Democratic and Popular Republic of Algeria
Ministry of Higher Education and Scientific Research
Ahmed Zabana University - Relizane

Faculty of Natural and Life Sciences

Department of Biological Sciences

Course handout:

Industrial Microbiology

Designed for Master's Year 2 students

Specialty: Applied Biochemistry

Authored by: Dr ADDI Nesrine

Academic Year 2025/2026

Preface

This course handout, titled Industrial Microbiology for M2 Applied Biochemistry Students, is authored by **Dr. ADDI Nesrine**, a Lecturer (Class A) in the Department of Biological Sciences at the Faculty of Natural and life Sciences, and a member of the Environmental and Sustainable Development Laboratory at the University of Relizane, Algeria. This course handout encompasses the course material for Industrial Microbiology designed for the second year of the Master's program in Applied Biochemistry at the Faculty of Natural and life Sciences, Department of Biological Sciences, at Ahmed Zabana University of Relizane.

This module is an essential and indispensable component of the curriculum for second years of the Master's program, as well as for students conducting research for their final thesis or even doctoral work. It equips students with the ability to successfully in the field of the use of microbiology in industry, and to use it for their final thesis, particularly in the context of startup.

Prerequisites: To derive maximum benefit from this course, students should possess foundational knowledge in microbiology, some notions in industry, and some laboratory procedures. Familiarity with microbiological concepts and sterile techniques will be advantageous.

Learning objectives:

The course aims to develop the following competencies in students:

- ✓ The ability to successfully perform microbiology in the laboratory.
- ✓ The ability to develop projects in industry, especially in startup projects.
- ✓ The ability to overcome challenges encountered during manipulation and select appropriate methods for their research.

Students will progressively build this complex skill by mastering knowledge, applying practical skills, and adopting a professional attitude.

By the end of this course, students will be able to:

- ❖ **In terms of knowledge:**
  - ✓ Understand the importance of microbiological control in industry.
  - ✓ Learn the terminology and basic concepts of microbiology, facilitating their learning and expanding their knowledge.
  - ✓ Know the basic principles of viruses and master their detection methods.

❖ **In terms of practical skills:**

- ✓ Easily manipulate micro-organisms in aseptic conditions, maintain cultures, and transition from primary to secondary cultures.
- ✓ Use appropriate protocols for different concepts of industry.

❖ **In terms of professional attitude:**

- ✓ Develop the capacity to create a project in industry.
- ✓ Respect the requirements of microbiology, such as culture conditions, sterilization, and aseptic techniques.
- ✓ Overcome challenges encountered during manipulation, such as contamination.

This course details five main areas in industrial microbiology: Introduction to the module, the microbiology control and his importance in industry, the fomenters and detailed examples of uses of them, notions on viruses and prion as well as immunological methods of virus detection.

List of figures

List of tables

Table of content

Chapter III: The fermenter

Chapter IV: Concepts about viruses and prion basics

Chapter V: Immunological and molecular techniques for virus detection

Chapter VI: Application exercises in industrial microbiology

Chapter VII: Course review

Introduction:

Industrial microbiology is a discipline that links microorganisms to industry. It is of great global importance and involves all fields. This discipline uses microorganisms (bacteria, yeasts, molds) to produce products of interest, whether in food (cheese, bread), agriculture (pesticides, hormones), the pharmaceutical industry (antibiotics, vitamins), or for environmental applications such as water treatment. Thus, microorganisms transform raw materials into desirable products through biological reactions, notably fermentation.

The purpose of using industrial microbiology is to innovate in industry and revolutionize the production system day by day, that is why we are interested in this discipline, especially for application in start-ups. Thus, the knowledge acquired in this course will allow the student to know the basics of how the industry works and to be able to carry out a start-up project.

The use of these microbiological processes is often more economical than traditional chemical syntheses. The ability to produce complex molecules that are difficult or impossible to obtain chemically is achievable through industrial microbiological processes, and the use of agri-food waste as substrates allows for environmental remediation.

The history of industrial microbiology begins with ancestral fermentation methods, using unknown microbes to transform agricultural raw materials. It developed thanks to the discovery of microbes in the 17th century and the work of Louis Pasteur demonstrating their role in fermentation. The 20th century saw the emergence of modern microbiology, with genetic engineering and biotechnology, expanding its scope from the level of traditional processes to that of numerous technological applications.

The course covers an introduction to industrial microbiology, and then focuses on microbiological control given the importance of its application in industry, then on the

fermenter in order to learn about the production system, through examples of application. Finally, viruses and their cytopathogenic study, and their detection methods will be discussed, without forgetting some notions on prion. The student will also produce a dairy product in the laboratory to learn about the industry and its principles. Finally, a trip to a dairy factory is organized. Thus, the student will be able to receive all the knowledge he may need in industrial microbiology.

I.1. Definition of industrial microbiology:

Industrial microbiology is the use of microorganisms in industry, and is necessarily accompanied by microbiological control. It is a scientific discipline that uses microorganisms (bacteria, yeasts, molds, etc.) to carry out biological transformations and synthesize a wide range of products, from food and beverages to pharmaceuticals, fuels, chemicals, and enzymes, while also playing a key role in waste treatment and bioremediation. It relies on bioconversion and fermentation processes, techniques that have been used for millennia, for example in cheese or beer production, to transform substrates into desired products. The applications of industrial microbiology are varied and span several sectors, such as food and beverage (Production of yogurt, cheese, bread, wine, beer, etc.) using yeasts and bacteria,

In pharmaceutical Industry, in chemicals and bioenergy (Manufacture of ethanol, organic acids, solvents, and other chemical compounds), in agriculture (Production of plant protection products, pesticides, and plant hormones), in environment (Wastewater treatment, bioremediation of polluted soils, biogas production, and many other disciplines.

I.2. Areas of application of industrial microbiology:

-Food industry: (flavoring agents, emulsifiers, lactic acid bacteria, etc.)

-Energy production: (ethanol, methane, etc.)

-Environment: (biological water purification, oil drilling, etc.)

-Agriculture: for the production of herbicides, insecticides, plant hormones, etc.

-Molecular biology: production of restriction enzymes, etc.

-Pharmaceutical industry: (antibiotics, vitamins, amino acids, insulin, growth hormone, and many other antitumor drugs).

I.3. Advantages of using industrial microbiology:

1/ Lower cost than chemical processes: example of enzymatic catalysis, etc.

2/ Feasibility

3/ Specificity of the reaction

4/ Production of complex molecules: Microorganisms can produce synthetic molecules that are impossible to obtain chemically.

5/ Waste utilization: They can recover industrial by-products or waste, contributing to a more circular economy.

6/ Sustainable processes: They offer ecological solutions for waste treatment and environmental restoration.

I.4. Microbial products of industrial interest:

Once the desired microorganism has been obtained, the formation of the desired products depends on its cultivation. The use of microorganisms in modern biotechnology is therefore based on the principles of mass culture and therefore involves:

– Management of microbial culture processes

– Knowledge of the factors limiting microbial growth.

Microbial products of interest are essentially:

• Vitamins and amino acids

• Intracellular enzymes

• Extracellular enzymes

• Exotoxins

• Bacteriocins

• Lipids

• Antibiotics

• Exocellular polymers (dextran, alginates, etc.)

In industrial microbiology, we use all of these products for different purposes, example: the use of bacterial enzymes in the cheese industry to vary the flavors of dairy products.

I.5. Microorganisms of industrial interest:

Microorganisms play an essential role in industry due to their ease of cultivation, rapid growth, ability to use inexpensive substrates (sometimes waste from the food industry), and adaptability to genetic manipulation. These characteristics make them valuable tools for many industrial applications. Among the microorganisms used in industry, we mainly find fungi such as yeasts and molds, as well as certain prokaryotes, such as bacteria, particularly the genus Streptomyces, and archaea. These microorganisms exhibit specific properties that make them suitable for specific industrial functions.

Examples of Microorganisms Useful in Industry

1. Archaea:

Archaea are unicellular prokaryotic microorganisms, lacking a nucleus and organelles. Originally considered extremophile bacteria, they are found in environments such as oceanic hydrothermal vents, volcanic hot springs, salt lakes, soils and wetlands, as well as in intestinal flora. Archaea play an important role in the carbon cycle and the nitrogen cycle.

- Industrial Applications of Archea:

1. Environment and Ecology:

- Methanogenic archaea are used in biogas plants to produce methane from organic waste.

- Some types of archaea are used for wastewater treatment, contributing to the degradation of organic compounds and water purification.

2. Food Industry:

Halophile archaea (adapted to saline environments) are used in the production of salt. Some archaea are used in the fermentation of food products.

3. Biotechnology:

Extremophile archaea (adapted to extreme environments) are studied for their unique abilities and enzymes that are resistant to extreme conditions. They can be used in industrial processes requiring harsh conditions, such as chemical production or waste treatment.

4. Scientific Research:

Archaea serves as study models to understand evolution and adaptation to extreme environments. Their study can help us better understand survival mechanisms and biological adaptations in extreme conditions, which may have implications for research on the origin of life.

5. Medicine and Pharmacology:

Archaea provide potential sources of new antibiotics and bioactive compounds. Some archaea are studied for their role in gut health and modulation of the microbiome.

2. Bacteria:

Bacteria are single-celled microorganisms found in many environments. Their small size and ability to reproduce rapidly make them ideal candidates for large-scale production.

They have different shapes (cocci, bacilli, coccobacilli, vibrios, spirals, etc.), as well as different ways of grouping (in clusters, beads, clusters, etc.). The nuclear apparatus of bacteria consists of a single chromosome (circular, self-contained, does not contain large repetitive regions) located in the cytoplasm. There may be extra chromosomal genetic material such as plasmid (double-stranded, circular, transmissible DNA). Depending on the

carbon and energy source, bacteria are classified into photo autotrophs, photo heterotrophs, chemo autotrophs, and chemo heterotrophs.

- **Applications of Bacteria:**

Here are some specific examples of bacterial applications:

1. Enzyme and Protein Production:

- *Bacillus subtilis* is used to produce enzymes such as amylase, protease, and lipase, which are used in various industries, including food, laundry, and biofuel production.
- *Escherichia coli* is used as a host in the production of recombinant proteins.

2. Dairy Product Production:

Lactic acid bacteria such as *Lactobacillus bulgaricus* and *Streptococcus thermophilus* are used in the production of yogurt, while *Lactococcus lactis* and *Leuconostoc* are used in the manufacture of cheese and other fermented dairy products.

3. Acid Production:

Lactic acid bacteria, particularly Lactobacillus acidophilus and *Lactobacillus casei*, are used to ferment sugars and produce lactic acid, which is used in the food, cosmetic, and pharmaceutical industries, while *Acetobacter* and *Gluconobacter* are the main microorganisms responsible for vinegar production.

4. Vitamin Production:

Some bacteria, such as *Propionibacterium freudenreichii*, are used to produce vitamin B12, which is used in dietary supplements and the pharmaceutical industry.

5. Hydrocarbon Degradation:

Bacteria such as *Pseudomonas aeruginosa*, is used to degrade hydrocarbons present in oil spills and oil spills.

6. Waste Degradation:

Bacteria such as *Bacillus* spp. and *Pseudomonas* spp. are used in wastewater treatment facilities to break down organic matter and remove pollutants.

7. Bioplastic production:

Bacteria such as *Cupriavidus necator* are used to produce "Polyhydroxyalkanoate" (PHA), a biodegradable bioplastic used in packaging and disposable products.

8. Drug Production:

Genetically modified bacteria, such as *Escherichia coli*, are used to produce therapeutic proteins such as insulin, growth hormones, and antibodies, which are used in the pharmaceutical industry.

3. Fungi:

3.1. Molds:

- These are generally multicellular organisms; most molds are heterotrophic, with some species having a mixed metabolism. Molds were responsible for the discovery of penicillin, the first truly effective antibiotic. It is produced naturally by molds of the genus *Penicillium.*

A/ *Aspergillus niger:*

This filamentous ascomycete fungus appears as a black mold on fruits and vegetables. *Aspergillus niger* is an economically important species because it is used in industrial fermentation to produce:

- Citric acid: used in the food industry as an acidifier and antioxidant to enhance flavors and preserve fruit juices.

- Gluconic acid: is a natural constituent of fruit juices that is widely used in medicines, food, detergents, textiles, leather, etc.

-Enzymes: such as glucose oxidase, catalase, and hydrolases (cellulase, xylanase, pectinase), which are the main enzymes used in the production of beer and sweetened beverages.

B/ *Penicillium*:

Penicillium are filamentous fungi. They are very common fungi in the environment and can be responsible for many types of degradation. Various species are cultivated industrially for the production of cheese (Penicillium roqueforti, Penicillium camembertii) and metabolite production: Penicillin-type antibiotics (*Penicillium notatum, Penicillium chrysogenum*), and Gluconic acid (from *Penicillium purpurogenum*).

3.2. Yeasts**:**

Yeast is a single-celled, asexually reproducing fungus capable of causing fermentation of animal or plant organic matter. Yeasts are often used in the food industry for the production of various products such as bread, fermented beverages, ethanol, dairy products, and beer. They play a crucial role in the fermentation of these foods, contributing to their flavor and texture.

