Chapter 3: Industrial culture media

1. Overview of Industrial Culture Media

What is Fermentation Media?

Fermentation media are nutrient solutions used to grow microorganisms in industrial fermentations. Their purpose is to provide all the nutritional requirements for the microorganism and to support the production of a target product, which could be **cell biomass** (like yeast) or a **metabolite** (like antibiotics or ethanol).

- **For primary metabolites or biomass**: The medium is designed to support maximum **growth**.
- **For secondary metabolites** (e.g., antibiotics): The medium is designed to first allow growth, then **limit one nutrient** to trigger production of the metabolite (since production is not linked to growth).

- The composition of culture media can vary depending on the specific needs of the target microorganisms and the desired final products.

- Culture media are often composed of carbon sources, nitrogen sources, mineral salts, as well as growth factors to optimize microorganism growth and the production of desired substances.

- The culture media used in industrial processes are generally made from raw materials.

- They are prepared in very large quantities in tanks, the volume of which can reach several cubic meters.

- Additionally, an anti-foaming agent and buffering chemicals may also be added to the medium.

- When biomass is the target product, the production medium must allow for optimal growth of the microorganism.
- For the production of secondary metabolites (such as antibiotics, whose biosynthesis is not linked to growth), media are designed to provide an initial period of cell growth, followed by conditions optimized for secondary metabolite production.
- Culture media can be: **synthetic, semi-synthetic, or complex**.
  Culture media can be **liquid** or **solid**.

2. General Components of Media

1.Water: Main component; must be clean and consistent.

Water is a major component in all culture media and is responsible for all metabolic reactions in industrial fermentation processes (except for solid-state fermentations).

•Its quality is very important for obtaining optimal and reproducible cultures (e.g., demineralized and deionized water are obtained through membrane filtration techniques).

2. Carbon source: Provides energy and building blocks for biosynthesis (e.g., sugars, oils, alcohols).

Types of Carbon Sources

$$Y_{carbon}\ (g/g) = \text{biomass produced (g)} / \text{carbon substrate utilized (g)}$$

•**Molasses**: Cheap, contains sucrose, vitamins, and minerals.

•**Malt extract**: Good for fungi and yeasts; contains sugars and nitrogen.

•**Starch**: From corn or potatoes; must be broken down into sugars.

•**Sulfite waste liquor**: From paper industry; used for yeast cultivation.

•**Whey**: Dairy byproduct; contains lactose.

•**Alkanes and alcohols**: Methanol and ethanol can be used but are toxic at high levels.

•**Oils**: High energy content; useful in fed-batch processes.

3. Nitrogen source: For proteins and nucleic acids (e.g., ammonium salts, yeast extract, corn steep liquor).

•For bacteria, the average nitrogen composition is 12.5%. To produce 5g of biomass per liter, 625 mg of nitrogen must be used.

•**Types of Nitrogen Sources:** Nitrogen is generally used in organic or inorganic form

•**Inorganic**: Ammonium salts, ammonia.

•**Organic**: Corn steep liquor, yeast extract, peptones, soya meal.

- **Corn steep liquor**: Rich in amino acids and minerals; used in penicillin production.
- **Yeast extract**: Contains vitamins and peptides; must be low in salt to avoid corrosion.
- **Peptones**: From hydrolyzed proteins; expensive but rich in amino acids.
- **Soya meal**: Slowly metabolized; good for antibiotic fermentations.

4. Minerals: Include phosphorus, sulfur, magnesium, and trace elements (iron, zinc, etc.).

Mineral salts are essential and have very diverse roles:

•**Constituent elements** of antibiotics (P, S, Cl…); and of biomass (Mg, P, Cl, Ca, Fe,…)

•**Responsible for osmotic pressure** (NaCl, KCl,….); **pH modifiers** ($CaCO_3$….)

