Biophysical and chemical influences on microbial growth

Microbes can be **damaged or killed** by elements of their **physical and chemical** environment.

These include:

- **Irradiation**
- **UV radiation**
- **Pulsed electric fields (PEF)**
- **Pulsed magnetic fields (PMF)**
- **Low temperatures**
- **High temperatures**

- **Ozone (O_3)**
- **Osmotic pressure**
- **Oxygen**
- **pH**
- **Nutritional requirements**

  (energy, carbon, nitrogen sources; minerals; growth factors; water)

Irradiation

- Ionising radiation **kills** cells indirectly by creating **reactive free radicals** (hydroxyl radical, superoxide anion radical, hydrogen peroxide, oxygen singlet, hypochlorite, nitric oxide radical, and peroxynitrite radical).

- These free radicals can attach to and chemically **alter sensitive macromolecules** in the cell leading to their inactivation.

- Most of the cell's macromolecules are affected by ionizing radiation, but **damage to the DNA is most often the cause of cell death.**

- DNA often contains **only a single copy** of its genes (in prokaryotes).

- **Proteins**, on the other hand, often have **several copies** so that damage of one will not lead to cell death, and in any case may always be **re-synthesized** provided the DNA has remained intact.

- Bacteria such as *Deinococcus radiodurans* are particularly **resistant to radiation**, but are not pathogenic.

- The **spores of gram-positive bacteria** contain **storage proteins that bind tightly to DNA**, possibly acting as a protective barrier to radiation damage resulting in very long survival.

UV radiation

- Ultraviolet radiation has been used as a **germicide** by both industry and medicine for more than a century.

- It **inactivates DNA** by making **thymine dimers** which cannot be copied**.**

Thymine Dimers are produced when adjacent thymidine residues are covalently linked by exposure to ultraviolet radiation. Covalent linkage may result in the dimer being replicated as a single base, which results in a frameshift mutation.

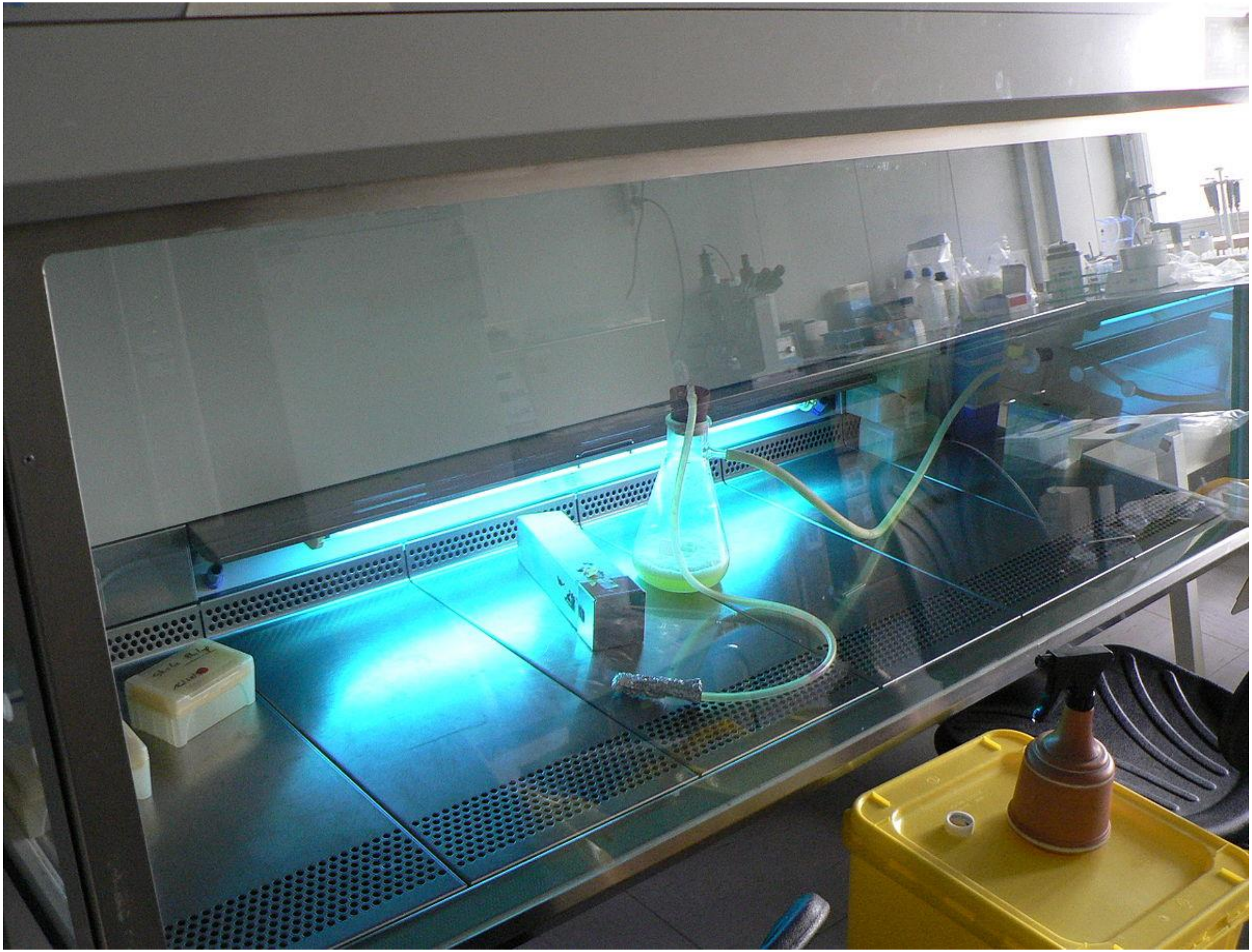
- UV-A radiation may produce **free radicals** that interacts with and oxidizes DNA bases.*