In human and Animal Health: yeasts are used in the pharmaceutical industry for the production of insulin and other drugs (antibiotics). They are also used as probiotics to improve the digestive health of monogastric and ruminant animals.

In cosmetics: Yeasts are used in the cosmetics industry for the manufacture of hair, nail, and skin products. Their beneficial properties are exploited in various personal care products.

Yeasts are also used in the production of industrial alcohols, biofuels, and antibiotics.

There are different types of yeast, such as the genus *Saccharomyces*, which is the most common and best-known type of yeast. It is widely used in the production of bread.

In production of Bioethanol: yeasts can ferment the sugars present in plant biomass to produce bioethanol, a biofuel used as an alternative to fossil fuels. Special yeast strains are selected and optimized to maximize bioethanol production from feed stocks such as sugarcane, corn, or cellulose.

In Enzyme production: Some yeasts produce enzymes of industrial interest, such as amylases, proteases, and lipases. These enzymes are used in various industrial processes such as detergent production, food processing, biofuel production, and the paper industry.

In pollution control: Certain yeasts are capable of degrading and detoxifying certain toxic chemicals and pollutants present in the environment. They can be used in bioremediation processes to degrade hydrocarbons, heavy metals, and other toxic substances present in soil, water and air.

4. Viruses:

A virus is an infectious agent that requires a host (obligate parasite). Viruses are classified according to the nature of the nucleic acid in their genome (DNA or RNA), the structure of the nucleic acid (single-stranded or double-stranded), and the shape of the nucleic acid (linear, circular, segmented, or not). They are tools used, for example, to enable a cell to acquire the ability to produce a protein of interest or to study the effect of introducing a new gene into the genome. Viruses also have specific industrial applications. Here are some examples.

- **Bacterial infection control:** Bacteriophages, which are viruses that specifically infect and destroy bacteria, are used in healthcare and the food industry to control bacterial infections. Bacteriophages can be used to combat antibiotic-resistant bacterial infections, particularly in medical, veterinary, and agricultural applications.
- **Genetic engineering:** Some viruses, such as Adeno-Associated Viruses (AAVs), are used as gene transfer vectors in gene therapy applications. These viruses are modified to carry specific therapeutic genes into target cells to treat genetic or acquired diseases.
- **Recombinant protein production:** Some viruses are used in large-scale recombinant protein production, which are harvested and purified for use in various applications, including pharmaceuticals and scientific research.

- Plant biotechnology: Plant viruses, such as the tobacco mosaic virus, can be used to produce transgenic plants. These viruses can be modified to carry genes of interest into plants, allowing them to develop desired traits, such as disease resistance or improved crop quality.
- Agricultural pest control: Some viruses are used as biological control agents against agricultural pests. For example the granulosis virus is used to control caterpillar populations in crops, providing an environmentally friendly alternative to chemical pesticides.

I.6. The Microbial Strain for Industrial Use:

I.6.1.Characteristics of the Ideal Strain:

A microorganism used in an industrial process must have characteristics other than its ability to produce the desired substance in high yield.

1. First, the organism must be capable of growing and forming products in large-scale culture.

2. It must produce spores (if it is a fungus or yeast) or another form of reproductive cell so that it can be easily inoculated into the large containers used to cultivate the producing organism on an industrial scale.

3. It must also grow rapidly and produce the desired product in a relatively short period of time.

4. It must also be able to grow in a liquid culture medium available in large quantities and at low cost. Many industrial microbiological processes use carbon waste from other industries as the main or supplemental ingredients for large-scale culture media. These include corn steep liquor and whey.

5. An industrial microorganism must not be pathogenic, particularly to humans or economically important animals or plants. Due to the high cell densities in industrial

microbial processes and the near impossibility of avoiding contamination of the environment outside the growth vessel, a pathogen would pose potentially disastrous problems.

6. Finally, an industrial microorganism must be amenable to genetic manipulation. Because increased yields are often achieved through mutation and conventional genetic selection techniques, a genetically stable and easily modified microorganism is therefore an undeniable advantage for an industrial process.

I.6.2. Obtaining strains:

Isolation and selection of industrial microorganisms "Screening Technique" is the first step in developing a productive strain consists of isolating the microorganisms in question from their natural habitat. They are present virtually everywhere, for example, in air, water, soil, plant and animal surfaces, as well as in plant and animal tissues. However, the most common sources of industrial microorganisms are soils and lake and river sludge. Often, the ecological habitat from which a desired microorganism is most likely to be isolated depends on the characteristics of the desired product and the development of the process. The sample is then inoculated onto an appropriate medium to isolate the desired organism. The next step is to isolate and purify the various clones obtained. Purification is carried out by depletion streaking. This technique allows the isolation of colonies and the production of pure cultures.

After isolation, the resulting microorganisms then undergo selection based on their biological and technological suitability:

- Biological criteria specific to the microorganisms and their application, for example, lactic ferments are selected based on their acidifying and flavoring properties, as well as their “bacteriocin” production.

- Non-pathogenic and do not produce undesirable metabolites such as toxins.

- Easy to genetically manipulate.

- Grow on inexpensive substrates (food industry waste).

- Do not have specific requirements for growth factors.

- Genetically stable, especially after preservation by freezing or freeze-drying.

- Able to withstand various technological processes.

- Resistance to bacteriophages

Microorganisms can also be obtained in the form of pure cultures, thus, when a strain is selected whith the selection criteria, it undergoes molecular identification based on 16S rDNA sequencing.

Strains can also be obtained from strain collections which are collections gathered and organized, for example, American Type Culture Collection (ATCC).

I.6.3. Strain Improvement:

The modification of bacterial strains aim is to improve their performance through genetic, metabolic, or physiological alterations, allowing the optimization of the production of substances, the conferring of useful resistances, or the increase of their effectiveness in various industrial and research applications. Methods include metabolic engineering, which targets specific cellular functions, and random mutagenesis, which induces genetic modifications without prior knowledge of the genome.

Engineered bacterial strains offer enhanced performance for optimal use in research and industry, whether to increase production, improve resistance to specific conditions, or confer new functionalities.

After identifying an organism that produces a valuable product, it becomes necessary to increase fermentation yield to minimize production costs. The objectives are therefore:

- Increase the production capacity (of the product) of the microorganisms.
- Improve substrate specificity and production speed.

- Improve new production routes.
- Improve resistance to adverse conditions (temperature, pH, toxin, bacteriophage).

➢ Strain Modification Strategies:

-Metabolic Engineering: This approach involves targeted modification of genes and enzymatic functions to optimize molecule production or improve bacterial performance.

-Random Mutagenesis: This technique induces genetic mutations without knowing their precise location in the genome, a method that has been highly effective in improving the industrial production of antibiotics.

Thus Modification and Improvement Techniques:

-Genetic Modification: The use of molecular biology and genetic engineering techniques allows the introduction of new genes or the modification of existing genes to achieve desired characteristics. Manipulation of genetic material not involving foreign DNA (conventional mutation), mutation frequency and mutant screening identification for a given growth factor or a mutant resistant to a chemical antimicrobial agent or a mutant capable of fermenting a carbohydrate. For strain mutagenesis, natural genetic recombination such as sexual and asexual reproduction is used, cell fusion induced by protoplast fusion, or in vitro genetic recombination.

-Selecting Natural Variants: This approach involves continuous genetic modification of the crop and optimization of culture media to select variants with improved properties, as demonstrated in the production of penicillin. The technique consists of: Choose the best-performing individual**,** cultivate it**,** select the best-performing offspring (2nd generation)**,** Continue for several generations.

➢ Application Examples:

-Industrial Product Production: Bacterial strains are modified to efficiently produce recombinant proteins or other products of interest to industry.

-Crop protection against pests: Bacteria, such as *Bacillus thuringiensis* , have been modified to increase their effectiveness in protecting crops against insect infestations and diseases.

I.6.4. Strain Preservation:

- For a desired shelf life: keep the strain to be preserved viable, available, and identical. In microbiology, where we work with single-celled organisms, preserving them viable identically means preserving them viable under conditions that maintain the genome's integrity.
- Preservation must obviously exclude contamination. Depending on the case, desired storage durations vary from a few days to several years, and the choice of a preservation technique will be strongly influenced by this duration parameter.
- It will be necessary to verify that a set of genetic and/or morphological and/or physiological and/or biochemical traits that characterize the pure strain to be preserved and that are of interest to users of the strain have been maintained identically (the traits are defined by the user based on the nature of their work on the strain to be preserved).
- Preservation methods involve one or more of the following techniques:

- Reduction of the growth temperature.

- Desiccation or dehydration of the culture medium.

- Limiting the nutrients available to the microorganism.

➢ Preservation Methods

The choice depends on the microorganism and the desired goal:

1. Methods based on reducing the growth temperature:

Preservation on agar with ordinary refrigeration (4-8°C) by successive subculturing. For aerobic microorganisms: agar slant. It is used for anaerobic microorganisms: deep agar + paraffin or oil. Storage for 3 to 12 months.

This is an inexpensive method because it does not require special equipment, but the refrigeration temperature does not completely limit the growth of microorganisms and therefore requires repeated subculturing of the strain, with the risk of contamination and mutation.

2. Preserving Microbial Strains by Freezing:

The principleis that low temperatures will stop all cellular chemical reactions by crystallizing water, thus ensuring perfect preservation. Reheating is sufficient to restart the process.

In practice, from -30°C, the kinetics of chemical reactions are very slow, changes are real but occur over many months. At -80°C, there is virtually no chemical change. At -196°C, nothing changes.

A method for preserving a strain by freezing must therefore include: a procedure for cooling: composition of the medium with the presence of a particular cryoprotectant, and a procedure for warming (particularly the kinetics of warming). The procedures will be more or less simple depending on the strains' ability to resist damaging effects.

• At -20°C, the temperatures are not low enough and the system evolves chemically, and not for the better: bacteria, yeasts, and molds can be preserved for a few months to a few years.

• At -80°C, chemical changes are very, very slow. Freezing in the presence of cryoprotectants such as glycerol which were very useful for preservation over several years. Especially since -80°C freezers are now accessible.

• At -196°C, in liquid nitrogen, we are also looking at possible storage over very long periods.

3. Preservation of Microbial Strains after Desiccation:

Water is the solvent for chemical reactions in living organisms. After desiccation, the chemical reactions will be stopped (or at least slowed down) and preservation is effective.

Some bacteria tolerate dehydration perfectly in a dry natural atmosphere. This can be facilitated by the use of a partial vacuum or desiccant agents. For the dehydration step, the microorganisms are spread on an inert support such as blotting paper, sand, or silica grains. After desiccation, storage must be perfectly dry, generally around 0-4°C (in a sealed container with a desiccator in a refrigerated environment). Revivification is carried out by immersion in culture medium.

3.1. Preservation of Microbial Strains after Freeze-Drying (Lyophilization):

Freeze-drying allows for advanced dehydration compatible with very long storage times. The strain to be preserved is: frozen and then dehydrated by sublimation of ice (evaporation of water directly from solid to vapor). This requires working in a cold environment under reduced pressure, the lyophilizate is then sealed and stored in the dark, generally in a cool atmosphere 0-4°C.

Lyophilization is not compatible with all microorganisms. In some cases it causes cellular and genetic damage, but when the practical conditions for a given strain are perfect, lyophilization is an excellent long-term storage method and is easy to manage (no

problems with managing liquid nitrogen or a -80°C freezer...). Entire collections of strains in major international strain libraries, are example of liophilization.

The revivification (rehydration) is achieved by adding culture medium to the ampoule of lyophilizate.

I.7. Microbiological Environmental Control:

Microbiological control is the set of analyses and procedures aimed at evaluating and controlling the presence of microorganisms (bacteria, viruses, molds, etc.) in products (food, pharmaceuticals, etc.) or environments. Its main objective is to guarantee the safety and quality of products by detecting contaminants, ensuring the absence of dangers to public health and verifying the effectiveness of cleaning and disinfection processes. Microbiological control is an essential tool for risk control, quality assurance and compliance with standards in various industries, thus ensuring product and consumer safety.

I.7.1. Environnmental monitoring objectives:

The objectives of microbiological control are multiple, but the main aim is to guarantee consumer safety by verifying the absence of pathogenic microorganisms, to evaluate and control the microbiological quality of products (food, pharmaceuticals, cosmetics), to control the efficiency of manufacturing processes, and to comply with regulatory requirements.

- **The main objectives are:**

-Consumer Safety: Detect and eliminate pathogenic germs (*Salmonella, Listeria, Campylobacter*) to prevent their introduction into the production chain and ensure safe products.

-Overall Quality: Assess the overall microbial load (mesophilic aerobic germs) as an indicator of the microbiological quality of the raw material.

-Hygiene Control: Identify microorganisms that indicate poor hygiene during production (coliforms, *Enterobacteriaceae*).

-Spoilage Prevention: Detect spoilage microorganisms that can degrade finished products, even those that pose no health risk.

-Critical Control Points: Establish contamination thresholds and monitor adverse trends to control production.

- **Types of control:**

-Checks as part of a facility qualification procedure: before starting operations in a new environment.

-Monitoring checks: For example, checks carried out at any time for monitoring purposes (example of monitoring a cheese production facility).

-Investigative control: As part of an epidemiological investigation, the search for the source of contamination is conducted in order to eliminate it.

