°C). *Thermoduric* organisms ordinarily live as mesophiles but can withstand short periods of exposure to high temperatures. Inadequate heating during canning or in pasteurization may leave such organisms alive and therefore able to spoil food. Examples include heat-resistant cysts such as *Giardia* or spore formers such as *Bacillus* and *Clostridium.*

Thermophiles or heat-loving organisms grow best at temperatures from 50°C to 60°C. *Bacillus stearothermophilus*, which usually is considered an obligate thermophile, grows at its maximum rate at 65°C to 75°C but can display minimal growth and cause food spoilage at temperatures as low as 30°C.

Hydrogen ion pH



Microbial growth and survival are also influenced by the pH of the habitat. The pH is defined as the degree of acidity or alkalinity (basicity) of a solution. The pH of pure water (7.0) is neutral, neither acidic nor basic. As the pH value decreases toward 0, the acidity increases, and as the pH increases toward 14, the alkalinity increases. The majority of organisms live or grow in habitats between pH 6 and 8 because strong acids and bases can be highly damaging to enzymes and other cellular substances. Most microbes do not grow at a pH more than 1 pH unit above or below their optimum pH.

According to their tolerance for acidity or alkalinity, bacteria are classified as:

- **Acidophiles**.
- **Neutrophiles**.
- **Alkaliphiles**.

<u>Acidophiles</u> or acid-loving organisms, grow best at a pH of 0.1 to 5.4. *Lactobacillus*, which produces lactic acid, is an acidophilic, but it tolerates only mild acidity. Some bacteria that oxidize sulfur to sulfuric acid, however, can create and tolerate conditions as low as pH 1.0.

<u>Neutrophiles</u> exist from pH 5.4 to 8.0. Most of the bacteria that cause disease in humans are neutrophiles.

<u>Alkaliphiles</u> or alkali-loving (base-loving) organisms exist from pH 7.0 to 11.5. *Vibrio cholerae*, the causative agent of the disease cholera, grows best at a pH of about 9.0. *Alcaligenes faecalis*, which sometimes infects humans already weakened by another disease, can create and tolerate alkaline conditions of pH 9.0 or higher. The soil bacterium *Agrobacterium* grows in alkaline soil of pH 12.0.

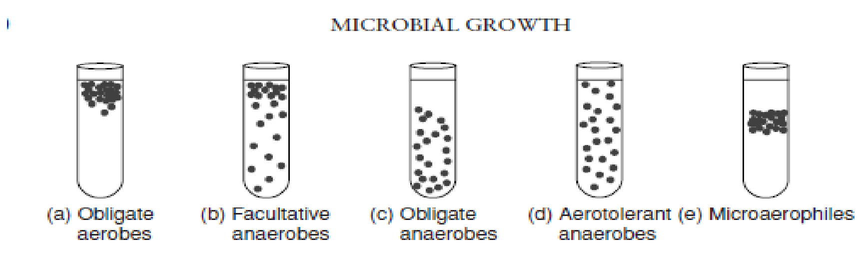
The effects of pH on organisms can, in part, be related to the concentration of organic acids in the medium and to the protection that bacterial cell walls sometimes provide. *Lactobacillus* and other organisms that produce organic acids during fermentation inhibit their own growth by producing acids such as lactic acid and pyruvic acid, which accumulate in the medium. Changes in pH can lead to denaturing of enzymes and other proteins and can interfere with pumping of ions at the cell membrane. Other organisms have relatively impervious cell walls that prevent the cell

membrane from being exposed to an extreme pH in the medium. These organisms appear to tolerate environmental acidity or alkalinity because the cell itself is maintained at a nearly neutral pH. Many bacteria often produce sufficient quantities of acids as metabolic by-products that eventually interfere with their own growth. To prevent this situation in the laboratory cultivation of bacteria, *buffers* are incorporated into growth media to maintain the proper pH levels. Phosphate salts are commonly used for this purpose.

Oxygen

Oxygen is present as a major constituent (20 per cent) of our atmosphere, and most life forms are dependent upon it for survival and growth. Bacteria, especially heterotrophs, can be divided into aerobes, which require oxygen to grow, and anaerobes, which do not require it. Among the aerobes, cultures of rapidly dividing cells require more oxygen than do cultures of slowly dividing cells. **Obligate aerobes**, such as *Pseudomonas*, must have free oxygen for aerobic respiration, whereas **obligate anaerobes**, such as *Clostridium botulinum, C. tetani*, and *Bacteroides*, are killed by free oxygen. Aerobic organisms require oxygen to act as a terminal electron acceptor in their respiratory chains.

Such organisms, when grown in laboratory culture, must therefore be provided with enough oxygen to satisfy their requirements. For a shallow layer of medium such as that in a petri dish, sufficient oxygen is available dissolved in surface moisture. In a deeper culture such as a flask of broth however, aerobes will only grow in the surface layers unless additional oxygen is provided (oxygen is poorly soluble in water). This is usually done by shaking or mechanical stirring. *Obligate anaerobes* cannot tolerate oxygen at all. They are cultured in special anaerobic chambers, and oxygen excluded from all liquid and solid media.



Microaerophiles appear to grow best in the presence of a small amount of free oxygen. They grow below the surface of the medium in a culture tube at the level where oxygen availability matches their needs. Microaerophiles such as *Campylobacter*, which can cause intestinal disorders.

