Chapter 1: Introduction

1. Importance of microorganisms in food

1.1 Microorganisms: three key roles in the food industry

Microorganisms (bacteria, yeasts, molds) play **a triple fundamental role** in the field of food. Understanding this dual nature—both beneficial and harmful—is essential for mastering food processing and preservation.

- **A. Spoilage agents**
  - **Definition:** Microorganisms responsible for the deterioration of the organoleptic characteristics of a food (taste, smell, texture, appearance). They render the product unfit for consumption without necessarily posing an immediate health hazard.
  - **Examples:**
    - **Lactic acid bacteria and others:** Acidification, unwanted fermentation, gas production, biofilm formation.
    - **Molds:** Visible mycelial growth, mycotoxin production, texture spoilage.
    - **Yeasts:** Packaging swelling, alcohol or unpleasant odor production.
  - **Consequence:** Major economic loss for the industry and the consumer.
- **B. Agents causing foodborne toxi-infections**
  - **Definition:** Pathogenic microorganisms or toxin-producers that, when ingested, cause illness in humans (gastroenteritis, systemic infections, sometimes fatal).
  - **Two main mechanisms:**
    1. **Infection:** The microorganism itself colonizes and infects the host (e.g., *Salmonella*, *Listeria monocytogenes*, *Campylobacter*).
    2. **Intoxication:** The preformed toxin in the food produced by the microorganism is ingested (e.g., toxins from *Staphylococcus aureus*, *Bacillus cereus*, *Clostridium botulinum*).
  - **Consequence:** Major public health risk, with strict legal and regulatory implications (microbiological criteria, HACCP plans).
- **C. Agents used in food manufacturing**
  - **Definition:** Microorganisms selected and controlled to transform raw materials and confer specific qualities to foods.
  - **Examples:**
    1. **Lactic starters:** Yogurt (*Lactobacillus bulgaricus*, *Streptococcus thermophilus*), cheeses (*Lactococcus lactis*), dry sausages.
    2. **Yeasts:** Baking, winemaking, beer production (*Saccharomyces cerevisiae*).
    3. **Molds:** Cheese ripening (*Penicillium roqueforti*, *P. camemberti*).
    4. **Biotechnological starters:** Production of enzymes, flavors, natural preservatives.
  - **Consequence:** They are at the heart of **bioprocesses**. Controlling them requires keeping them alive and active, which can conflict with the goals of inactivating undesirable flora.

2. Importance of understanding mechanisms and factors of destruction/survival

2.1 Why study inactivation mechanisms?

Microbial inactivation is not simply an "all or nothing" event. It is the result of accumulated damage to the vital structures and functions of the cell. Understanding these mechanisms allows us to:

- **Optimize processes:** Adjust the treatment intensity (time, temperature, radiation dose, disinfectant concentration) to achieve the inactivation goal with maximum efficiency and minimal cost.
- **Predict and Model:** Develop reliable mathematical models of destruction (survival curves, D-value, z-value) to predict the effectiveness of a treatment under different conditions.
- **Explain Resistances:** Understand why some microorganisms (bacterial spores, certain osmotolerant yeasts) are more resistant to given treatments.
- **Develop New Technologies:** Design combinatorial methods (hurdle technology) that act synergistically on different cellular targets.
- **Avoid Under- or Over-Processing:** Insufficient treatment leaves sanitary risks, while excessive treatment degrades quality and wastes energy.

2.2 Factors influencing destruction or survival

The effectiveness of an inactivation treatment depends on both:

- **The microorganism (Intrinsic factor):** Species, strain, growth phase (exponential vs. stationary), physiological state (vegetative cell vs. spore), prior history (adaptive stress).
- **The food environment (Intrinsic factor of the medium):** Water activity (Aw), pH, composition (lipids, proteins, salts), presence of natural inhibitors.
- **The applied treatment (Extrinsic factor):** Nature (heat, cold, high pressure, radiation, chemicals), intensity, duration, application kinetics.

3. Physiological aspects of microorganisms related to survival

3.1. Composition and structure of microbial cells

3.1.1. Two major structural types

Microorganisms are divided into two fundamental categories based on their cellular organization, a distinction with major implications in biology, medicine, and industry:

1. **Prokaryotes** (cells without a true nucleus):
    - **Bacteria** (Domain *Bacteria*)
    - **Archaea** (Domain *Archaea*)

The genetic material (DNA) is free in the cytoplasm, in a region called the *nucleoid*.

2. **Eukaryotes** (cells with a nucleus):
    - **Fungi** (Yeasts, molds), **Protozoa**, **Microscopic algae**.

DNA is contained within a membrane-bound nucleus. They possess membrane-bound organelles.

This course will focus primarily on the bacterial (prokaryotic) structure.