•**Precipitation agents** (NH_4-SO_4-2); **Reaction catalysts** (Mg^{+2}, Mn^{+2}, $Fe^{+2/+3}$, Zn)

5. Vitamins: Some microbes cannot synthesize them and need supplements (e.g., biotin).

6. Oxygen: For aerobic fermentations; supplied as air or pure oxygen.

7. Antifoams: Prevent foam that can block filters and cause contamination.

8. Buffers: To maintain pH; acids or bases may be added during fermentation.

9. Precursors, inducers, inhibitors: Added at specific times to guide or enhance product formation.

- ✓ **Precursors** are intermediate molecules that serve as starting substrates for reactions during the biosynthesis of secondary metabolites such as antibiotics.
  They are often added in a controlled manner and in a relatively pure form. For example:

•Phenylacetic acid is added for the side chain in penicillin production.

•D-threonine is used as a precursor in the production of L-isoleucine by *Serratia marcescens*.

- ✓ **Inducers**: Trigger gene expression in genetically modified microbes or plant cells.
- ✓ **Inhibitors**: Block unwanted metabolic pathways.
- ✓ **Permeability modifiers**: Help release intracellular products (e.g., surfactants).

The main factors that affect the final choice of individual raw materials are as follows.

1- **Cost and availability**: ideally, materials should be inexpensive, and of consistent quality and year round availability.

2 - **Ease of handling in solid or liquid forms**, along with associated transport and storage costs, e.g. requirements for temperature control.

3 - **Sterilization requirements** and any potential denaturation problems.

4 - **Formulation, mixing, complexing and viscosity** characteristics that may influence agitation, aeration and foaming during fermentation and downstream processing stages.

5 - **The concentration of target product attained**, its rate of formation and yield per gram of substrate utilized.

6 - **The levels and range of impurities**, and the potential for generating further undesired products during the process.

7 - **Overall health and safety implications**

Media Formulation Strategy

•Start with **stoichiometry** to determine how much carbon, nitrogen, and other elements are needed.

•Use the **elemental composition** of the microorganism (e.g., $C_4H_7O_2N$) to estimate nutrient needs.

•Choose ingredients based on:

- Cost and availability
- Ease of sterilization
- Impact on product yield and recovery
- Safety and consistency

3. Sterilization of Culture Media and Added Compounds

Sterilization of the culture medium, the bioreactor, and substrates added during fermentation must be carried out to avoid unwanted contamination. Indeed, the constituents of culture media, water, and equipment contain a certain number of microorganisms in vegetative form or as spores. They must be eliminated before inoculation without altering the components of the culture medium.
Heat treatment is the most widely used method for sterilizing the medium, generally by autoclaving or with hot steam. Filtration is also used.

3.1. Culture Medium Sterilization

Two types of culture medium sterilization processes can be distinguished:
•Batch sterilization process;
•Continuous sterilization process.

Problems Related to Sterilization

The sterilization conditions determined for the culture medium must eliminate all vegetative and sporulated forms of microorganisms and prevent the destruction of the nutrient components of the culture medium.
Two problems can arise during culture medium sterilization:
•**Heat-labile compounds** that are denatured by heat;
•**Carbonyl groups** of reducing carbohydrates and **amino groups** of amino acids and proteins undergoing **Maillard-type reactions**.

3.1.1. Batch Sterilization:

It is carried out at a temperature of 121 °C.

The sterilization duration depends on the volume and nature of the culture medium.

Two main methods:

•**In situ sterilization** directly in the bioreactor.

•Sterilization in separate equipment (batch sterilizer).

Advantages of batch sterilization with a separate sterilizer:

•**Time savings:** the medium can be sterilized while the bioreactor is being cleaned.

•**Reduced bioreactor corrosion,** as it is not exposed to high temperatures.

•**Possibility of sterilizing concentrated media,** using smaller equipment.

Disadvantages:

•Higher cost.

•Increased risk of contamination during the aseptic transfer of the sterilized medium to the bioreactor.

Industrial Preference:

Despite the advantages of batch sterilization, industries often prefer continuous sterilization due to its additional benefits.