-Educational controls: To visualize the presence of microorganisms in an environment.

- **Indicators to control:**

- Pathogen screening: Detection of microorganisms that are hazardous to health, such as *Salmonella, Listeria*, or *E. coli.*
- Microbial flora enumeration:

-Mesophilic aerobic bacteria: Indicator of the overall microbiological quality of a food.

-*Enterobacteriaceae* and colibacteria: Indicators of compliance with hygiene regulations during production.

- Physicochemical tests: Certain physicochemical parameters (water content, pH, acidity) can reflect microbial presence and be used as an alternative or in addition to culture analyses.

I.7.2. Limits of microbiological control:

Limitations of microbiological control include the natural variability of microorganisms, limitations inherent in analytical methods (uncertainty, sampling), and physicochemical factors of the products. Their interpretation can be complex due to the uneven distribution of microorganisms in the food and variations in their state. Limits must also take into account the potential health risk, the product's ability to support their growth, and the relevance of the criterion to protect the consumer.

- There are three levels of microbiological control:

1. Target level: This is the quality level that aims to ensure and maintain normal operating conditions within a controlled environment.

2. Alert level: This is the level that provides an initial warning in the event of a deviation from normal conditions. When this alert threshold is exceeded, additional investigations must be undertaken to verify the observed results and ensure that the process and/or environment are still under control.

3. Action level: This is the level that must trigger an immediate response, including analysis of the causes of the malfunction and implementation of remedial measures.

I.7.2.1. Factors influencing the limits of microbiological control:

-Microorganism variability: Microorganisms vary depending on the species, strain, and metabolic state. This complicates determining the accuracy and measurement range of analyses.

-Uneven distribution: Microorganisms are generally not evenly distributed throughout the food, which can affect the representativeness of the sample.

-Product properties: Product characteristics (such as pH, water activity, or the presence of alcohol) influence the growth of microorganisms, but controls are still necessary even in products that do not support their growth.

-Microflora changes: Changes in the microbial population during storage and distribution must be taken into account, as their numbers can vary.

I.7.3. Microbiological control of raw materials:

Microbiological control of raw materials is an essential process, particularly in the food, pharmaceutical, and cosmetic industries, aimed at ensuring consumer safety and the safety of finished products. This control allows the detection of potentially dangerous microorganisms (pathogens) or indicators of poor hygiene (such as *Enterobacteria*) present in raw materials, which could alter product quality. It focuses on the identification of germs, the assessment of the total microbial load, and the verification of the absence of dangerous contamination through validated methods, complying with pharmacopoeia standards and current regulations.

The pathogenic microorganisms that we need to control are mainly: *Salmonella, E.coli, Vibrio Cholerae, Listeria monocytogenes.*

I.8. Industrial fermentation:

This fermentation involves using microorganisms as a means of production in an industrial process, based on microbial fermentation. For this industrial production, it is necessary to carry out cultures of microorganisms in very large quantities in tanks whose volume can reach

several cubic meters. These installations are called "fermenters" (Fig. 1). A fermenter is a container used to cultivate microorganisms (such as yeast or bacteria) to produce substances or biomass, or to modify an environment. It is an essential piece of equipment in the food, pharmaceutical, and biotechnology industries. It is often equipped with temperature control, agitation, and sensor systems to maintain optimal conditions for cultivating microorganisms, which transform organic substances into products such as alcohol or acids.

Figure 1: Fermenter (Raypa, 2020).

I.8.1. Use of microorganisms in fermenters:

Microorganisms in fermenters are bacteria, yeasts, or molds that are grown in a container called a fermenter or bioreactor to produce substances of interest or process raw materials. Yeasts are commonly used in bread and beverages, bacteria in dairy products, and molds in cheeses or the production of antibiotics like penicillin. The fermenter provides a controlled and sterile environment, with agitation and temperature, pH, and oxygen control systems to optimize the growth and production of microorganisms.

➢ Types of Microorganisms used in fermenter:

-Bacteria:

- ✓ Lactic Acid Bacteria : Used in the production of yogurt, cheese, and other dairy products.
- ✓ Acetic Acid Bacteria: Involved in the fermentation of certain foods.

-Yeasts: *Saccharomyces cerevisiae*: for bread yeast, the best-known, is essential for alcoholic fermentation in the production of gas for bread.

-Molds: Used to process cheeses and to produce antibiotics such as penicillin with *Penicillium.*

➢ Principle used by microorganisms:

Fermenter production is primarily based on microbial fermentation, which is a metabolic process that converts carbohydrates into acids, gases, and alcohol. The goal is to extract some of the chemical energy while oxidizing coenzymes.

- ***Fermentation equation:***

$C_6H_{12}O_6 + 2\ NAD^+ + 2\ ADP + 2\ P_i \rightarrow \quad 2\ ATP + 2\ NADH + 4\ H^+ + 2\ H_2O + 2\ CH_3COCOO^-$

- ***Example of fermentation in a fermenter: lactic acid fermentation:***

Lactic acid fermentation is a metabolic pathway, carried out by certain bacteria (lactic acid bacteria) and certain animal cells, which convert carbohydrates such as glucose and other hexoses into lactate "$CH_3CHOHCOO$-" with the production of a small amount of metabolic energy in the form of ATP.

I.8.2. Operation and Control of fermenter:

-Maintaining Optimal Conditions: A fermenter provides a controlled environment for growing microorganisms.

-Temperature Control: A heating and cooling system maintains the ideal fermentation temperature, such as 37°C for specific microorganisms or other temperatures for brewing beer.

-Agitation and Aeration: An agitation system ensures proper mixing of nutrients and microorganisms, while an aeration system can introduce oxygen if necessary.

-Parameter Monitoring: Sensors continuously measure temperature, pH, oxygen levels, etc., and transmit this data to a control unit, which can adjust conditions to maintain stable fermentation.

- Important Considerations:

-Cleaning: It is crucial to thoroughly clean and sterilize the fermenter and utensils before use to avoid contamination with unwanted strains.

-Filling Level: Sufficient space must be left in the tank for products that can rise, such as sourdough starter or yogurt, to prevent overflow.

I.8.3. Applications of fermenter:

Fermenters have many and varied applications, spanning sectors such as food (wine, beer, yogurt, bread), healthcare (antibiotics, vaccines, enzymes), chemistry (biofuels, solvents, acids), and the environment (water treatment, pollution control). These devices enable the cultivation of microorganisms for the production of biomass, metabolites, and end products essential to many industries.

➢ In the food industry:

-Dairy products: Fermenters are used to produce cheese, yogurt, and kefir.

-Beverages: They are essential for making wine, beer, and other alcoholic beverages.

-Bread products: They are used for making bread and pastries.

-Fermented foods: They are used in the production of sauces.

-Flavorings and additives: Fermenters produce flavorings, vitamins, organic acids, and texturizing agents.

- **In the healthcare and biotechnology industries:**

-Medicines: Production of antibiotics (such as erythromycin), vaccines, and therapeutic proteins.

-Diagnostics: Production of antibodies used for diagnosis.

-Therapies: Production of insulin, hormones, interferon, and other growth factors.

-Cell cultures: Propagation of animal and plant cells for therapeutic applications, such as burn care.

- **In the chemical and energy industries:**

-Biofuels: Production of ethanol and other biofuels from renewable resources.

-Solvents and chemicals: Production of solvents, acids (citric, lactic), and enzymes.

-Biopolymers: Production of texturizing agents and other biopolymers.

- **In the environmental industry:**

-Wastewater treatment: Microorganisms cultivated in fermenters contribute to water pollution control.

-Pollution control: They are used for the biodegradation of pollutants, for example, during oil spills.

-Energy production: Anaerobic digestion, a fermentation process, is used to produce energy.

II.1. Microbiological control:

Microbiological testing of food products verifies the absence of harmful microorganisms and the level of spoilage microorganisms to ensure food safety. It involves testing to detect pathogens, such as bacteria, as well as counting flora that indicate hygiene. This testing is essential for validating existing hygiene measures and monitoring the progress of manufacturing processes.

The purpose of microbiological testing is to ensure that products are not contaminated by harmful microorganisms. It is essential to perform this test. Common contamination germs only affect food and have hygiene repercussions if they are present in large numbers. Consequently, the test aims to detect batches of food products whose contamination flora population levels exceed the threshold tolerated by current standards.

II.2. Main objectives of microbiological control:

- ✓ **Ensuring food safety:** Ensuring the absence of pathogenic (disease-causing) microorganisms and their toxins in food.
- ✓ **Validating hygiene procedures:** Verifying that hygiene measures (cleaning, disinfection, etc.) are effective in preventing contamination.
- ✓ **Ensuring product safety:** The primary goal is to prevent the presence of pathogenic microorganisms (such as *Salmonella* or *Listeria*) that could pose a risk to human health during consumption, use, or contact with the products.
- ✓ **Assessing hygienic and general quality:** This control verifies that good hygiene practices are followed during manufacturing and that the overall microbiological quality of the product is good, by analyzing the total number of germs present.

- ✓ **Controlling the production chain:** Controlling critical points in manufacturing helps identify and manage contamination risks throughout the process, from raw material sourcing to the finished product.
- ✓ **Complying with regulatory requirements:** Microbiological controls are essential to comply with current legal standards and ensure that products can be legally marketed.
- ✓ **Protecting the marketability of products:** By preventing the proliferation of unwanted microorganisms, product spoilage, deterioration and loss of market value are prevented.

II.3. Who are concerned by microbiological control:

All food industry companies are affected, including:

- Food manufacturers.
- Restaurants and food service businesses (bakers, delicatessens, caterers, etc.).
- Beverage producers.

II.4. Why is this control important:

The objective of microbiological control is to ensure consumer safety by guaranteeing that products (food, medicines, cosmetics) are not contaminated by pathogenic microorganisms and that their hygienic quality is satisfactory, all while complying with current regulations. It also makes it possible to assess the microbiological quality of raw materials and finished products, and to control critical points on production lines to prevent spoilage and product losses. To summarize, microbiological control is a legal requirement for food companies. It protects consumers by ensuring healthy products and safeguards the company's reputation.

II.5. Parameters to be monitored:

To avoid the risk of manufacturing accidents and to identify the sources of contamination, all components used in the manufacturing of the product must be subjected to microbiological analysis. In the food industry, this control concerns:

Raw materials: before they enter the factory, and even the origin of the raw materials, such as milk-producing animals.

Water: used for washing and processing products. In all cases, water used in the food industry must be potable, including that used for washing premises, utensils, and the production line.

The surfaces of the premises and utensils: those involved in storage, cutting, or any other transformation process.

The air: ambient air in the processing and treatment workshops and storage areas.

The unit's personnel: those involved from the reception of the raw material to the storage of the finished product.

Packaging and wrapping equipment.

II.6. Microbiological control procedure:

Sample Collection: Samples are collected under strict hygienic conditions.

Sample Preparation: The sample is ground or mixed with a sterile diluent to form a suspension.

Dilution: The suspension is diluted in a cascade fashion.

Placing: Dilutions are inoculated onto specific culture media (surface or depth).

Incubation: Plates are incubated for a specified period.

Counting and Identification: After incubation, colonies are counted using a colony counter and can be identified using microscopic, biochemical, or molecular techniques.

II.7. Microbiological control in factory:

To carry out microbiological control in a factory, it is necessary to define a sampling plan including surfaces, products and personnel, to take samples under aseptic conditions, to transport them quickly to the laboratory for analysis by conventional or rapid methods (PCR, culture), and to interpret the results in relation to the limit values set by the pharmacopoeias or the needs of the company. The aim is to guarantee product safety and to identify malfunctions in hygiene processes.

1. Sampling Plan and Objectives:

Define Targets: Determine the surfaces to be tested (work surfaces, equipment, personnel), intermediate and finished products, water, and air.

Set Frequency: Establish a testing frequency appropriate to the risk level and the nature of the product.

Identify Indicators: Look for microorganisms that indicate contamination (bacteria, fungi), or specific pathogens if their presence is intolerable.

2. Collecting Samples

Use aseptic techniques: Use swabs, sponges, or air samples to collect samples from surfaces.

Follow good practices: Personnel must wear appropriate hygiene equipment (gloves, etc.) and follow strict procedures to avoid contamination.

Packaging samples: Transport samples in sterile, labeled containers, protected from light, and maintained at an appropriate temperature.

3. Laboratory Analysis:

Pre-analysis: The pre-analytical phase includes sample reception, identification, and conditioning prior to analysis.

Analysis:

- **Conventional Methods:** Culture on selective media allows for the identification and enumeration of microorganisms.
- **Rapid Methods:** Molecular techniques such as PCR can be used for rapid detection, particularly for pathogen detection.

Incubation: Cultures are incubated at an appropriate temperature (example: 24 to 48 hours) before colony identification.

4. Interpretation of Results:

Comparison to Limits: Results are compared to limit values established by pharmacopoeias or the manufacturer.

Risk Analysis: Identify potential causes of contamination using tools such as the Ishikawa diagram, and implement corrective actions.

5. Continuous Controls and Maintenance:

Process Monitoring: Systematically track the effectiveness of infrastructure measures and hygiene procedures.

Regularity: A regular monitoring program is essential to detect trends and maintain product safety throughout production.