  These oxidized bases don't pair correctly during replication, causing **mutations**.

    One example of this is a G to T transversion** mediated by reactive oxygen species.

* There are three types of UV radiation, UV-A, UV-B and UV-C.

** Transversion refers to a point mutation in DNA in which a single (two ring) purine (A or G) is changed for a (one ring) pyrimidine (T or C), or vice versa.



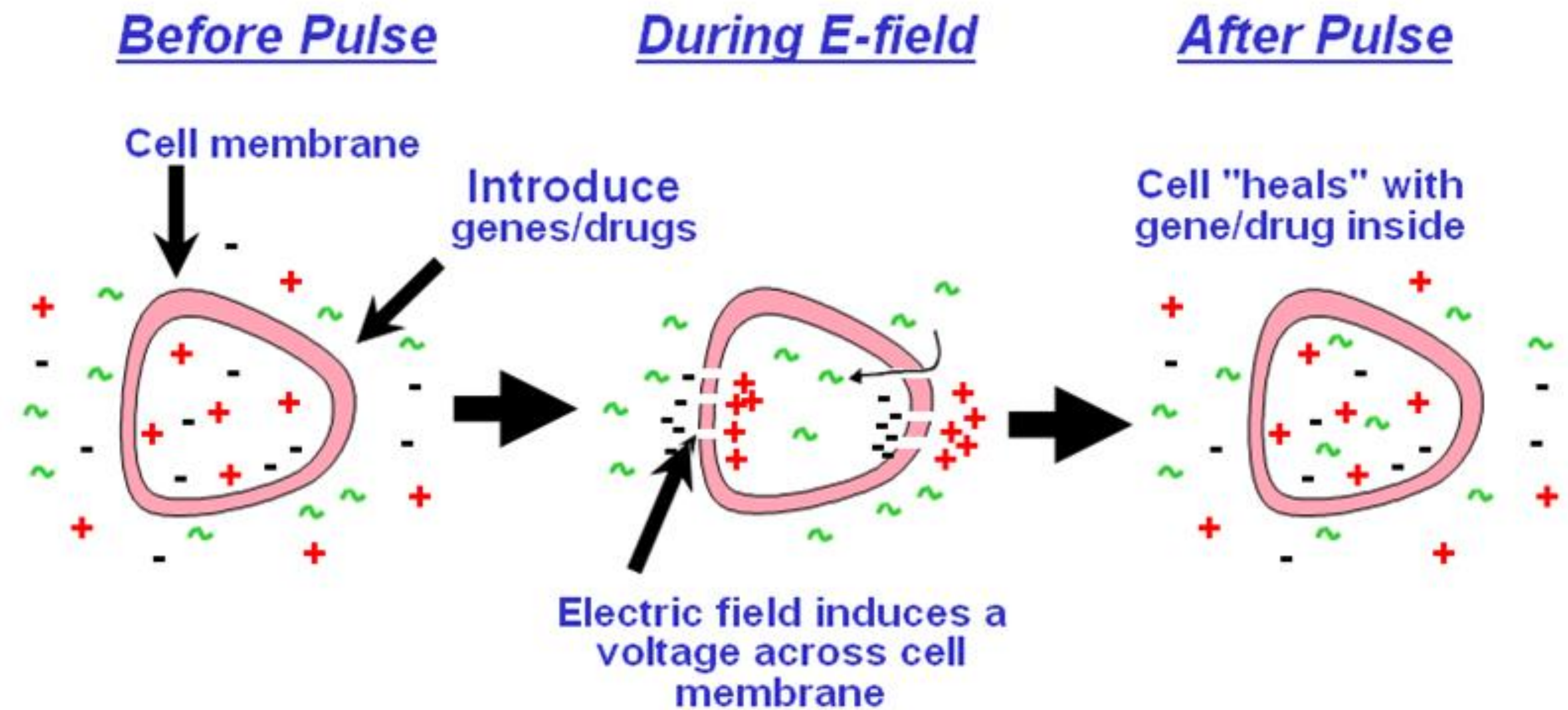
Pulsed electric fields (PEF)