3.1.2. Continuous Sterilization Processes

The major advantage of continuous sterilization is that it is possible to reach temperatures higher than 121 °C in order to reduce the sterilization time; the heat-sensitive nutrients in the culture medium (e.g., growth factors) are then not denatured because the sterilization time is shorter. Furthermore, this brief time limits the production of Maillard reaction compounds.

There are two techniques for continuous sterilization.

A. Indirect Continuous Sterilization with Spiral Heat Exchangers:

•Uses spiral stainless steel heat exchangers.

•The culture medium is heated by a thermal fluid, then cooled.

•Avoids fouling caused by suspended particles.

•Transfer to the bioreactor must be aseptic.

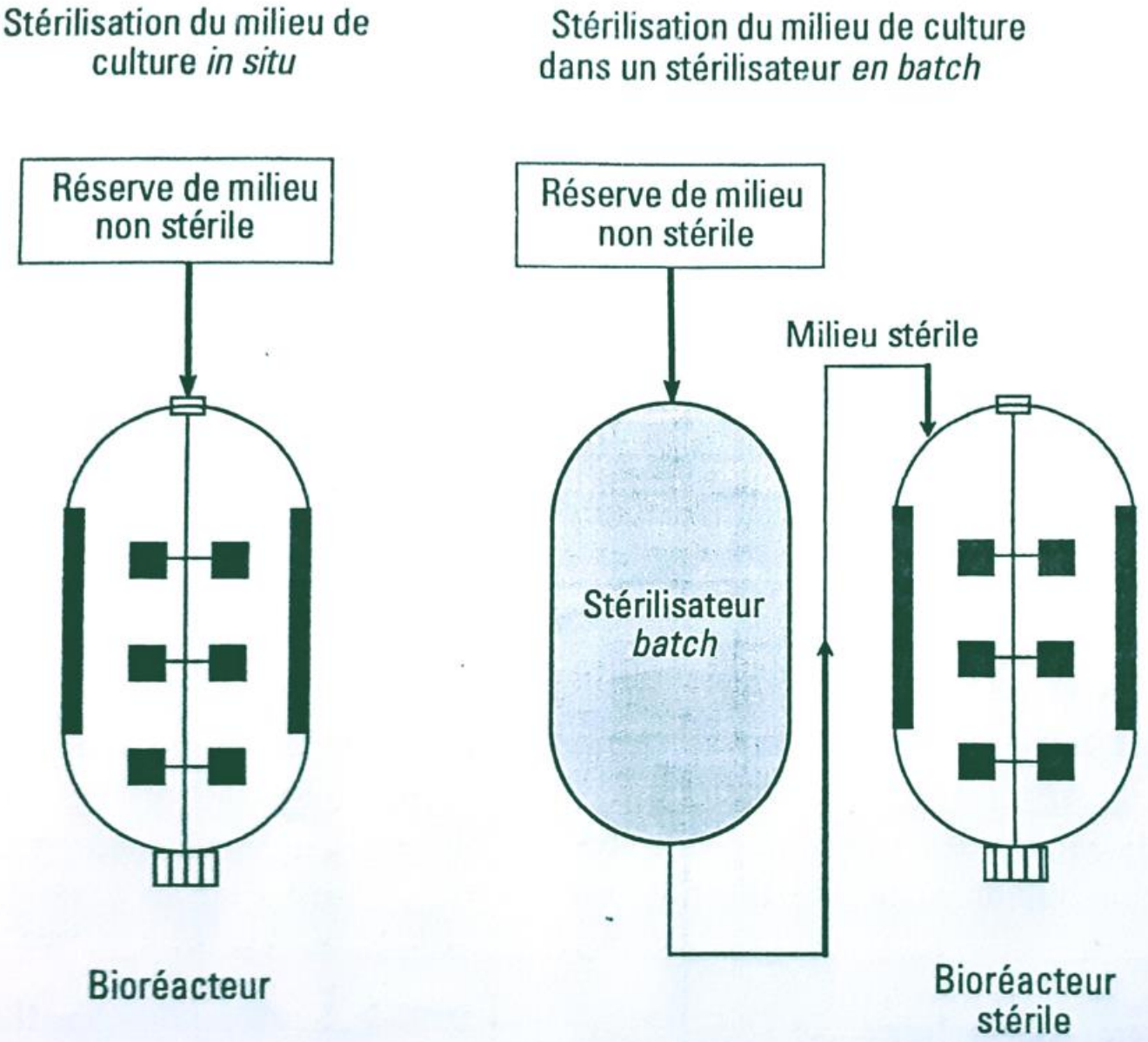
B. Continuous Sterilization by Direct Steam Injection:

•Steam is injected directly into the culture medium.

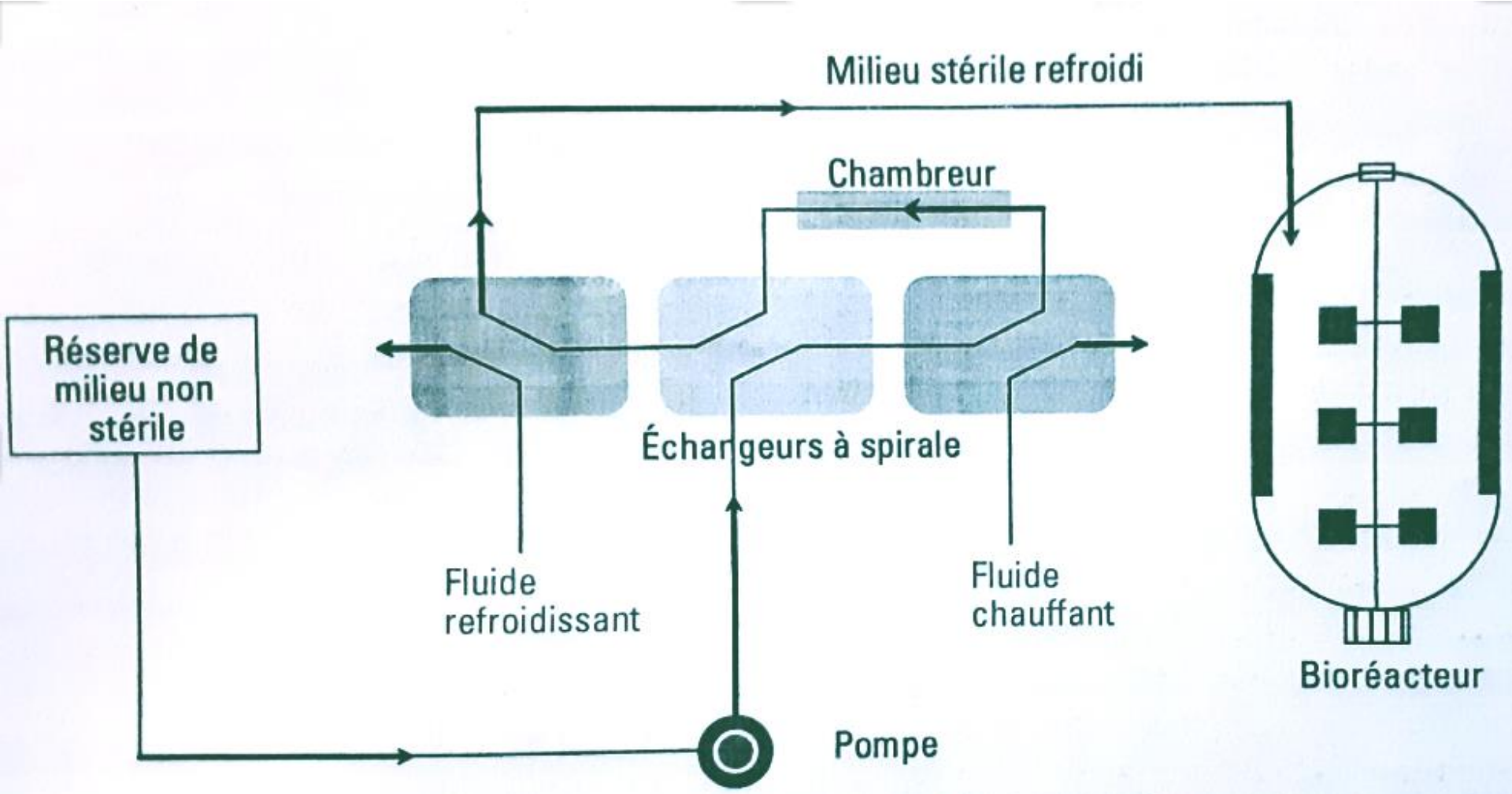
•Reduced cost, rapid temperature increase, applicable to media containing particles, easy maintenance.