II.8. Microbiological control of an industrial product:

To carry out microbiological control of an industrial product, it is necessary to define the risks and frequency of testing, choose appropriate analysis methods (such as membrane filtration,

plate method or PCR) depending on the product, carry out the tests by accredited laboratories, and compare the results with the limits set by regulations or the manufacturer's standards. The steps include sampling, laboratory analysis (detection and quantification of germs, endotoxins, etc.), and interpretation of the results to ensure product quality and safety.

1. Define the control plan:

- **Identify risks:** Determine potential microbial contamination based on the type of product (food, medication, etc.).
- **Set the testing frequency:** Establish an appropriate frequency for analyses, which depends on the production stages and the type of risk.

2. Choose the analytical methods:

Traditional culture methods:

- **Membrane filtration:** Used to quantify microorganisms in liquids.
- **Plate count:** Involves diluting the sample, plating it onto a solid culture medium, incubating, and then counting the colonies.

Most probable number (MPN) method: Used when plate or filtration methods are not suitable for the product.

Molecular methods (PCR):

Useful for detecting and quantifying microorganisms more quickly and specifically, by identifying the DNA or RNA of pathogens.

Other analyses:

- **Endotoxin measurement:** Essential for injectable products or medical devices.
- **Preservative effectiveness testing (PET):** To verify the presence and effectiveness of preservatives.

3. Collect the sample:

- **Sampling:** Obtain a representative sample of the product under aseptic conditions to avoid contamination.

4. Perform laboratory analyses:

- **Analytical phase:** Perform tests according to validated protocols.
- **Quality control of media and reagents:** Use high-quality culture media and ensure their sterility.
- **Count microorganisms:** Use a colony counter to count the colonies after incubation.

5. Interpret the results:

- **Compare with limits:** Compare the results obtained with the limit values set by the pharmacopoeia or the manufacturer's specifications.
- **Ensure quality:** Verify that the results ensure the safety, efficacy, and purity of the product before its release.

II.8.1. Example of microbiological control of industrial product:

Industrial microbiological testing, for example for pharmaceutical or food products, may include the counting of germs (bacteria, yeasts, molds) on the finished product and during manufacturing, the analysis of the water and air quality in production areas, as well as the search for specific pathogens. These analyses are carried out using culture methods on solid or liquid media, after aseptic preparation of the sample, in compliance with Good Manufacturing Practice (GMP) standards. The results are compared to limit values set by regulations (pharmacopoeias, health standards) to guarantee the safety and quality of the product.

- **For a food product, an inspection could be carried out as follows**:

1. **Water quality control:** Analyze the microbiological quality of the water used in the manufacturing process.
2. **Surface sampling:** Take samples from the surfaces of equipment, production lines, and personnel using swabs or sponges to assess the cleanliness of work areas.
3. **In-process product analysis:** Take product samples at various stages of production to verify compliance with Good Manufacturing Practices.

4. Finished Product Analysis: Perform a count of total microorganisms (aerobes, yeasts, and molds) and test for specific germs (pathogens such as salmonella or fecal coliforms).

5. Analysis Method:

- **Sample Preparation:** Perform aseptic sample preparation and product dilution.
- **Inoculation:** Inoculate the dilutions onto Petri dishes filled with appropriate culture media.
- **Incubation:** Incubate the dishes at the appropriate temperature to allow the microorganisms to grow.
- **Counting:** After incubation, count the microbial colonies.

II.9. Microbiological control of a meat product:

Microbiological control of meat products is essential to ensure their safety and technological quality, involving the search for pathogens (*Salmonella, Listeria, E. coli*) and the evaluation of spoilage flora. Methods include traditional and automated laboratory analyses, molecular biology techniques such as PCR, and simulation software to anticipate the behavior of microorganisms. Strict regulations set tolerance limits, and corrective actions such as

improving hygiene or recalling non-compliant products are necessary in the event of detection.

The aims of this control are to preserve food safety: Detect pathogenic microorganisms (bacteria, yeasts, molds) capable of causing serious illnesses, such as *Salmonella* or *Listeria monocytogenes*, as well as their toxins. To conserve Technological Quality: Identify spoilage germs (lactic acid bacteria, yeasts) that can affect the shelf life and marketability of the product. To verify the effectiveness of hygiene measures, applied technologies, and storage conditions for meat products.

➢ Control Methods :

- Traditional and Automated Microbiology: it allows to the enumeration of microorganisms and species identification from meat or raw material samples.
- Molecular Biology (PCR): is a rapid and sensitive technique for amplifying and detecting DNA fragments specific to pathogenic microorganisms.
- NGS (Next Generation Sequencing): allows for precise identification of the microorganisms responsible for spoilage and understanding of the origin of contamination.
- Simulation Software: can simulate the evolution of microorganisms based on parameters such as temperature, pH, and product packaging.

II.10. Actions in Case of Non-Compliance:

A case of microbiological non-conformity occurs when the results of an inspection reveal that established criteria for the presence of microorganisms have been exceeded, whether pathogenic microorganisms (dangerous to health) or microorganisms responsible for spoilage (making the product unsaleable). Managing these cases requires a rapid

investigation to identify the cause of the non-conformity, such as a technological defect, environmental contamination, or poor hygiene practices, in order to implement appropriate corrective measures.

A. Definition and Types of Non-Conformities:

Hygienic non-conformity: Presence of pathogenic microorganisms (bacteria, viruses, molds) that pose a risk to consumer health.

Technological non-conformity: Presence of microorganisms (often yeasts or molds) that impair the marketability of a product without necessarily being hazardous to health, but that render it unmarketable.

B. The causes:

Technological failure: The technology used to ensure the microbiological stability of the product is ineffective.

Environmental contamination: Microorganisms can be present on surfaces, in the air, or in water and contaminate products.

Poor hygiene practices: Manufacturing processes do not comply with hygiene regulations, allowing the proliferation of microorganisms.

Factors intrinsic to the product: Certain parameters (temperature, presence of oxygen, water activity) can promote microbial growth

C. Non-compliance plan:

1. Identify the source of the non-compliance: Conduct a thorough investigation to determine the origin of the problem (product, equipment, environment, process).
2. Implement corrective actions: Adapt technology, hygiene controls, improve storage conditions, etc., depending on the identified cause.

3. Follow-up: Verify the effectiveness of the measures taken through additional microbiological controls.

D. Application of the plan:

- Analysis and Diagnosis: If pathogens are detected, the source of the contamination must be identified and hygiene measures (cleaning, disinfection) and temperature control must be reinforced.
- Corrective Action Plan: An action plan must be implemented to correct non-compliances.
- Recall the product: In the event of proven contamination, products must be withdrawn or recalled, and the relevant authorities notified.
- Follow-up and Reanalysis: Follow-up analyses are required to verify the effectiveness of corrective actions before production and sale can be reauthorized.

III: The fermenter

III.1. Definition of a fermenter:

A fermenter, or bioreactor, is a device in which microorganisms (yeasts, bacteria, microscopic fungi) are propagated for the production of biomass or metabolites. These include small laboratory fermenters and large factory fermenters.

Bioreactors are used in the manufacture of numerous products such as yogurts, food additives, vaccines, antibiotics, antibodies, and vitamins.

III.1.1. Fermenter Components:

The fermenter consists of:

• A tank, also called a reactor: Its volume can range from a few liters (laboratory fermenters for research, teaching, small-scale cultivation, etc.) to several cubic meters in the case of industrial units.

• A syringe with a catheter or tubing to inject a solution.

• A stirring system with one or more impellers, depending on their size.

• Sensors for measuring temperature (thermometer), pH (pH meter), and concentration.

• A computer-controlled control system to record and monitor all operating parameters. Figure 2 below details the components of the fermenter.

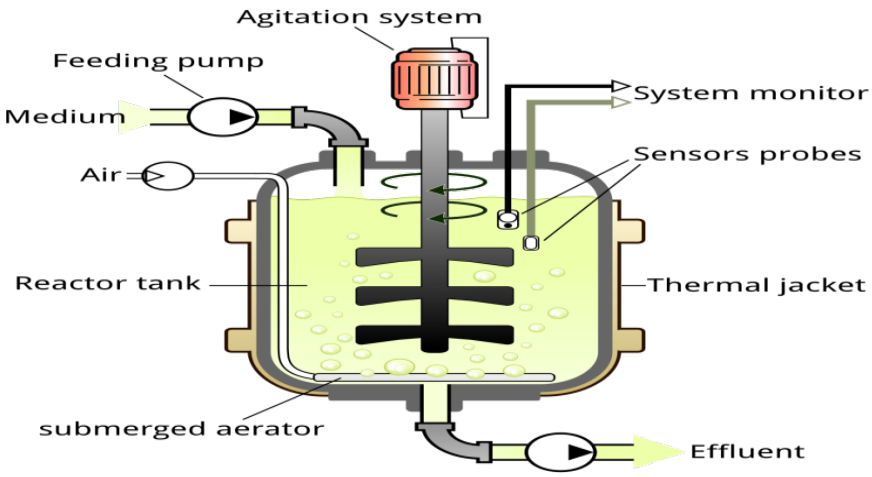


Figure 2: fermenter compounds (Biology Reader, 2025)

III.1.2. Fermenter Uses:

Food and Beverage: For the production of dairy products (cream, butter, cheese, etc.), meat products (mortadella), yeast (bread, pastries, etc.), flavorings, vitamins, organic acids (lactic, citric, etc.), and texturizing agents.

Healthcare and Biotechnology: for production of antibiotic, vaccine, and antibody production, hormone, insulin, and growth factor production. Also for cell culture for gene therapy and burn treatment applications.

Agriculture: Silage production for animal feed preservation and biopesticide production.

Industry: Industrial alcohol, solvent, and biofuel production, and soil remediation and wastewater treatment.

In fine chemistry: production of enzymes, solvents, and biofuels.

- ***Example of using Bacillus amyloliquefaciens in a fermenter:***

This bacterium has the ability to solubilize phosphorus. This bacterium is particularly useful when the soil has a high phosphorus requirement. It is used for vegetable crops such as

lettuce, cauliflower, and artichokes. Its use is required in soils with high pH, where phosphorus tends to be fixed by calcium.

This bacterium is cultivated in fermenters in order to be used as a soil fertilizer.

III.1.3. Bioreactor's sterilization:

To cultivate the selected cells or microorganisms, and only these, the bioreactor must be completely sterilized before introducing the inoculum. In the case of small fermenters, the tank is prepared and then sterilized in an autoclave at 120°C. These small fermenters are therefore often referred to as "autoclavable."

Conversely, in the case of larger fermenters, sterilization is performed on-site, before culturing, by circulating steam. These are referred to as "in situ sterilizable." Some sophisticated units have automatic sterilization sequences.

III. 1.4. Fermenteur feed mode:

III.1.4.1.Batch feeding mode:

The tank is filled with sterilized culture medium, followed by the inoculum. Fermentation then proceeds without the addition of additional medium. The volume remains constant, and productivity is relatively low. At the end of fermentation, the fermenter is emptied and its contents replaced.

III.1.4.2. Batch feed mode (discontinuous mode):

When growth is in stationary phase, sterile culture medium is added. The volume in the tank then increases over time. Feed batching saves time, increases productivity, and allows for the possibility of modifying the medium during cultivation. However, the risk of contamination is high.

III.1.4.3. Continuous power supply mode:

The addition of sterile medium begins when the cells enter the stationary phase of growth.

The suspension is homogeneous throughout the tank. The feed rate is constant when a certain cell concentration is reached in the tank. In theory, it's not necessary to empty the tank, but mutations and contamination require emptying. Productivity here is much higher than in batch mode.

III.1.5. Industrial fermentation:

Industrial fermentation processes use microorganisms to convert substrates into desired products, such as biofuels, fermented foods, acids, and enzymes. The three major types are alcoholic fermentation, which produces ethanol; lactic acid fermentation, which produces lactic acid; and acetic acid fermentation, which produces acetic acid. These processes involve reactors called fermenters, rigorous sterilization, controlled conditions (temperature, pH), and harvesting and product extraction steps.

III.5.1. Common Types of Fermentation in Industry:

1. Alcoholic fermentation:

Carried out by yeasts, it transforms sugars into ethanol and carbon dioxide. It is used to produce biofuels and alcoholic beverages.

Alcoholic (or ethanolic) fermentation is an anaerobic (oxygen-free) biochemical process in which yeast converts sugars (such as glucose) into ethanol (alcohol) and carbon dioxide (CO_2). This reaction, which releases energy (ATP) for the yeast, is essential in the production of bread dough lighter.

➢ The detailed process:

1. Carbohydrates -> Pyruvate: Yeasts absorb sugars and convert them to pyruvate via glycolysis, producing energy (ATP) and NAD+.

2. Pyruvate -> Acetaldehyde: In the absence of oxygen, pyruvate loses a carboxyl group, forming acetaldehyde and releasing carbon dioxide.

3. Acetaldehyde -> Ethanol: Acetaldehyde is then reduced to ethanol through the oxidation of NADH to NAD+, allowing glycolysis to continue.

➢ Optimal Conditions:

Temperature: The optimal temperature varies: approximately 25-30°C for red wine and 18-20°C for white wine.

Oxygen: Although anaerobic, fermentation requires a small initial amount of oxygen for yeast growth.

