FDE 328
INDUSTRIAL MICROBIOLOGY

Effects of Environmental Conditions on Microbial Growth

Effects of Environmental Conditions on Microbial Growth

- Growth and development of microorganisms are greatly affected by the chemical and physical conditions of their environment.

- Important environmental factors affect microbial growth;

1. Temperature
2. pH
3. Oxygen
4. Solutes, water activity
5. Radiation
6. Hydrostatic pressure

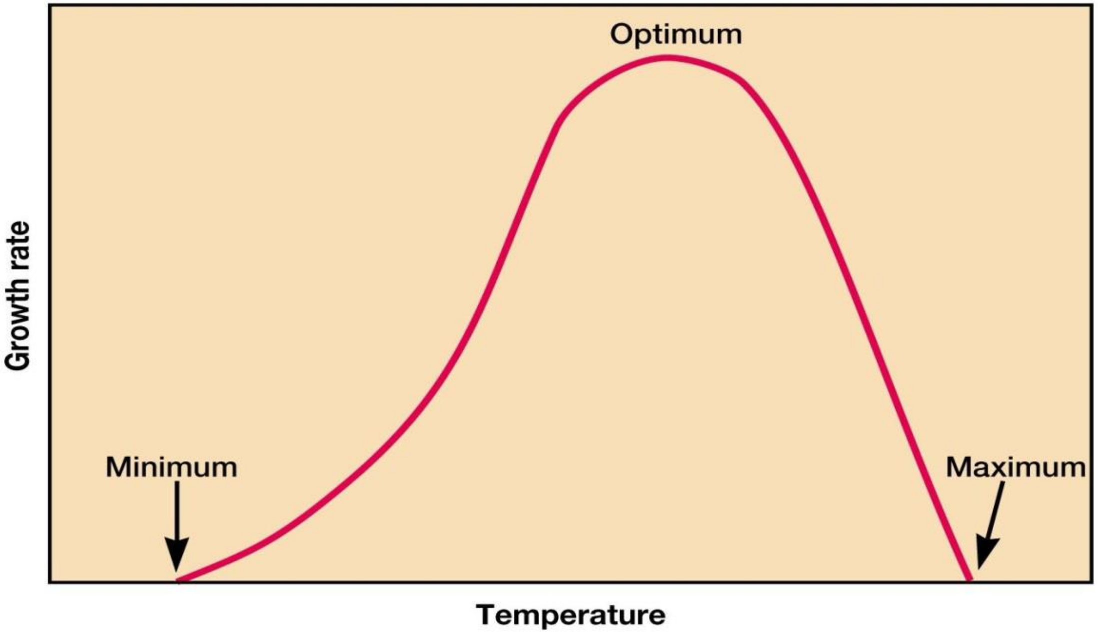
Effects of temperature on growth

- Temperature is an important factor affecting the performance of cells.
- As the temperature rises, the rate of chemical reactions increases.
- This rate doubles for every 10°C rise in temperature.
- Thus, cells should grow faster as the temperature is raised.
- However, there are maximum limits beyond which some temperature-sensitive macromolecules (proteins, nucleic acids and lipids) will become denatured, and hence, non-functional.
- There is also a minimum temperature for growth, below which the lipid membrane is not fluid enough to function properly.
- All organisms have an optimum temperature for growth and different groups of microorganisms have evolved to grow over different temperature ranges.
- A typical microorganism, referred to as **stenothermal**, can grow over a temperature range of approximately 30°C, and **eurythermal** organisms grow over even wider ranges.