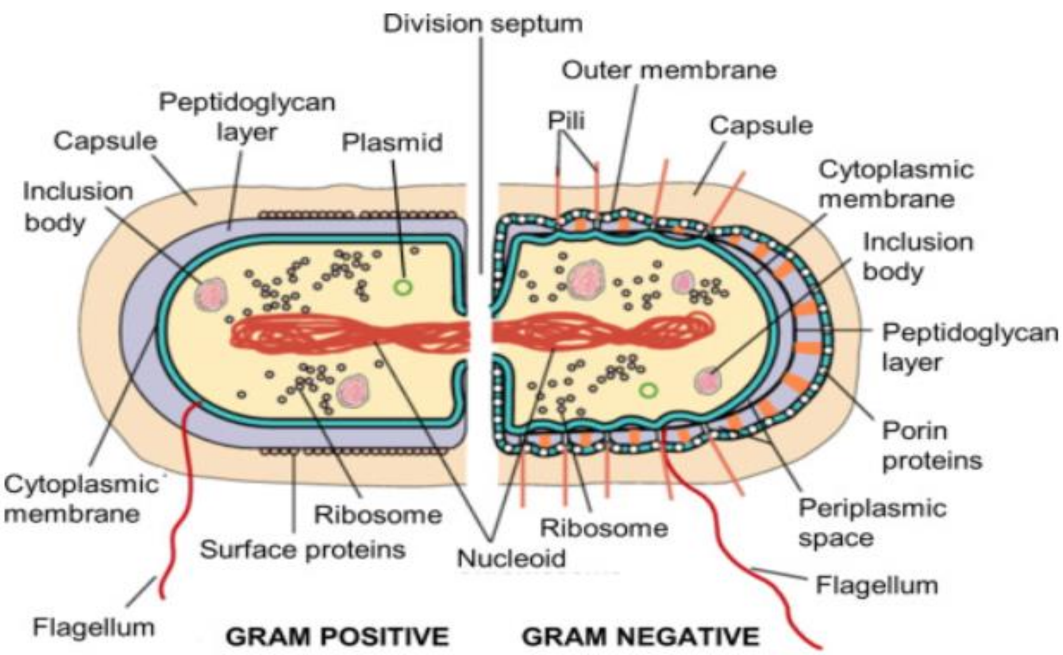
3.1.2. General chemical composition of a bacterial cell

A bacterial cell is composed of ~70% water. The remaining 30% (dry weight) is distributed as follows:

- **Proteins** (≈50% of dry weight): Enzymes, structural proteins, transporters.
- **Nucleic Acids** (≈20%):
  - **DNA** (≈3-4%): Genetic information, a single circular chromosome (+ plasmids).
  - **RNA** (≈15-17%): rRNA (ribosomes), tRNA, mRNA for protein synthesis.
- **Polysaccharides** (≈15-20%): Cell wall (peptidoglycan), glycogen, capsules.
- **Lipids** (≈10%): Cytoplasmic membrane, lipid reserves.
- **Others** (mineral salts, ions, various metabolites).

3.1.3. Cellular structures and their functions

We distinguish between **essential** structures (present in all bacteria) and **facultative** structures (variable depending on species or conditions).



A. Essential structures (required for life)

1. The cytoplasmic membrane

- **Structure**: A phospholipid bilayer (fluid mosaic model) with embedded proteins. No sterols (except in *Mycoplasma*).
- **Crucial functions**:
  - Selective barrier: Regulates exchanges (nutrients in, waste out).
  - Site of the respiratory chain and ATP synthase (Energy production) in aerobic bacteria.
  - Site of synthesis for cell wall components (peptidoglycan, LPS).
  - Chromosome segregation during division.

2. The cytoplasm

- **Fundamental substance** (cytosol) containing soluble components: enzymes, nutrients, ions.
- **Ribosomes** (70S): Sites of protein synthesis. Composed of rRNA and proteins.
- **Nucleoid**: Region containing the single, circular, supercoiled bacterial chromosome. **No nuclear membrane**.

3. The cell wall

- A rigid structure, external to the membrane. It is the target of major antibiotics (penicillin, cephalosporins).
- Its composition differentiates bacteria into two major groups, identifiable by **Gram staining**.

3.1.4. Cellular envelopes:

Table: The Gram (+) / Gram (-) difference.

Characteristic	Gram-Positive Bacteria (G+)	Gram-Negative Bacteria (G-)
Gram Stain	Violet (retains the crystal violet-iodine complex)	Pink/Red (retains the counterstain, safranin)
Wall Thickness	**Thick** (20-80 nm)	**Thin** (2-3 nm)
Structure	**Single, thick layer of peptidoglycan** (40-90% of the wall). Extensive cross-linking.	**Thin layer of peptidoglycan** (5-10% of the wall). Weak cross-linking.
Periplasmic Space	**Limited** (between membrane and thick wall).	**Highly developed**. Compartment between the two membranes, rich in enzymes (hydrolytic, transport).
Membranes	One: the cytoplasmic membrane.	**Two membranes**: 1. Cytoplasmic (inner) membrane. 2. Outer membrane (unique to G-).
Specific Components	**Teichoic acids** (linked to peptidoglycan or the membrane). Role in adhesion, ion regulation.	**Lipopolysaccharides (LPS)** anchored in the outer membrane. Composed of: - Lipid A (endotoxin, toxic). - Core polysaccharide. - O-specific chain (specific antigen).
Permeability	Relatively permeable to molecules.	**Effective barrier** due to the outer membrane and porins (specific protein channels). Natural resistance to some antibiotics, detergents, dyes.
Examples	*Staphylococcus aureus*, *Bacillus subtilis*, *Lactobacillus* spp., *Streptococcus thermophilus*.	*Escherichia coli*, *Salmonella*, *Pseudomonas aeruginosa*, *Neisseria gonorrhoeae*.