•Risk of foam formation and dilution of the medium by condensed steam.

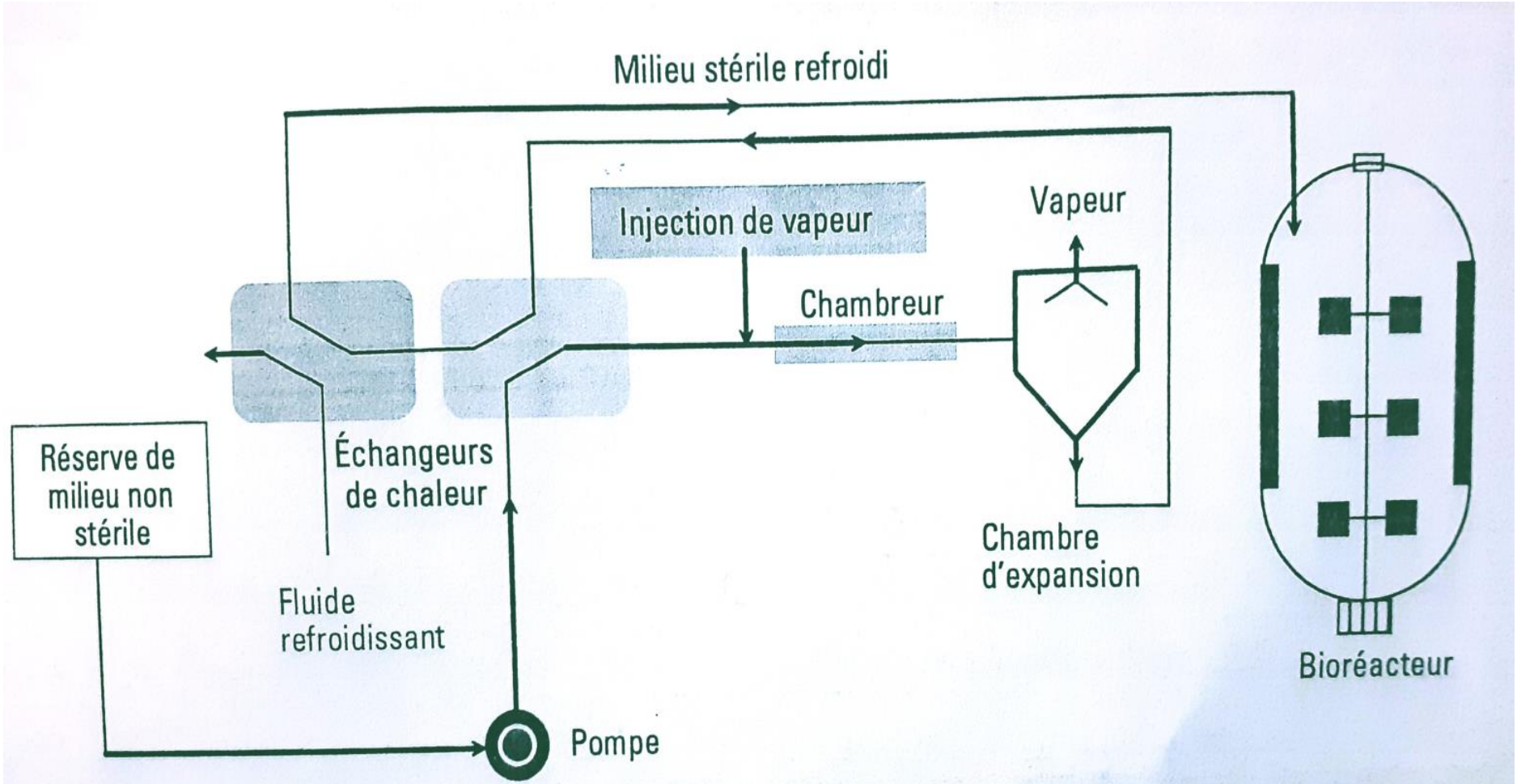
•Steam quality is crucial to avoid altering the medium composition.