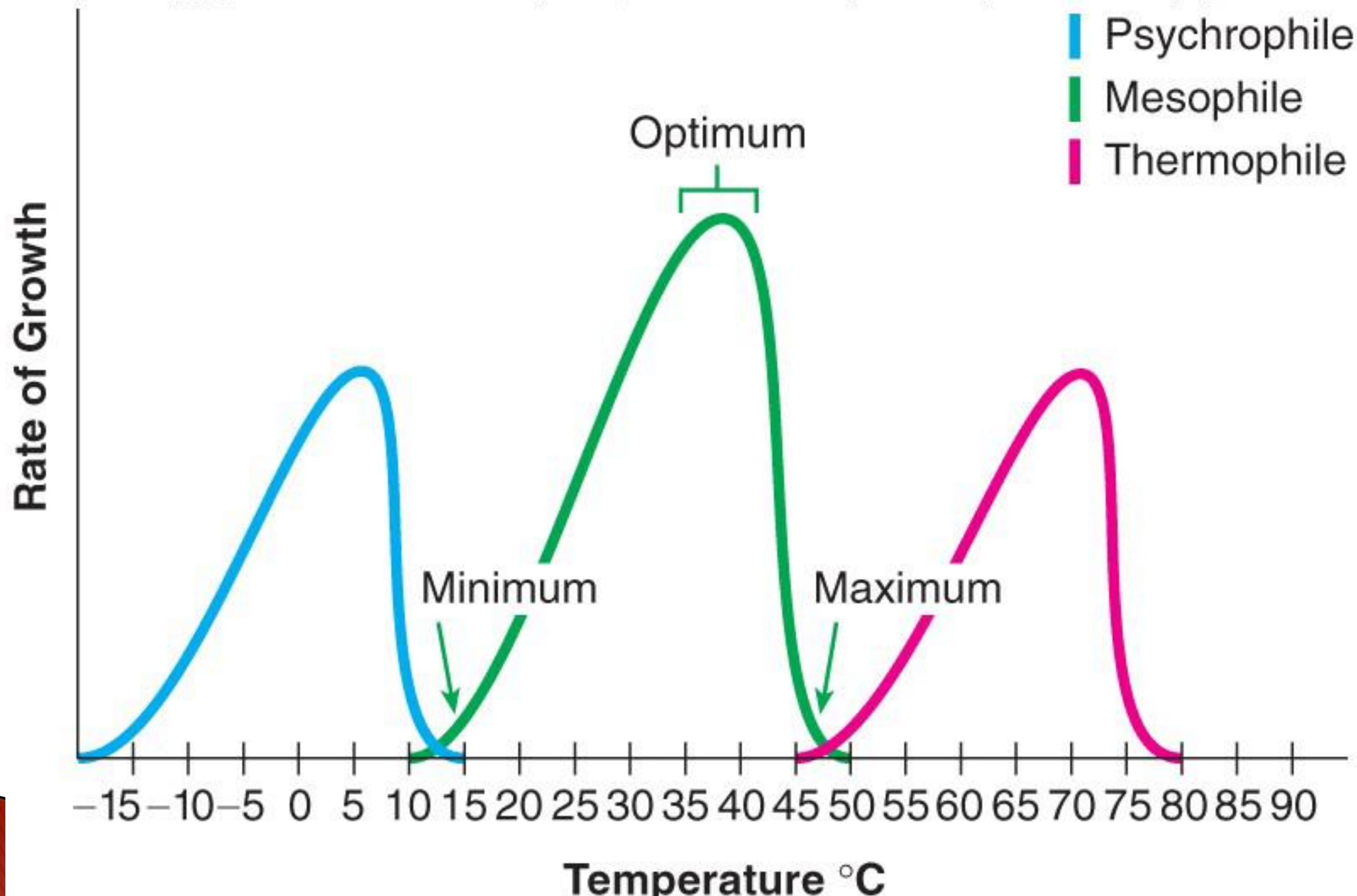
Effects of temperature on growth

Organisms exhibit distinct cardinal growth temperatures

- minimum
- maximum
- optimum



Effects of temperature on growth



Effects of temperature on growth

According to the optimum growth temperatures, microorganisms are classified into five main groups.

- **Psychrophiles**
- **Psychrotrophs**
- **Mesophiles**
- **Thermophiles**
- **Extreme (hyper-) thermophiles**

Effects of temperature on growth

- **Psychrophiles** usually have their optimum temperatures below 15°C and these organisms are often killed by exposure to room temperature. They are able to function at low temperature because their membranes contain a high proportion of unsaturated fatty acids. These molecules remain fluid at the lower temperatures, whereas membranes containing saturated fatty acids become non-functional.

- **Psychrotrophs** are sometimes referred to as facultative psychrophiles. They grow most rapidly at temperatures above 20°C, but are capable of slow growth at temperatures down to around 0°C. Several are responsible for the spoilage of refrigerated products.