- General functions of the cell wall (for both G+ and G-):
  - Determines cell shape (coccus, rod, spiral).
  - Protects against osmotic pressure (prevents lysis in hypotonic media).
  - Site of action for β-lactam antibiotics.
  - Role in adhesion to surfaces.
  - Carries antigenic determinants (induces an immune response).

3.1.5. Facultative (or accessory) structures

1. The capsule or slime layer (Glycocalyx)

- An external, viscous, usually polysaccharide (sometimes polypeptide) envelope.
- **Functions**: virulence (protects against phagocytosis), adhesion (biofilm formation), protection against desiccation.

2. Flagella

- Long, thin protein appendages (flagellin) responsible for motility. Their arrangement (polar, peritrichous) is characteristic.

3. Pili or Fimbriae

- Protein appendages shorter and more numerous than flagella.
- **Functions**: Adhesion to surfaces (common pili), genetic transfer during conjugation (sex pili).

4. Spores (or endospores)

- Resistant structure produced by certain genera (*Bacillus*, *Clostridium*).
- Dormant, highly resistant to heat, radiation, chemical disinfectants.
- Allows survival in extreme conditions for long periods.
- Is not a mode of reproduction.

5. Plasmids

- Small, circular, double-stranded DNA molecules, independent of the chromosome.
- Often carry genes conferring advantages: antibiotic resistance, virulence factors, ability to degrade specific substrates.
- Replicate autonomously, transferable between bacteria (conjugation).

3.2 Cellular homeostasis: maintaining vital balance

Homeostasis is a cell's ability to maintain constant internal conditions despite variations in the external environment. Its disruption is a major cause of inactivation.

- **A. Maintenance of intracellular pH (pHi)**
  - **Principle:** The cytoplasmic pH is maintained around neutrality (pHi ~7.5) even if the external medium (pHe) is very acidic or very alkaline. This is crucial for enzymatic activity.
  - **Mechanisms:**
    1. **Barrier to proton (H+) penetration:** The cytoplasmic membrane is poorly permeable to H+.

2. **Active transport systems:**
   - **Active ejection of H+** via ATPase pumps (F0F1) or respiratory chains.
   - **Ion exchangers:** E.g., the K+/H+ antiport (K+ entry, H+ exit).
- **Related stress:** In an acidic food (low pHe), H+ accumulate inside the cell if the ejection mechanisms are overwhelmed. The drop in pHi denatures proteins and nucleic acids, inhibiting metabolism. Lactic acid bacteria are adapted to this stress.

- **B. Maintenance of the transmembrane potential (ΔΨ)**
  - **Principle:** Difference in electrical charge across the cytoplasmic membrane (generally negative inside, positive outside). It is a "biological battery."
  - **Roles:** It is essential for:
    1. **Active transport** of many nutrients (co-transport with ions).
    2. **ATP production** via ATPase (flow of H+ protons down the electrochemical gradient).
    3. **Flagellar motility.**
  - **Target of treatments: Ionophores** (e.g., nisin, some organic acids) create pores in the membrane, allowing ions (H+, K+) to diffuse freely. This dissipates the ΔΨ, blocking transport and energy production, leading to cell death.

- **C. Maintenance of Cytoplasmic Turgor (Osmotic Pressure)**
  - **Principle:** The rigid cell wall prevents the cell from bursting under the effect of internal pressure generated by water influx. This balance between internal pressure and wall resistance creates turgor, essential for growth and division.
  - **Adaptation mechanisms in a hypertonic medium (low Aw, high in salt or sugar):**
    1. **Accumulation of compatible solutes:** The cell synthesizes or imports organic molecules (proline, glycine betaine, trehalose) that accumulate in the cytoplasm without disrupting cellular functions. This balances osmotic pressure without causing water loss.
  - **Related stress:** In a hypotonic medium, water enters massively. Without a strong wall (e.g., protoplasts), the cell lyses. In a very hypertonic medium, if the cell cannot adapt, water exits, the cytoplasm retracts (plasmolysis), and metabolism stops (dormant state or death).

Microbial inactivation most often results from multiple and synergistic lesions that compromise the **integrity of cellular structures** (envelope) and, critically, **disrupt homeostasis**. The success of an inactivation method depends on its ability to target and overwhelm the cell's defense and repair mechanisms.