- Strong electric field pulses* applied to cells cause their membranes to develop pores (**electroporation**), **increasing membrane permeability** resulting in undesirable migration of chemicals.

- Pulses of **low intensity** may result in the increased production of secondary metabolites and a **build-up of resistance** (antibiotics are secondary metabolites).

- PEF treatment is an adequate process for inactivation of microbes in **acids** and other **thermosensitive media**, but holds inherent dangers of developing resistance because of incomplete destruction.

* Pulse means supply in short bursts not continuous.


Before Pulse
During E-field
After Pulse
Cell membrane
Introduce genes/drugs
Cell "heals" with gene/drug inside
Electric field induces a voltage across cell membrane

Pulsed magnetic fields (PMF)

- A 2004 study found that *E. coli* is susceptible to pulsed magnetic fields with a survivability figure of **1 in 10,000**.
- As with PEF, cell walls become **porous** with resultant cell death.
- Enzymes such as lactoperoxidase, lipase and catalase are **readily inactivated**, though with varying degrees of susceptibility.

Low temperatures

- Keeping food at **low temperature** to preserve its quality has been used since time immemorial. This is because microbes cannot multiply efficiently.

- Low temperature **curb the spoiling effect** of microorganisms in food, **but can also preserve some pathogens** unharmed for long periods of time.

- Some microorganisms are killed by **physical trauma**, others are sub-lethally injured, and may recover to become infectious.

High temperatures

- **Extreme** temperatures **destroy viruses and vegetative cells** (normal growing cells) that are active and metabolizing.

- **Organic molecules** such as proteins, carbohydrates, lipid and nucleic acids, as well as cell walls and membranes, are **damaged by excessive heat.**

- Food for human consumption is routinely heated by **baking, boiling and frying** to temperatures which **destroy most pathogens.**

- **Autoclaves** generate **steam at higher than boiling point** and are used to sterilize laboratory glassware, surgical instruments, and medical waste.

- Autoclaving is necessary to kill **bacterial and fungal spores**.

- Life on earth, with regard to temperature, exists wherever **water remains in a liquid state.**

- Thus, psychrophiles **grow in solution** wherever water is **supercooled below 0 °C.**

- **Extreme thermophilic archaea** (hyperthermophiles) have been identified growing near **deep-sea thermal vents at temperatures up to 120 °C.**

Microorganisms are usually classified according to their **optimum temperature range**:

- **Psychrophiles** grow best at cooler temperatures ranging from -**5°C to 20°C**.
- These bacteria can be found growing in **refrigerators**, on **snow**, in the **Arctic** and **Antarctic** regions, and **deep in the ocean**.
- **Psychrotrophs** are organisms that **grow slowly at temperatures below 20°C.**

- **Mesophiles**, which include **most disease-causing forms** of bacteria, grow best at temperatures ranging from **20°C to 40°C**.

- **Thermophiles**, which include bacteria growing in **hot springs** and **thermal vents**, prefer warmer temperatures ranging from **40°C to 80°C.**

- **Hyperthermophiles**, which can be found in **geysers** and **volcanoes**, grow best at temperatures **above 80°C.**

Ozone (O_3)

- Microorganisms suffer a **reduction in viability** on contact with ozone which **effect their cell walls**.

- **Gram-negative bacteria** are **more vulnerable** to ozone than gram-positive organisms.