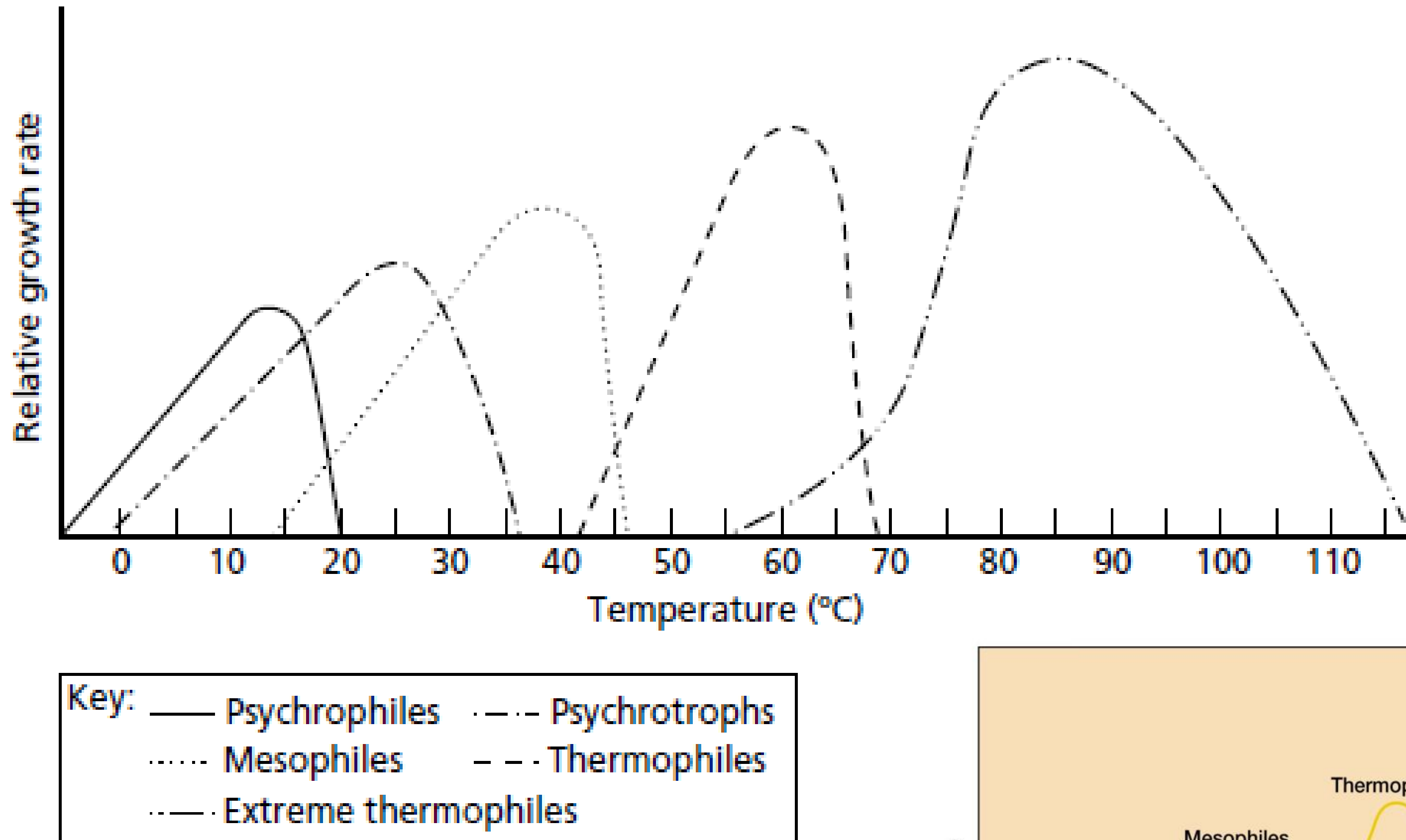
- **Mesophiles** have optimum growth temperatures in the range 20–45°C and a minimum around 15–20°C.