2. Lactic acid fermentation:

Used by lactic acid bacteria, it converts glucose into lactic acid. It is found in the production of yogurt and many fermented foods.

Lactic acid fermentation is a biochemical process, carried out by lactic acid bacteria in the absence of oxygen, which converts carbohydrates (such as lactose in milk) into lactic acid. This process, used in the production of fermented dairy products (yogurts) and pickled vegetables, acidifies the environment, which increases the preservation of foods, modifies their flavor and texture, and can have health benefits by balancing the intestinal flora.

Process:

-Carbohydrate source: They transform the carbohydrates present in the food (example of lactose in milk).

-Lactic acid production: In the absence of oxygen, these carbohydrates are converted into lactic acid.

-Environmental acidification: The lactic acid produced lowers the pH of the product.

Common Applications:

-Dairy Products: Milk is transformed into yogurt through acidification caused by lactic acid, which coagulates the milk proteins.

-Fermented Vegetables: Vegetables are chopped with salt and canned. Lactic acid fermentation produces the acid that gives them their characteristic flavor and preserves them.

Benefits:

-Storage: Lactic acid inhibits the growth of unwanted bacteria, extending the shelf life of foods.

-Flavor and Texture: It imparts unique organoleptic properties to fermented dairy products and vegetables.

-Gut Health: Lactic acid bacteria, which are probiotics, can help maintain a balanced gut microbiota, which can have positive effects on digestion and the immune system.

3. Acetic acid fermentation:

Acetic acid fermentation is an aerobic bio-oxidation process in which bacteria of the genus Acetobacter convert ethanol (alcohol) into acetic acid, the main component of vinegar, in the presence of air and water. This process, although called "fermentation," is actually an oxidative metabolism that takes place under aerated conditions to produce vinegar from alcoholic solutions.

➢ Mechanism of Acetic Acid Fermentation:

1. Substrate: The process begins with a solution containing ethanol (ethyl alcohol).

2. Microorganisms : Acetic acid bacteria of the genus Acetobacter are the agents responsible for this transformation.

3. Conditions: The presence of oxygen (air) is essential, as these bacteria have an aerobic metabolism.

4. Reaction: Acetobacter bacteria oxidize ethanol to acetic acid, creating vinegar. The reaction can be represented as: Ethanol + Oxygen → Acetic Acid + Water.

5. Biofilm Formation: These bacteria can form a biofilm called mother of vinegar in the presence of air.

4. Other types of fermenttion:

Industrial fermentation can also produce organic acids, enzymes, antibiotics (example of penicillin), and bio-based molecules of interest. Thus, We also have malolactic fermentation (which converts malic acid into lactic acid, which is important in wine), butyric fermentation (which produces butyric acid), and propionic fermentation (which produces propionic acid and CO2). These processes, carried out by various microorganisms, are used to preserve food, transform products, and improve their organoleptic and nutritional qualities.

- **Malolactic fermentation**: Bacteria convert highly acidic malic acid into milder lactic acid, improving the flavor and stability of the wine.
- **Butyric fermentation**: Carried out by bacteria of the genus Clostridium, it produces butyric acid and has industrial applications.
- **Propionic fermentation**: Produces propionic acid and CO2, a process used in the production of certain cheeses.

These fermentations are used by humans for a variety of applications, ranging from preserving foods (such as vegetables and dairy products) to making beverages and baked goods.

III.1.6. Example of antibiotic production in bioreactors:

III.1.6.1. stages of antibiotic production:

To produce an antibiotic, we mainly go through the following steps.

1/Inoculation Preparation:

Inoculation is the entire biomass required to seed a fermenter. Inoculation preparation is based on the cultivation of genetically modified strains. For this, we must prepare a suitable culture medium for the culture.

It can be done by culture method which is a production processes use batch culture, or in batch mode, which is a production which continues until the medium is exhausted or unfavorable physiological conditions occur.

The production environment must first ensure significant growth to lead to a high cell concentration at the time of production. It must then ensure the maintenance of cell vitality and optimized antibiotic production. It must therefore provide energy sources and ensure the desired physicochemical conditions (pH, temperature and oxygenation).

2/Propagation:

Propagation consists of a series of cultures called "pre-cultures" in media of increasing volume. The objective is rapid growth and the production of significant biomass. One of the goals of propagation is to bring the cells into a physiological state conducive to good antibiotic production. The media must provide monosaccharides, polysaccharides, fatty acids, triglycerides, and proteins (these compounds provide carbon and energy sources).

During the initial growth phase, rapidly catabolizable energy sources can be added to ensure rapid growth (glucose for example). During this phase, cells use slowly catabolizable energy

and carbon sources (example of lactose for the production of penicillin; dextrin or starch for the production of macrolides).

Fatty acids and their derivatives are often provided by oils in the form of triglycerides. The most commonly used oils are soybean, peanut, corn, and rapeseed.

In addition to their role as an energy source, fatty acids exert relevant physicochemical actions: emulsion formation, foam reduction, and modification of membrane permeability. Ammonium is the best source of nitrogen to ensure rapid growth. As with glucose, ammonium salts are added to promote this phase while monitoring the concentration to avoid a drop in production.

These complex sources fulfill multiple functions, like soy flours serve as a source of nitrogen, they also provide nucleic acids, vitamins, trace elements, lipids, sulfur and phosphorus.

3/Extraction:

After fermentation, the antibiotic is present at relatively low concentrations in a complex mixture including cells, environmental elements, and numerous metabolites. The antibiotic must therefore be separated, and for this, we use several methods of separation, among them, the following methods.

-Liquid-solid separation:

This separation step is performed by decantation, filtration, or, more specifically, centrifugation. This method is often used.

-primary extraction

From the culture, if there has been no prior separation of the solid-liquid phases, extraction with a suitable solvent is performed after treatment with an acid or base to obtain an ionic form of the antibiotic.

The solvent must be chosen based on its ability to solubilize the antibiotic (a good partition coefficient between water and solvent), it's chemical neutrality with respect to the product (low degradation), a good degree of selectivity (less impurity extraction), a moderate cost and physical properties that facilitate it's disposal or reuse.

4/ Purification :

This uses more refined techniques than extraction to specifically remove impurities. This step represents a significant portion of the production cost.

We can use Liquid-liquid separation with different solvents. The costs are relatively low. When this method cannot be used, purification can be performed using membrane processes such as nanofiltration or gel filtration.

These processes make it possible to select or eliminate some molecules present in the mixture. If purification can't be achieved using the above techniques, chromatographic separations must be used. These methods lead to high levels of purity, which can be used in the medical field, but the chromatography steps are expensive.

II.1.6.2. Factors influencing the production of antibiotics:

The composition of the medium can regulate production by blocking or accelerating enzyme biosynthesis genes. The environments must allow the precursors necessary for the synthesis of antibiotics to be provided without limitation. For good control of biosynthesis, we regulate certain factors in medium, such as fatty acids, nitrogen sources and trace elements. we also must control : pH, temperature and aeration, these factors play a very important role in addition to the possibility of genetic modification of microbial strains.

-Regulation by fatty acids:

Fatty acids play an interesting role. They are present in oils and can be added in their pure form. Example: The addition of methyl oleate (1%) to the production medium

improves the production of nigericin (an antibiotic) by more than 70%.

-Regulation by nitrogen sources:

High concentrations of ammonium or rapidly metabolized nitrogen compounds in the environment suppress the biosynthesis of many antibiotics. Therefore, nitrogen sources must be added in very small quantities. During the production phases, slowly metabolized nitrogen sources must be used.

-Regulation by trace elements:

A whole series of elements: cofactors for the growth of microorganisms are necessary at very low concentrations (traces): Mn, Fe, Co, Ni, Cu, Zn, Mo. Some of them play an important role both quantitatively and qualitatively in the biosynthesis of antibiotics. The most important metal ions for the production of antibiotics are: Mn, Fe, and Zn.

-Regulation of pH:

pH plays a vital role in the production of secondary metabolites. Small variations in pH can have significant effects on strain productivity.

-Regulation of temperature:

It should be noted that while the temperature that allows microorganisms to grow is around 25°C, the temperature that allows antibiotic synthesis is only 5 to 10°C.

-Aeration Regulation:

All antibiotic production occurs under aerobic conditions. Optimal oxygen concentrations are not always the same for growth and for metabolite synthesis. The conditions are also different from one antibiotic production to another, examples: for the production of cephalosporin, we need more oxygen, whereas for the production of penicillin, we decrease the oxygen concentration.

-Improvement by modification of strains:

Improvement strategies aim to increase the final product concentration and reduce the production of undesirable co-metabolites. Improvement strategies use genetic modification of microorganisms.

III.1.7. Example of production of dairy product:

To make cheese or yogurt, we go through several steps, based on the principle of fermentation by lactic acid bacteria (also called lactic ferments). Lactic acid bacteria used in industry are prepared in a series of steps and then packaged for subsequent use in fermenters.

III.1.7.1. Steps to obtain industrial strains:

1-Create a bacterial culture collection strains:

they are preserved in cryovials and stored at -80°C to ensure genetic stability. Strain identity and culture purity are confirmed by laboratories.

2-Preparation of the inoculum (in the laboratory):

The microorganism is propagated in stages using a culture medium in 1- to 10-liter bottles.

It is then incubated at its own optimal temperature. Each bacterium has a specific nutrient and production process tailored to each strain to ensure optimal effectiveness. Step duration: 1-7 days/step (depending on the strain).

3-Fermentation:

The inoculum is transferred to a sterilized medium in the fermenter, duration of each stage: 1-5 days/stage.

4-Biomass harvest:

After cooling, the live bacteria/yeasts are separated from the culture medium and washed by centrifugation to obtain a pure bacterial cream. For Penicillium products, there is an intermediate sieving step to separate the spores from the mycelium.

5-Concentrated reservoirs:

Additional ingredients are added to help to the maintain of cells in optimal condition during final processing (primarily freezing and drying).

6-Freeze-drying:

The bacterial concentrate is frozen at -50°C and vacuum-dried in a freeze-drying chamber.

At this stage, the product has reduced from approximately 75% moisture content to 2% moisture content. Duration of each stage: 72-120 hours/stage.

7-Granulation:

The dried product is ground through a sieve (Fig.3). When the material is pushed through the very fine holes of the sieve, it is reduced to a uniform particle size.

Figure3: A sieve (SYT, 2025).

8-Storage:

The ground bacterial concentrate is stored at -25°C to keep it stable until it is time to mix it.

9-Laboratory Testing and packaging:

Before being used in packaging, the bacteria are sent to the laboratory to be counted and tested to ensure that it is free of contaminants. Finally, the mixed product is packaged in laminated aluminum foil pouches (10 to 100 g) to maximize shelf life. Each batch has a separate lot number.

III.1.7.2. Steps of production of yogurt:

1/Milk Preparation:

By Pasteurization, this is most often done to eliminate microorganisms present in milk that are undesirable for humans. Pasteurization involves heating the milk to a temperature of 72°C for 15 seconds. After that, the milk is cooled and maintained at a temperature of 43°C, the temperature at which the enzymes present in the lactic ferments will best perform their task.

2/Fermentation and inoculation:

Inoculation thus consists of introducing specific lactic ferments into the milk, so that it takes on a new consistency. The rules for making yogurt are therefore strict: to make yogurt, two

types of lactic ferments must be introduced: Lactobacillus bulgaricus and Streptococcus thermophilus.

3/Steaming (post-fermentation treatment):

Once inoculated, the milk is poured into jars. The sealed jars are then placed in a warm room for 3 hours to allow the ferments to multiply and transform the milk into yogurt.

The diagram below (fig.4) summarizes the main stages of yogurt production.

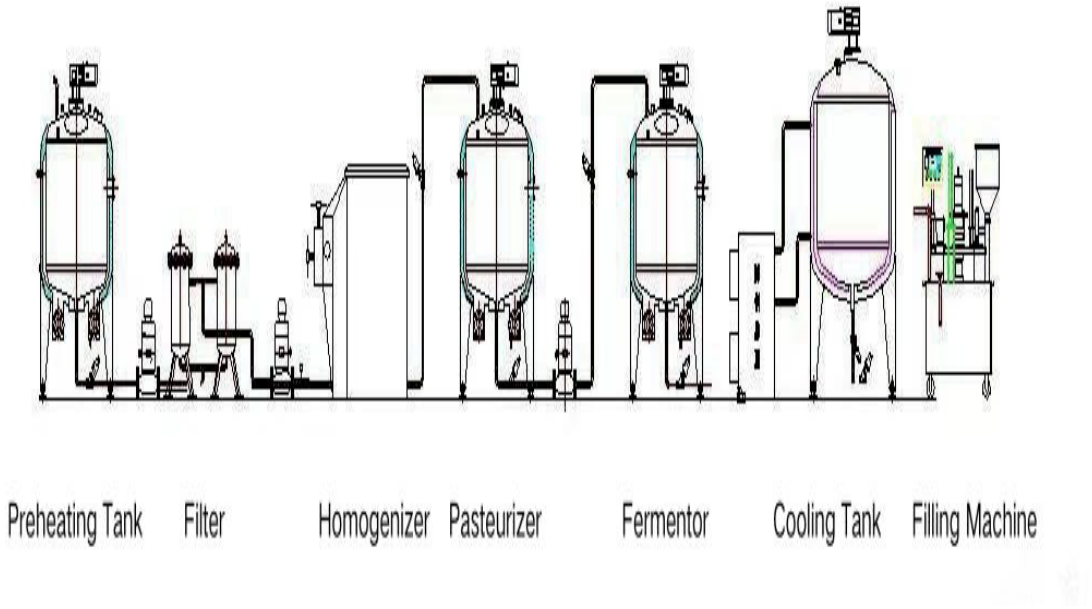


Figure 4: Diagram of steps of yogurt production (Andino, 2011).