Schémas de principe de la stérilisation in situ et de la stérilisation en stérilisateur en batch



Stérilisation du milieu de culture en continu indirecte à l'aide d'échangeurs de chaleur à spirale



30 *Stérilisation du milieu de culture en continu, directe par injection de vapeur*

3.2. Sterilization of Compounds Added During Fermentation

The methods used depend on the volume of solution to be injected during fermentation. If the volume is small, then filtration will be used. On the other hand, if the supplement volume is large, the continuous sterilization method will be used.

3.3. Sterilization of Contaminated Medium After Fermentation

After fermentation, sterilization of the contaminated medium can be carried out using the methods described above: batch sterilization or continuous sterilization.

3.4. Cleaning In Place and Sterilization of the Bioreactor

3.4.1. Cleaning In Place

Industrial fermenters need to be cleaned in place after use and before new sterilization (CIP method, Cleaning In Place).

3.4.2. Sterilization of the Bioreactor

Two cases can be distinguished:

•If the culture medium is sterilized *in situ*, then the bioreactor is sterilized at the same time as the culture medium;

•If the culture medium is sterilized in a batch sterilizer or if it is sterilized by a continuous sterilization process (indirect or direct), then the bioreactor must be sterilized before the addition of the sterile culture medium. This sterilization is carried out with steam under a pressure of 1 bar for 20 minutes.

4. Criteria for Choosing Raw Materials

The main factors that affect the choice of industrial culture media are as follows:

- **Price**
- **Abundance and availability**
- **Transportation cost**
- **Ease of waste treatment after use**
- **Uniformity of raw material quality**
- **Adequate chemical composition**
- **Ease of handling and sterilization**

5. Composition Optimization

Objective: Promote microorganism growth and productivity:

•Identification of the best carbon and nitrogen sources.

•Selection of necessary mineral salts and trace elements.

•Determination of specific growth factors for each microorganism.

Methods:

•In-depth studies to explore different nutrient combinations.

•Use of analytical techniques (spectroscopy, chromatography) to analyze and quantify compounds.

Importance of Balance: An excess or deficiency of nutrients can harm microbial growth.

Result: Optimal formulation of culture media for maximum microorganism growth.

6. Use of Selective Media

Definition: Specific formulations that promote the growth of certain microorganisms while inhibiting that of others.

Applications:

•Isolation, identification, and culture of specific microorganisms in complex samples.

•Purification and enrichment of microbial cultures.

Composition:

•Inhibitory agents (antibiotics, dyes) to prevent the growth of unwanted microorganisms.

•Examples: Media for Gram-positive bacteria or specific yeasts.

Advantages:

•Facilitates the study of microorganisms of interest.

•Optimizes production processes in biotechnology.

•Controls contamination and improves fermentation yields.

Importance: A valuable tool in industrial microbiology for targeted and efficient cultures.

7. Culture Maintenance Media

- Used to preserve industrial strains.
- Designed to maintain viability and genetic stability.
- May include selective agents to retain specific traits.