Capnophiles or carbon dioxide–loving organisms, they thrive under conditions of low oxygen and high carbon dioxide concentration.

Facultative anaerobes ordinarily carry on aerobic metabolism when oxygen is present, but they shift to anaerobic metabolism when oxygen is absent. *Staphylococcus* and *Escherichia coli* are facultative anaerobes; they often are found in the intestinal and urinary tracts, where only a small amount of oxygen is available.

Aerotolerant anaerobes can survive in the presence of oxygen but do not use it in their metabolism. *Lactobacillus*, for example, always captures energy by fermentation, regardless of whether the environment contains oxygen.

Compared with other groups of organisms defined according to oxygen requirements, facultative anaerobes have the most complex enzyme systems. They have one set of enzymes that enables them to use oxygen as an electron acceptor and another set that enables them to use another electron acceptor when oxygen is not available.

In contrast, the enzymes of the other groups defined here are limited to either aerobic or anaerobic respiration. Obligate anaerobes are killed not by gaseous oxygen but by a highly reactive and toxic form of oxygen called **superoxide** (O_2^-). Superoxide is formed by certain oxidative enzymes and is converted to molecular oxygen (O_2) and toxic hydrogen peroxide (H_2O_2) by an enzyme called **superoxide dismutase**. Hydrogen peroxide is

converted to water and molecular oxygen by the enzyme **catalase**. Obligate aerobes and most facultative anaerobes have both enzymes. Some facultative and aerotolerant anaerobes have superoxide dismutase but lack catalase. Most obligate anaerobes lack both enzymes and succumb to the toxic effects of superoxide and hydrogen peroxide.

MOISTURE

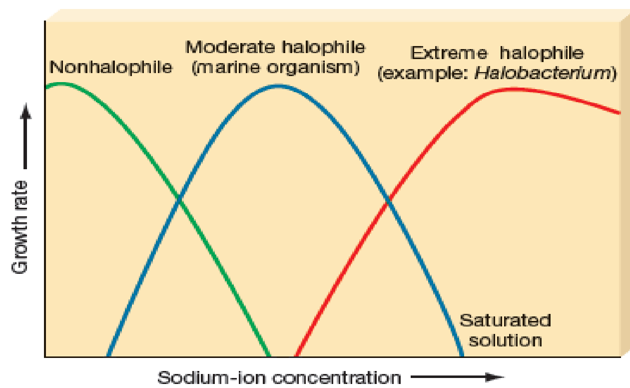
All actively metabolizing cells generally require a water environment. Unlike larger organisms that have protective coverings and internal fluid environments, single celled organisms are exposed directly to their environment. Most vegetative cells can live only a few hours without moisture; only the spores of spore-forming organisms can exist in a dormant state in a dry environment.

Osmotic pressure

The cell membrane allows water to move by osmosis between the cytoplasm and the environment. Osmosis is the diffusion of water across a semipermeable membrane from a less concentrated solution to a more concentrated one, equalising concentrations. The pressure required to make this happen is called the *osmotic pressure*.

If a cell were placed in a hypertonic solution (one whose solute concentration is higher), osmosis would lead to a loss of water from the cell

and undergo **plasmolysis** or shrinking of the cell. This is the basis of using high concentrations of salt or other solutes in preserving foods against microbial attack. In the opposite situation, water would pass from a dilute (hypotonic) solution into the cell, causing it to swell and burst. The rigid cell walls of bacteria prevent them from bursting; this, together with their minute size, makes them less sensitive to variations in osmotic pressure than other types of cell.



(a)

In bacteria, the rigid cell wall prevents cells from swelling and bursting, but the cells fill with water and become *turgid* (distended). Most bacterial cells can tolerate a fairly wide range of concentrations of dissolved substances. Their cell membranes contain transport systems that regulate the movement of dissolved substances across the membrane.

Bacteria called **halophiles** or salt-loving organisms, require moderate to large quantities of salt (sodium chloride). Their membrane transport systems actively transport sodium ions out of the cells and concentrate

potassium ions inside them. Two possible explanations for why halophiles require sodium have been proposed. One is that the cells need sodium to maintain a high intracellular potassium concentration so that their enzymes will function. The other is that they need sodium to maintain the integrity of their cell walls. Halophiles are typically found in the ocean, where the salt concentration (3.5%) is optimum for their growth. Extreme halophiles require salt concentrations of 20% to 30% (**Figure 6.16**). They are found in exceptionally salty bodies of water, such as the Dead Sea, and sometimes even in brine vats, where they cause spoilage of pickles being made there. Obligate *halophiles* such as *Halobacterium* and *Halococcus.*

RADIATION

Radiant energy, such as gamma rays and ultraviolet light, can cause mutations (changes in DNA) and even kill organisms. However, some microorganisms have pigments that screen radiation and help to prevent DNA damage. Others have enzyme systems that can repair certain kinds of DNA damage. The bacterium, *Deinococcus radiodurans* can survive 10,000 Grays (Gy) of radiation. The Gy is a unit of measurement for absorbed dose of radiation. 5 Gy will killa human, and 1,000 Gy will sterilize a culture of *E. coli*. Bacteria that can withstand high levels of radiation may be valuable for use in cleaning up contaminated sites.