III.1.7.2.1.Role of Lactic Acid Cultures:

These lactic acid cultures have the property of fermenting milk to produce yogurt. They digest lactose and produce lactic acid. These bacteria contained in lactic acid cultures must remain alive when the yogurt is consumed.

They also facilitate digestion, particularly for people who have difficulty digesting lactose.

Yogurts are thus said to have digestive properties that are beneficial in cases of intestinal disorders.

III.1.7.2.2.Type of fermentation used:

Lactic acid fermentation is the decisive step in transforming liquid milk into a gel. The two lactic acid bacteria used: *Streptococcus thermophilus* and *Lactobacillus bulgaricus* subsp.

At the beginning of fermentation *S. thermophilus* grows rapidly. They absorb lactose and transform it into L(+) lactic acid, which causes a drop in pH (acidification). They also initiate the development of the *Lb. bulgaricus* population by producing compounds capable of stimulating their growth, such as formic acid and carbon dioxide.

In return, *Lb. bulgaricus* hydrolyzes the casein using a proteinase attached to its walls, which, after further enzymatic reactions, produce other amino acids (especially valine). These compounds, which are essential for bacterial growth, are not present in sufficient quantities in milk at the start of fermentation. *S. thermophilus*, which has a lower proteinase activity, therefore benefits from the presence of lactobacillus.

Thanks to this joint action between the two bacteria (*S. thermophilus and Lb. bulgaricus*), both species grow rapidly and metabolize lactose into lactic acid so that fermentation is completed in 3 to 4 hours, whereas each would have spent 12 to 16 hours to obtain the same acidity.

These two bacterial species also synthesize polysaccharides that they excrete outside the cell (called exopolysaccharides). These are polymers of galactose, glucose, and rhamnose, produced in varying quantities depending on the strain. The presence of these compounds is highly sought after because they increase the viscosity of yogurt and give the product a creamy consistency, appreciated by consumers.

III.1. 7.3.Example of production of cheese:

We use propionic fermentation in cheese making. In this type of production, thus, propionic acid (propanoic acid), ethanoic acid, CO2, and hydrogen are formed. as it is shown in the equation below

- $C_6H_{12}O_6$.....> $CH_3CH_2COOH + CH_3COOH + CO_2 + H_2$

Propionic fermentation uses a wide variety of substrates: sugars, glycerol, lactic acid, and malic acid. Propionic fermentation, with lactic acid as its substrate, plays a major role in cheesemaking. It is used in the production of hard cheeses (Comté, Gruyère, and Emmental), to which it gives a characteristic flavor.

The bacteria that perform this process are the Propionibacterium genus, which converts lactate from the reduction of pyruvate into propionate, acetate, and CO2.

Propionic acid and its salts are known for their antifungal properties. Propionibacterium freudenreichii is essential as a ripening starter in the production of Emmental cheese. Propionate and acetate contribute to the cheese's nutty, mild flavor, and CO2 is responsible for opening the cheese: the holes formed are called cheese eyes (fig.5).

Figure 5: Formation of holes in cheese (La boxe fromge, 2025).

III.1.7.3.1. Steps of production of cheese:

Cheese making involves 5 main stages:

1/curdling of milk:

To make cheese, the milk must first be curdled (fig.6). During this process, the milk coagulates and transforms into a smooth homogeneous gel “the curd”. This coagulation of milk occurs under the action of a mixture of lactic acid bacteria Including ferments, or natural coagulants of animal or plant origin called "rennet," or a combination of the two (Jun *and al.,* 2021).

Figure 6 : The curdled milk (Producteurs du lait, 2020).

2/ Working with the Curd:

The curd is then sliced, broken, and fragmented. The larger of the fragments makes them containing more water. Conversely, the smaller fragments contain low quantity of moisture. Thus, the dough of cheese will be firm.

The grains obtained are then transferred into molds to promote draining (fig.7).

Figure 7: Draining the milk curd (Producteurs du lait, 2020).

3/ Draining and molding:

Draining determines the quality of a cheese. This is a key step during which the curd is drained of it's whey (lactoserum), or part of it's water. On average, 10 liters of milk yield about 1 kg of cheese.Draining can be slow, accelerated-pressed, or pressed-heated, this step lasts between 12 and 48 hours.

A/ Slow draining: which is done by turning, is based solely on gravity. The curd is divided into cubes and placed in a mold where the whey drains simply and quietly. It is used for soft cheeses.

B/The accelerated-pressed method: indicates that the curd cubes are placed in a mold, then mechanically pressed to promote the draining of the whey (fig.8).

Figure 8 : Mechanical pressure sieve (STY, 2025).

C/ The heated-pressing technique: it maintains the curd grains between 40°C and 55°C before being placed in the mold. This type of draining is used for semi-firm and firm cheeses.

4/ Salting:

After unmolding, the cheese will be immersed in a saline solution for a period of time specific to each cheese. Salt acts as a conservative and antiseptic agent. It can also be added to the

cheese mass just before pressing, this is the case for cheddar curds and blocks of fresh cheese (fig.9).

Figure 9 : Salting cheese (Producteurs du lait, 2020).

5/cheese ripening:

The cheese is then placed in a room called a ripening room (fig.10) for maturation. The conditions found in ripening rooms: humidity and ambient temperature (which varies between 8°C and 16°C), These conditions are crucial to the quality of the finished product. It is during this stage that the texture, crust, color and flavors of the cheese develop and become uniform.

Figure 10 : Ripening room (Producteurs du lait, 2020).

The diagram below (Fig. 11), summarizes steps of cheese manufacturing.

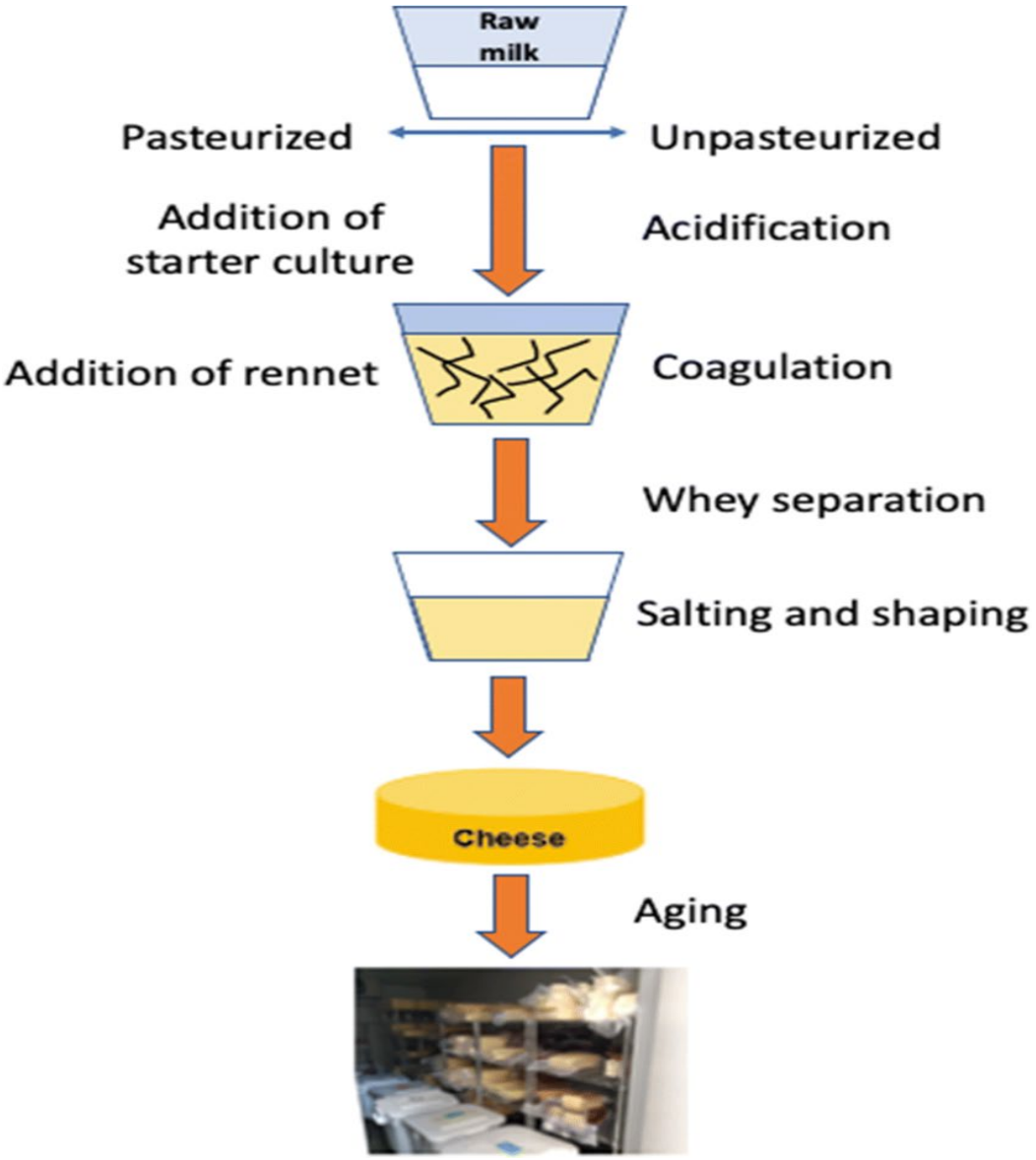


Figure 11: Diagram summarizing steps of cheese manufacturing (Jun *and al.*, 2021).

III.1.8. Example of Industrial soap making :

Industrial soap making is based on the hot process of saponification, where fatty substances (oils) and NaoH are mixed and heated for several days until a soap paste is obtained. NaoH transforms all the oils and is then removed by rinsing with salt water, which also separates the glycerin. The soap paste is then refined, mixed with additives such as perfumes and colorants, and extruded to form regular bars of soap.

➢ Key ingredients:

1. **Fat:** a vegetable oil (olive oil, argan oil, coconut oil, jojoba oil, etc.), shea butter, or animal fat.
2. **Strong base:** NaoH (for solid soap) or potash (for liquid soap).
3. **Water:** to dissolve.

The diagram below (Fig. 12) summarizes steps of soap manufacturing.

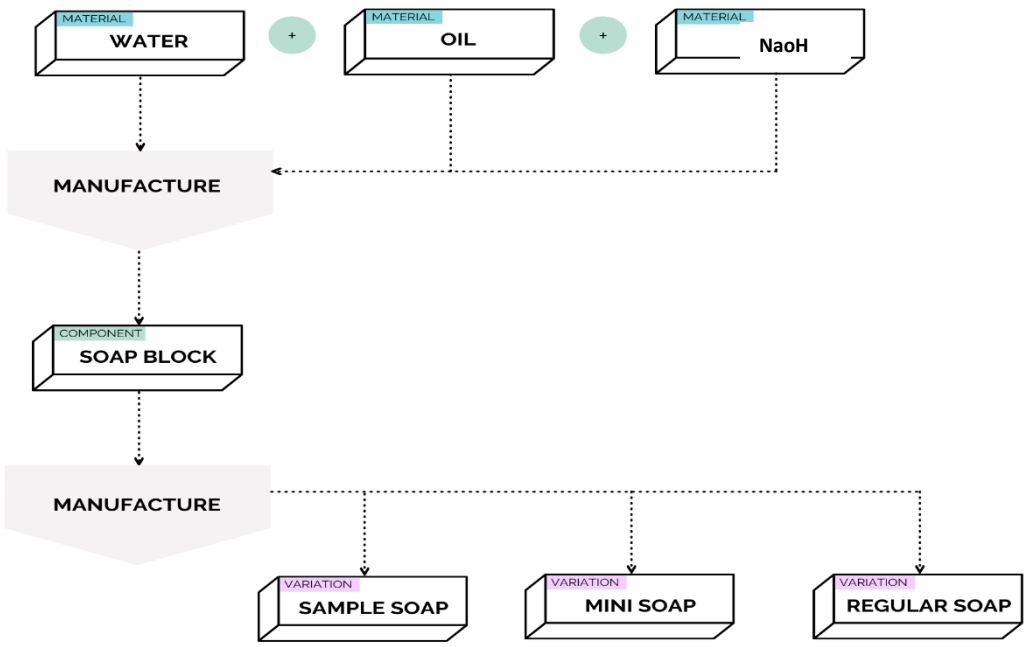


Figure 12: Diagram summarizing steps of soap manufacturing (Craftybase, 2025).

III.1.8.1. The step-by-step process:

Preparation of ingredients: Fatty substances (vegetable or animal oils) and hydroxide of sodium are prepared.

Hot saponification: The oils and soda are poured into a large cauldron and heated to a high temperature, reaching up to 100 degrees. The chemical saponification reaction occurs, transforming the fatty substances into soap paste.