- Ozone acts by **increasing** cell membrane permeability.

Osmotic pressure

- Osmosis is the **diffusion of water across a membrane** from an area of **higher water concentration** (lower solute concentration) to lower water concentration (higher solute concentration).

- Osmosis is powered by the **potential energy of a concentration gradient** and does not require the expenditure of metabolic energy.

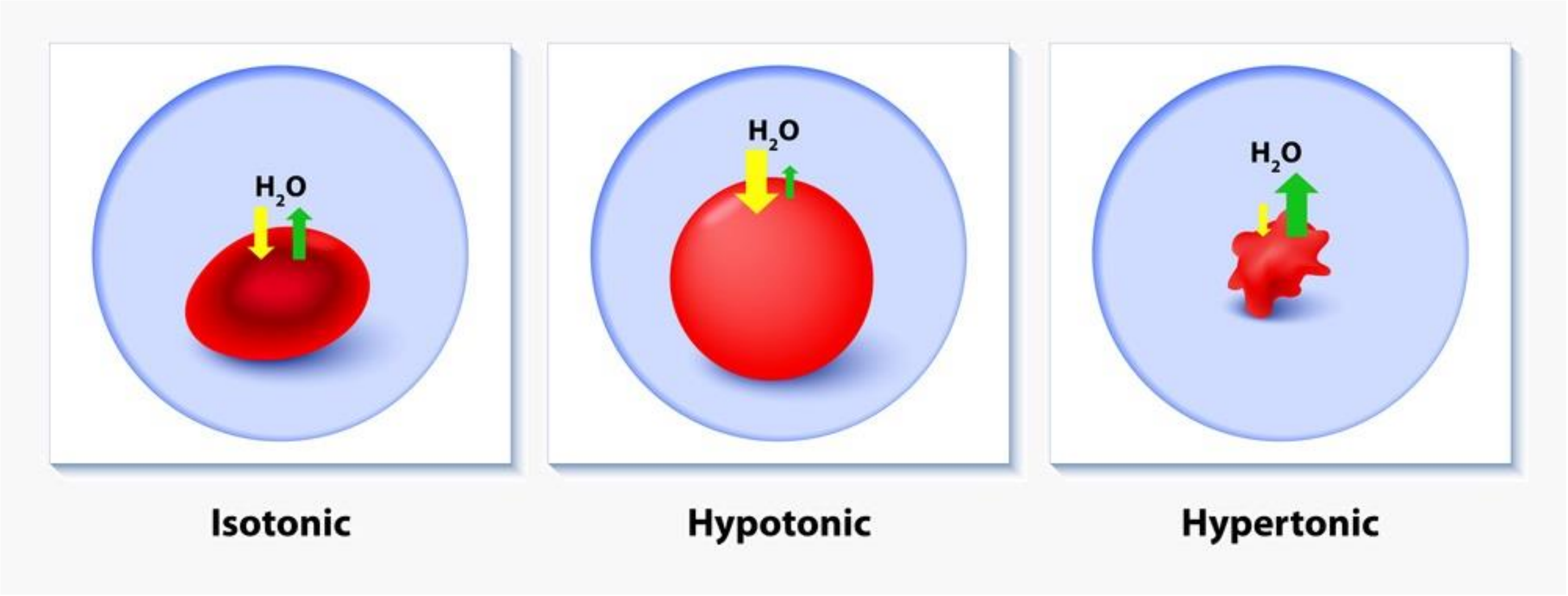
A cell can find itself in one of three environments:

- **isotonic**
- **hypertonic**
- **hypotonic**

- In an **isotonic environment**, both the **water and solute concentration are the same inside and outside the cell** and water goes into and out of the cell at an equal rate.

- If the environment is **hypertonic**, the **water concentration is greater inside the cell** while the solute concentration is higher outside. Water **goes out** of the cell.

- In an environment that is **hypotonic**, the **water concentration is greater outside the cell** and the solute concentration is higher inside. Water **goes into** the cell.



- Most bacteria require **an isotonic or a hypotonic** environment for optimum growth.

- Organisms that can grow at **relatively high salt concentration** (up to **6%**) are said to be **osmotolerant**.