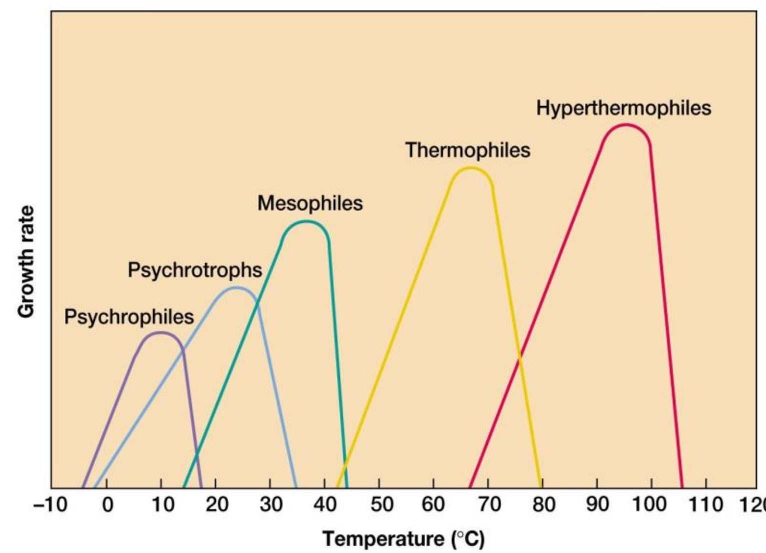
Effects of temperature on growth

- Organisms with optima above 50°C are termed **thermophiles**. Several algal, protozoal and fungal species have temperature maxima up to 55°C. However, it is only certain prokaryotes that are truly thermophilic, as no eukaryotic microorganisms grow at such high temperatures.

- Some **extreme (hyper-) thermophiles** can grow at 100°C or higher. For example, *Pyrolobus fumarii* has an optimum of 106°C and continues growing up to 113°C. All of these are archaeans, which are mainly anaerobic sulphate reducers or have other metabolism with lower requirements for thermolabile cofactors such as NADH and NADPH. They have potential industrial uses in coal desulphurization, metal leaching, methane generation and the production of commercial enzymes, especially DNA polymerases and restriction endonucleases.

Temperature ranges for microbial growth





Effects of pH on growth

- As with temperature, every microorganism has a pH optimum and a pH range over which it grows.
- Generally, fungi tend to grow at lower pHs (pH 4–6) than most bacteria.
- True **acidophiles** have pH optima between 1 and 5.5, and often have mechanisms for the exclusion of protons in order to maintain their internal pH at a higher level.
- However, the majority of microorganisms are **neutrophiles**, growing between pH 5 and 9, as most natural environments fall within this range.
- **Alkalophiles**, such as species of *Bacillus* and *Micrococcus*, have pH optima between 8.5 and 11.5, but take up protons to maintain their internal pH at a lower value.

Effects of pH on growth

- In most fermentations, pH can vary substantially.
- Often, the nature of the nitrogen source can be important.
- If ammonium is the sole nitrogen source, hydrogen ions are released into the medium as a result of the microbial utilization of ammonia, resulting in a decrease in pH.
- If nitrate is the sole nitrogen source, hydrogen ions are removed from the medium to reduce nitrate to ammonia, resulting in an increase in pH.
- Also, pH can change because of the production of organic acids, the utilization of acids (particularly amino acids), or the production of bases.
- Thus, pH control by means of a buffer or an active pH control system is important.

Effects of solutes and water activity on growth

- Actual water availability to a microorganism is indicated by the term **water activity** (a_w).

- This is the ratio of the vapour pressure of water in the solution surrounding the microorganism, P_{soln}, to the vapour pressure of pure water:

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

- i.e. pure water has an a_w of 1.

- Microbial species vary in their tolerance to dry conditions and environments of high osmotic strength.

- Most bacteria, with a few exceptions, cannot grow below an a_w of 0.9, which can provide a valuable means of food preservation.

- Many fungi that cause biodeterioration of stored grain (a_w = 0.7), including *Aspergillus restrictus*, are **xerotolerant** and can grow at low moisture levels.

- However, truly **xerophilic filamentous fungi** and osmophilic yeasts, e.g. *Zygosaccharomyces rouxii*, have evolved to inhabit environments where the a_w can be as low as **0.6**. Some of these species may not grow under conditions of high water availability.