Rinsing and separation: The paste is rinsed with salt water to remove excess soda. The salt water also precipitates and carries away the naturally produced glycerin.

Refining and addition: The remaining soap paste, stripped of glycerin and excess soda, is washed and dried. It is then grated into small shavings and mixed with fragrances and colorants.

Extrusion and cutting: The paste is passed through an extruder (or extruder) to form long, even bars of soap. These bars are then cut to the desired size.

Molding and Packaging: The cut bars are molded and stamped, then packaged before shipping.

III.1.8.2. Key differences from handmade soap:

Hot process soap making: The industrial method uses heat to accelerate the reaction, allowing for the rapid production of very large quantities of soap.

Glycerin extraction: In the industrial process, glycerin, a natural moisturizer, is systematically separated from the soap by rinsing with salt water.

Production time: The industrial process is very fast, with the soap ready to use in about a week, as opposed to several weeks of drying and curing for handmade soap.

III.1.9.Example of biopurification:

An example of biopurification is an activated sludge treatment plant where aerobic microorganisms degrade organic pollution in wastewater, forming flocs that are separated by settling. Other examples include trickling filters, reed beds, and lagooning, methods that use natural mechanisms, such as the action of bacteria in beds or basins, to purify wastewater. The diagram of figure 13, shows the main steps of activated sludge process (Kenki, 2020).

III.1.9.1. Steps of biopurification:

1. Activated Sludge Treatment:

-Principle: Bacteria present in the wastewater are fed and reintroduce air into the tank to form flocs (or activated sludge).

-Process: The flocs agglomerate, settle to the bottom after treatment, and the purified water is released into the natural environment.

-Equipment: The key stages are the aeration tank and the secondary settling tank, which separates the sludge.

2. Trickling Bed:

Principle: A support (rocks, biomedia) on which bacteria settle and form a biofilm.

Process: The wastewater passes through this bed where the biofilm degrades organic pollutants.

3. Lagooning:

Principle: Uses basins where wastewater is naturally treated through physical and biological processes. The Procedure is that water passes through these basins, undergoing settling and treatment by aquatic flora and fauna before being released into the natural environment.

Treatment in basins, primarily the aeration basin, involves bacteria that consume organic pollution from wastewater in the presence of oxygen, an essential process in wastewater treatment. Other types of basins exist, such as the primary settling tank, where solids settle, or the lagoon basin for natural treatment, as well as phytoremediation basins using plants.

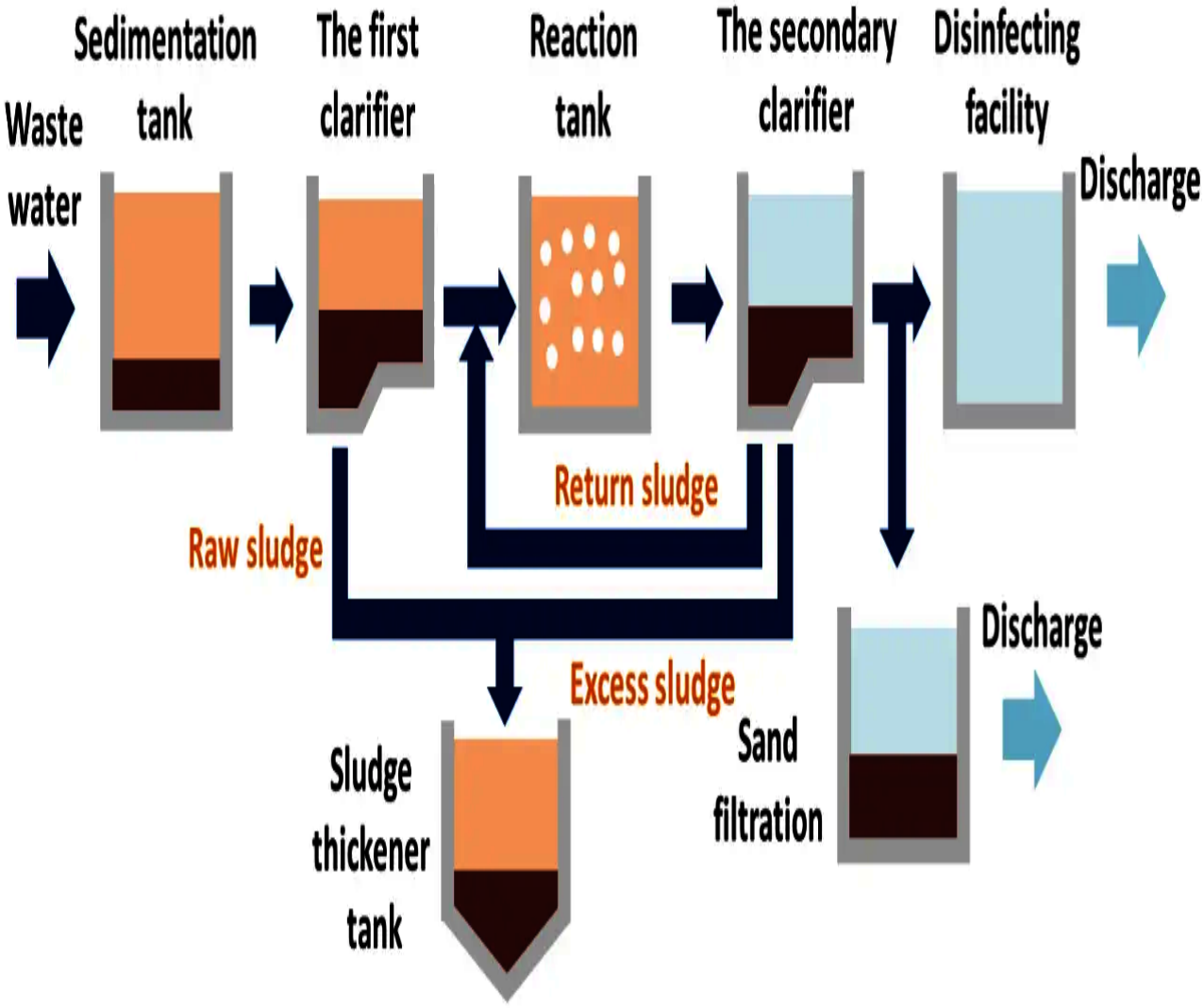


Figure 13: Diagram of conventional activated Sludge (Kenki, 2020).

➢ The Role of Wastewater Treatment:

-Water Treatment: Wastewater treatment basins treat domestic, industrial, and storm water wastewater to remove impurities before releasing it into the natural environment.

-Wastewater Treatment: They use physical, chemical, and biological processes to eliminate pollution.

➢ Different Types of Basins:

-Aeration basin (or biological reactor) (Fig.11): This is the heart of the wastewater treatment. Bacteria are cultivated here to degrade organic matter in the presence of oxygen, which they use as food. This process aerates the water, as the bacteria consume dissolved oxygen, and it transforms soluble pollutants into solid particles (bacterial sludge).

-Primary clarifier: After pretreatment (screening and grit removal), the water passes into this basin where the heavier solid particles settle to the bottom. This sludge is then scraped and removed.

-Lagoon pond: A natural purification technique, where ponds create "buffer" environments to treat polluted water using ecological processes.

-Phyto purification pond: An ecological solution that uses plants and their filtering properties to decontaminate wastewater.

Figure 14: bioreactors of water purification (Gettyimages, 2025).

➢ The Overall Process in the Basins:

1/Pretreatment: The water passes through screens to remove large debris.

2/Primary Treatment: The water settles in a basin, forming sludge that is discharged.

3/Secondary Treatment (Aeration Basin): Bacteria degrade pollutants using oxygen.

4/Clarification: After the aeration basin, the sludge separates from the water in another basin.

5/Discharge: The purified water is then returned to the natural environment.

➢ Why are these examples considered biopurification:

They all rely on the use of microorganisms (bacteria, protozoa, etc.) to break down pollutants.

They recreate the natural self-purification mechanisms of waterways, in the presence of oxygen (aerobic conditions).

III.1.10. Manufacturing baby milk powder:

Infant Formula process of milk requires an intense focus on cleanability, hygienic design, safety, repeatability in process parameters and reliable equipment. The production of Infant Formula requires a high focus on product safety, traceability of raw materials and quality which often leads to many energy-consuming steps in terms of heat treatment, and this is an expensive setup. The powder processing line can be designed in different ways. The design of the process is based on our experts' knowledge and experience.

Manufacturing baby milk powder involves a wet mix process where raw ingredients are dissolved in water, followed by pasteurization, homogenization, evaporation, and spray drying to create the powder. Heat-sensitive ingredients like vitamins are often added after these high-heat stages, and the final product is packaged under controlled atmospheric conditions to ensure safety and stability.

III.1.10.1. Steps of manufacturing baby milk powder:

1.Ingredient preparation: Raw materials like milk powders, whey, vegetable fats, lactose, and other nutrients are weighed and prepared. Heat-sensitive ingredients such as vitamins may be mixed in separately or added later.

2.Wet mixing: Ingredients are mixed with water or skimmed milk to create a liquid base.

3.Pasteurization: The liquid mixture is heated to kill harmful bacteria, followed by homogenization, which breaks down fat globules for a stable, uniform product.

4.Evaporation: Water is removed from the concentrated liquid in a vacuum evaporator to increase the total solids content.

5.Spray drying: The concentrated liquid is sprayed into a hot chamber, where the heat evaporates the remaining water, leaving fine powder particles.

6.Cooling: The powder is cooled to prevent clumping and maintain quality.

7.Final mixing and packaging: Heat-sensitive vitamins and other nutrients are often added to the cooled powder. The final product is then mixed thoroughly and packaged in an inert, controlled atmosphere, usually with nitrogen, to prevent oxidation and extend shelf life.

➢ Challenges in Infant Formula Mixing:

Preparation of the premix is subject to a number of problems when using agitators and conventional powder/liquid blending systems:

- The powders are very cohesive and must be added at a controlled rate to reduce lump formation.
- Conventional systems do not produce sufficient shear to break down agglomerates.
- Long processing times are required to complete dispersion and achieve a lump-free premix for homogenization.
- The vigorous agitation required to disperse the powder can lead to foaming.
- The process must be carried out in the most hygienic manner possible. Problems can arise with conventional systems as they tend to allow a build-up of partially hydrated powder on vessel walls and mixer shafts, etc. raising potential hygiene issues.

III.11. Manufacturing face cream:

Manufacturing face cream involves creating a stable emulsion of oil and water phases, typically through a multi-step process of formulating, mixing, and emulsifying. Key steps include heating and combining the separate oil and water phases, which contain ingredients like emulsifying waxes, oils, butters, humectants, active ingredients, and preservatives. Once

combined and cooled, the mixture is homogenized, preserved, and then filled and packaged (Fig.15).

Figure 15: Manufactured face cream (Shutterstock, 2025).

III.11.1. Steps of manufacturing of face cream:

According to Teixeira *and al* (2022), the main steps of face crem manufacturing are:

1. Formulation and design: Conceptualize the product, develop the formula, and determine the types and quantities of ingredients. This includes selecting the appropriate oils, butters, water-soluble ingredients like glycerin or aloe vera, emulsifying waxes, and preservatives.

2. Preparation of phases:

- **Oil Phase**: Combine and heat oils, butters, and emulsifying waxes until they are melted and homogenous. Some ingredients may be dry-blended first.
- **Water Phase**: Prepare a separate phase with water-soluble ingredients like glycerin and water, and stabilizers like Carbopol.

3. Heating: Heat both the oil and water phases to a consistent temperature, typically between 45 and 85 °C depending on the formulation.

4. Emulsification: While maintaining the correct temperature, slowly combine the heated oil phase into the water phase and mix thoroughly. A high-shear mixer is essential for creating a stable emulsion where oil and water particles are evenly dispersed.

5. Cooling and adding additives: Continue to mix the emulsion as it cools. Once the temperature drops below a certain point (below 45°C, add heat-sensitive ingredients such as preservatives, fragrances, or active ingredients.

6. Quality control and finishing: Conduct quality control checks, including pH balancing, and add batch coding and labeling.

7. Packaging: Transfer the finished cream into airtight containers, such as pump bottles, to prevent contamination and maintain freshness.

The Figure below (Fig.16) shows the main steps of manufacturing of face cream.