- Those that require relatively high salt concentrations for growth, like some of the Archea that require **sodium chloride concentrations of 6-18%** or higher are **halophiles.**

Oxygen

- Microbes display a **great diversity** in their ability to use and to tolerate oxygen.
- In part, this is because of the **paradoxical nature** of oxygen which can be **both toxic and essential to life**.
- An **obligate aerobe** is an organism that **requires oxygen to grow**.
- Through **cellular respiration**, these organisms use oxygen to metabolize substances like sugars or fats to obtain energy.

- Examples of **obligately aerobic bacteria** include *Mycobacterium tuberculosis* and *Nocardia asteroides*.

- With the exception of the yeasts, **most fungi are obligate aerobes**.

- Also, almost **all algae are obligate aerobes**.

- **Obligate anaerobes** are microorganisms that are killed by normal atmospheric concentrations of oxygen **(21% O_2).**

- Oxygen tolerance **varies** between species, some capable of surviving in **up to 8%** oxygen, others losing viability unless the oxygen concentration is **less than 0.5%.**

- The anaerobes do not have enzymes to **neutralize** free oxygen species. These enzymes include catalase and superoxide dismutase (SOD).

- An important distinction needs to be made here between the **obligate anaerobes** and the **microaerophiles**.

- **Microaerophiles**, like the obligate anaerobes, are damaged by normal atmospheric concentrations of oxygen.

- Microaerophiles, however, **require some oxygen** (typically 2-10% O_2) for growth.

pH

- Each organism has a **pH range** within which growth is possible, and most have well defined pH optima.

- Most **natural environments** have pH values between **5–9**, and most organisms have pH optima in this range.

Microorganisms can be placed in one of the following groups based on their **optimum pH** requirements:

- **Neutrophiles** grow best at a pH range of 5 to 8, e.g., almost pathogenic bacteria
- **Acidophiles** grow best at a pH below 5.5, e.g., *Helicobacter pylori*, *Pyrodictium occultum*
- **Alkaliphiles** grow best at a pH above 8.5, e.g. *Nitrosomonas, Nitrobacter*

Nutritional requirements

- In addition to a proper physical environment, microorganisms also depend on an available **source of chemical nutrients**.

- Microorganisms are often **grouped** according to their **energy source** and their **source of carbon**.

Energy source

- **Phototrophs** use **radiant energy (light)** as their primary energy source.

- **Chemotrophs** use the **oxidation** and **reduction** of chemical compounds as their primary energy source.

Carbon source

Carbon is the **structural backbone** of the organic compounds that make up a living cell.

Based on their source of carbon, bacteria can be classified as **autotrophs** or **heterotrophs**.

1. **Autotrophs:** Require only carbon dioxide as a carbon source. An autotroph can synthesize organic molecules from inorganic nutrients.

2. **Heterotrophs:** Require organic forms of carbon. A heterotroph cannot synthesize organic molecules from inorganic nutrients.

Nitrogen source

- Nitrogen is needed for the **synthesis** of such molecules as **amino acids, DNA, RNA** and **ATP**.

- Depending on the organism, **nitrogen, nitrates, ammonia,** or **organic nitrogen compounds** may be used as a nitrogen source.

Minerals

1. Sulfur

- Sulfur is needed to **synthesize sulfur-containing amino acids** and **certain vitamins**.

- Depending on the organism, **sulfates, hydrogen sulfide,** or **sulfur-containing amino acids** may be used as a sulfur source.

2. Phosphorus

- Phosphorus is needed to **synthesize phospholipids, DNA, RNA,** and **ATP.**

- **Phosphate ions** are the **primary source** of phosphorus.

3. Potassium, magnesium, and calcium

- These are required for **certain enzymes** to function.

4. Iron

Iron is a part of **certain enzymes**.

5. Trace elements

- Trace elements are elements required in **very minute amounts**, and like **potassium, magnesium and calcium**; they usually function as **cofactors** in enzyme reactions.
- They include **sodium, zinc, copper, molybdenum, manganese,** and **cobalt ions**.
- **Cofactors** usually function as **electron donors or electron acceptors** during **enzyme reactions**.

Growth factors

- Growth factors are **organic compounds** such as amino acids , purines , pyrimidines , and vitamins that a cell must have for growth but **cannot synthesize itself**.

- Organisms having **complex nutritional requirements** and needing many growth factors are said to be **fastidious**.

Water

- Water is the **most essential factor** for bacterial growth.

- Available water in the culture media determines the **rate of metabolic and physiological activities** of bacteria.

- **Sugar, salts** and other substances are **dissolved** in water and are made available for bacteria.


Thank You!