- Marine bacteria are optimally adapted to the salt concentration in sea water (*aw* = 0.98) and possess enzymes that have a specific requirement for sodium ions. True **halophiles**, e.g. *Halobacterium halobium*, found in areas such as the Dead Sea and Salt Lakes, will not grow in salt concentrations less than 3 mol/L. These are archaeans that have highly modified cell walls and unusual membrane lipids.

Effects of High Substrate Concentration:

- High substrate concentrations that are significantly above stoichiometric requirements are inhibitory to cellular functions.
- Inhibitory levels of substrates vary depending on the type of cells and substrate.
- Glucose may be inhibitory at concentrations above 200 g/L (eg, ethanol fermentation by yeast), probably due to a reduction in water activity.
- Certain salts such as NaCl may be inhibitory at concentrations above 40 g/L due to high osmotic pressure.
- Some refractory compounds, such as phenol, toluene, and methanol, are inhibitory at much lower concentrations (eg, 1 g/L).
- Typical maximum noninhibitory concentrations of some nutrients are glucose, 100 g/L; ethanol, 50 g/L for yeast, much less for most organisms; ammonium, 5 g/L; phosphate, 10 g/L; and nitrate, 5 g/L.
- Substrate inhibition can be overcome by intermittent addition of the substrate to the medium.

Effects of oxygen on growth

- Microorganisms are classified into five main groups on the basis of their requirements for oxygen.

1. **Obligate aerobes** grow only in the presence of oxygen, as it is required as the terminal electron acceptor for electron transport in aerobic respiration.
2. **Facultative anaerobes** function with or without oxygen, but grow more efficiently when oxygen is available.
3. **Microaerophiles** require some oxygen for the biosynthesis of certain compounds, but cannot grow at normal atmospheric oxygen concentrations of 21% (v/v). They must have lower oxygen levels of 2–10% (v/v).
4. **Aerotolerant anaerobes** essentially ignore oxygen and grow equally well in its presence or absence.
5. **Obligate anaerobes** cannot tolerate oxygen; exposure to it results in their death.

Effects of oxygen on growth

- In order to grow obligate anaerobes in the laboratory, procedures to exclude oxygen from the culture must be used and all manipulations must be performed in an anaerobic atmosphere. Suitable procedures include:

1. the use of media containing reducing agents, such as thioglycollate, which chemically combine with oxygen;

2. physical removal of oxygen from a growth chamber, by pumping out the air with a vacuum pump, and then flushing the vessel with nitrogen gas ± carbon dioxide;

3. the use of GasPak jars, which involves sealing the microbial cultures inside the jar, along with a palladium catalyst and a hydrogen gas generating system; the oxygen is removed through its reaction with the hydrogen to form water; and

4. anaerobic cabinets for the growth and manipulation of the organisms, which are flushed with a gas mixture of nitrogen : carbon dioxide : hydrogen (80 :10: 10) and have a ‘scrubber system’ to remove residual oxygen.

Effects of radiation on growth

- Visible and ultraviolet (UV) light are parts of the electromagnetic spectrum, which extends from strong radiation (γ-rays) to very weak radiation (heat and radio waves).

- Even visible light, particularly the more energetic violet and blue regions, can be damaging. It may generate singlet oxygen, a very powerful oxidizing agent, which can cause damage to cellular components and may kill some microorganisms.

- UV light, which is most harmful at 260nm, causes specific damage to DNA, notably the formation of thymine dimers.

- Ionizing radiation, such as γ-rays emitted from the excited nucleus of ^{60}Co, generates free radicals, OH• and H•, and hydrated electrons. Resulting damage includes breakage of hydrogen bonds in functional molecules, oxidation of many chemical groups and breakage of DNA strands.

Effects of hydrostatic pressure on growth

- In nature, many microorganisms are never subjected to pressures in excess of 1 atm (101.3 kPa or 1.013 bar) and higher pressures generally inhibit microbial growth.

- For example, in cider production, yeast performance is impaired where fermenter depths are greater than 14.5m, which produce hydrostatic pressures above 1.5atm.

- However, many marine organisms must be **barotolerant**, as they live at depths in oceans where they may be subjected to pressures of up to several hundred atmospheres, and only fail to grow at above 200–600atm. Some may even grow better under these conditions and are truly **barophilic**, capable of living under 700– 1000atm.