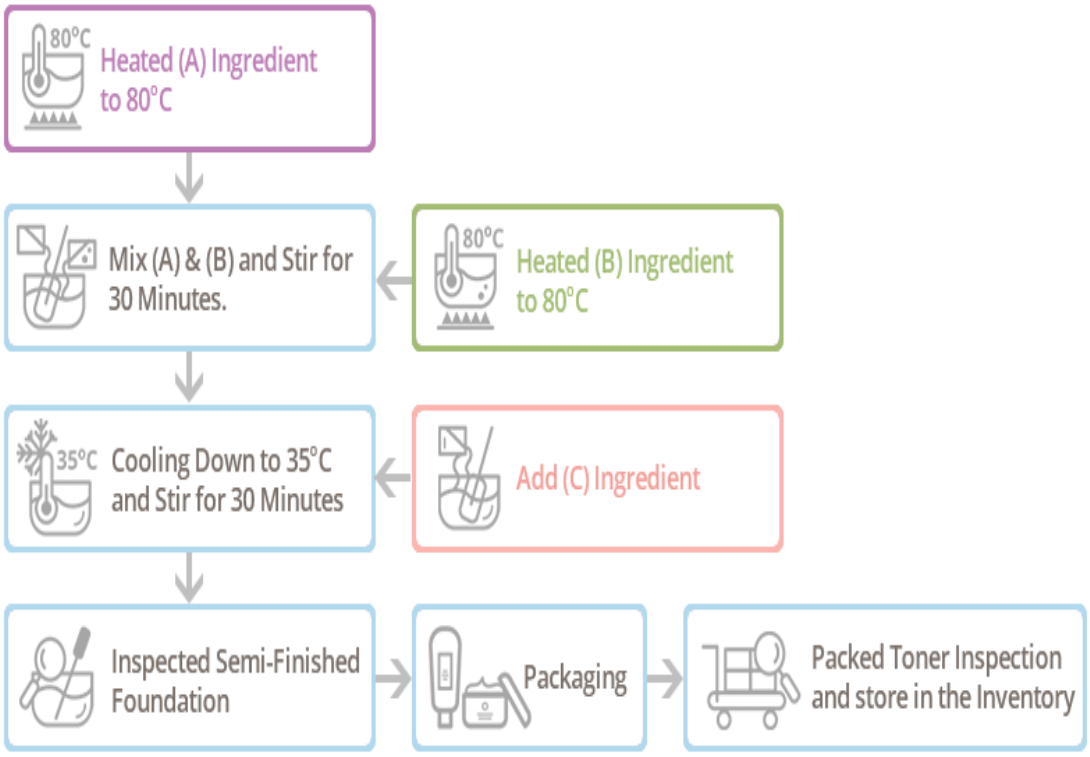


Figure 16: Diagram of manufacturing of face cream (Biocrown, 2024).

IV.1. Definition of viruses:

A virus is a type of infectious agent, a microorganism capable of causing disease by invading a host cell and replicating within it. Unlike bacteria, viruses are not cells but molecular assemblies that require the cellular machinery of a living being (host cell) to reproduce. They can transmit disease by attaching to receptors on the cell, entering it, and hijacking the cell's metabolism for their own replication.

A virus is characterized by its inability to reproduce by mitosis, by succissiparity, or by meiosis. Therefore, to replicate its nucleic acid, it depends on a host cell that it must infect to hijack and use its metabolism: a virus is necessarily an intracellular parasite. It is composed of one or more nucleic acid molecules (DNA or RNA, single or double stranded). Viruses change form during their life cycle; they pass through two stages.

- Characteristics of a Viral Infectious Agent:

-Nature: Viruses are obligate infectious agents, meaning they must parasitize a living cell to replicate.

-Structure: They consist of at least one nucleic acid (DNA or RNA) and are often encapsulated in a protein shell called a capsid.

-Replication: A virus attaches to a host cell, enters it, and uses the cell's constituents and metabolism to produce new viral particles.

-Phases: The virus has two phases: an extracellular phase in the form of an infectious viral particle, and an intracellular phase where it replicates by parasitizing cellular functions.

IV.1.2. Stages of life's viruses:

1/extracellular form:

This is an independent material unit called a "virion." This is when it has a capsid. In the extracellular form, viruses are particulate, infectious objects, consisting of at least one nucleic acid, often enclosed in a protein capsid.

2/ Intracellular form:

In the intracellular form (inside the host cell), viruses are genetic elements that can replicate by disrupting the host cell's metabolism. Viruses can integrate into the host genome chromosome.

- **General Stages of the Viral Life Cycle:**

-Attachment (or Adsorption): The virus attaches to the host cell surface by binding to specific receptors.

-Penetration: The virus or its genetic material enters the cell.

-Uncoating: The viral genetic material (DNA or RNA) is released from its capsule (capsid) inside the cell.

-Genome Replication: The virus uses the host cell's machinery to copy its own genetic material.

-Viral Component Synthesis: The virus directs the cell to produce proteins and other components needed to form new viruses.

-Assembly: The viral components (new copies of the genome and proteins) assemble to form new viral particles.

-Release: The new viral particles leave the host cell, either by lysis (cell bursting) or without killing the cell, to infect other cells.

- **Specific Cycles:**

-Lytic cycle: This is the active replication phase of the virus, where it reproduces rapidly, often destroying the host cell in the end.

-Lysogenic cycle: The viral genetic material integrates into the host cell's genome and may remain dormant for some time before switching to active replication mode.

These stages may vary slightly depending on the type of virus, but the general principle remains the same: a virus must enter a cell and use its resources to reproduce.

IV.1.3. Virus Structure:

Viruses primarily contain a capsid, which contains nucleic acid (DNA or RNA). The capsid is a lipid envelope. Some capsids sometimes contain enzymes (example of reverse transcriptase, an enzyme in HIV). Viruses also contain receptors to attach to host cells. Figure 17 shows virus structure. Based on their structure, viruses are classified into four (4) types of viruses.

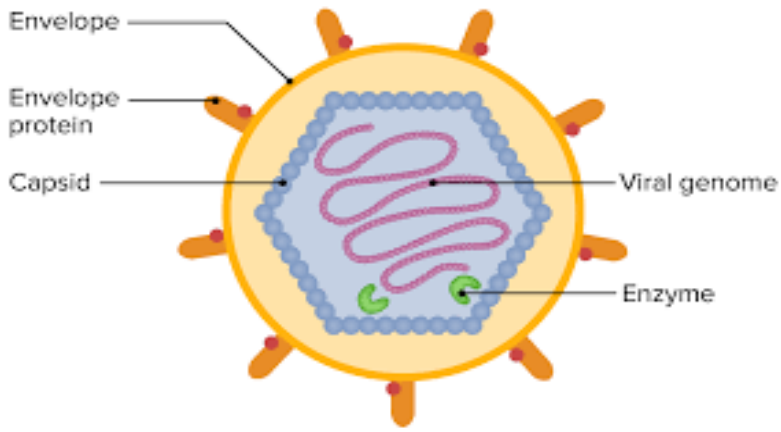


Figure17: Structure of viruses (General Microbiology, 2019).

IV.1.4. Types of viruses:

1/ Icosahedral viruses:

The icosahedral capsid gives the virus a spherical appearance (fig.18). An icosahedral virus has a capsid that is shaped like an icosahedron, a regular polyhedron with 20 equilateral triangular faces, 12 vertices, and 30 edges. This structure gives the virus a spherical appearance. The capsid is composed of protein subunits called capsomeres, organized into pentons at the vertices and hexons on the faces and edges.

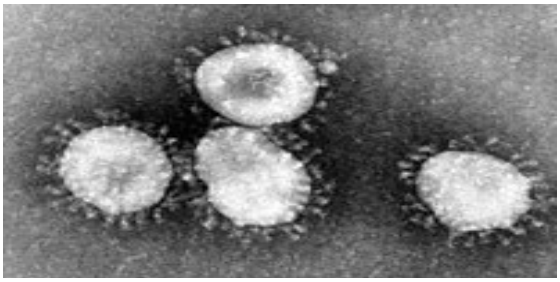


Figure 18: Electron microscope photo of an icosahedral virus (Shutterstock, 2003)

2/helical virus

These viruses are long, hollow cylinders composed of a type of protomer coiled in a helical spiral (fig.19) to form rings called capsomeres. The genetic material is housed inside the tube.
A helical virus is a virus whose capsid (protein envelope) is shaped like a hollow cylinder or rod. Protein subunits, called capsomeres, coil in a spiral to form this tubular structure, protecting the nucleic acid of the virus inside. Tobacco mosaic virus is a classic example of a helical virus.

- Main Characteristics of helical virus:

-Shape: Cylindrical or filamentous.

-Structure: The capsid is formed by the helical assembly of many identical proteins.

-Genetic Material: Nucleic acid (DNA or RNA) is contained within the hollow tube formed by the capsid.

- Examples: The tobacco mosaic virus, the rabies virus, and the Ebola viruses are examples of helical viruses.

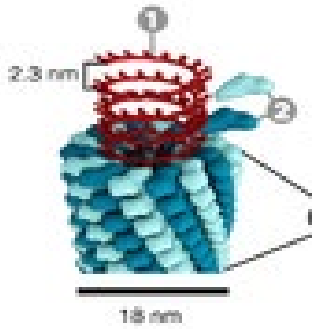


Figure 19 : Helical structure (Shutterstock, 2003).

3/Enveloped Viruses:

In addition to the capsid, some viruses are capable of surrounding themselves with a membrane structure borrowed from the host cell. This membrane envelope is composed of a lipid bilayer that may contain proteins encoded by the viral genome or the host genome. Example: Influenza viruses.

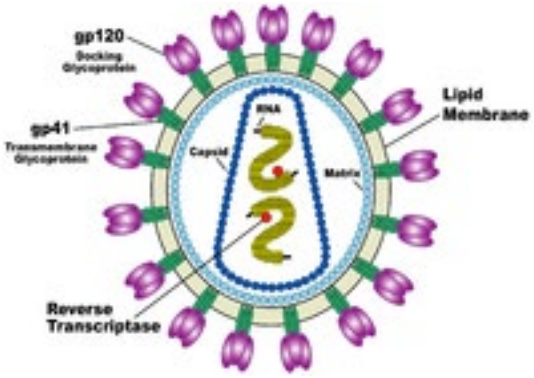


Figure 20: Enveloped viruses Structure (Shutterstock, 2003).

4/ Complex viruses:

These complex viruses have an icosahedral head attached to a helical tail (fig.21). A complex virus is one whose capsid (protein envelope) structure cannot be described by simple helical or polyhedral symmetry. These viruses incorporate a wider variety of components and more elaborate architectural structures, such as the absence of simple symmetry, the integration of two types of symmetry, or structures like "legs." Bacteriophages and poxviruses are examples of complex viruses.

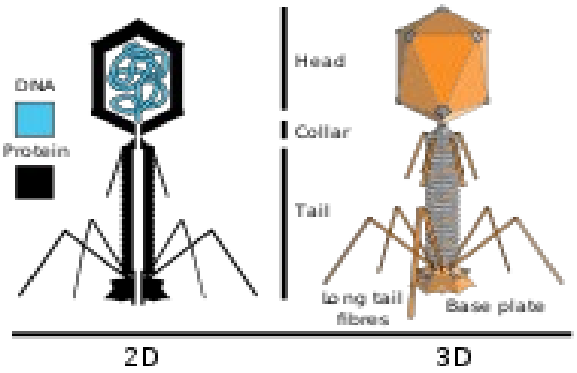


Figure 21: Structure of complex viruses (Shutterstok, 2003).

➢ Characteristics of complex viruses:

-Non-standard structure: Their capsid does not follow a simple geometry such as a helicoid or an icosahedron.

-Assembly of varied components: They contain accessory proteins or non-protein elements, which differentiates them from simple viruses, which have a more uniform structure.

➢ Examples of complex viruses:

- Bacteriophages: These viruses that infect bacteria often possess a complex, even binary, structure, combining helical and icosahedral elements, with "leg-like" structures for attachment.

- Poxviruses: These are large viruses with unusual morphology and complex structures, such as the smallpox virus, which do not fit into the categories of helical or icosahedral symmetry.
- Rotaviruses: Some of these may have a triple icosahedral capsid, making them highly resistant in the environment and in the digestive tract.

IV. 1.5. Cytopatic effect:

IV.5.1. Definition of the cytopathic effect:

These are the morphological and metabolic alterations caused by viruses. Viral replication mobilizes most of the host cell's biochemical resources, thus impacting the latter. There are several possible manifestations of the cytopathic effect depending on the viruses considered, such as: rounding of cells and detachment of groups of cells, fusion of infected cells forming polymorphonuclear giant cells, formation of inclusion bodies, as with poxviruses.

- Example of the cytopathic effect of a viral infection: The herpes virus "herpes":

Primary infection: The virus multiplies at the point of entry, the oral or nasal mucosa, reaches the sensory nerve endings, and is transported via the neuronal pathway. This primary oral infection is symptomatic in only 10% of individuals. Figure 22 shows symptoms of herpes infection.

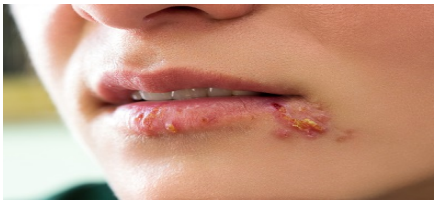


Figure 22 : Herpes infection (Diariopharma, 2018).

IV. 2. Prion Basics

IV.2.1. Definition of Prion:

A prion is a pathogenic protein structure (fig.23). Prion do not have nucleic acid (DNA or RNA) to carry infectious information. Prions are abnormally folded infectious proteins that cause rare and neurodegenerative diseases in humans and animals. This abnormal form of the cellular prion protein (PrPC) induces the misfolding of normal PrP, creating aggregates (Fig.24) that accumulate in the brain, destroying neurons and forming spongy tissue. Prion diseases, which include Creutzfeldt-Jakob disease (CJD) in humans and bovine spongiform encephalopathy (BSE) in animals, are incurable and progress rapidly to death.

Prions are extremely resistant to conventional disinfection methods.

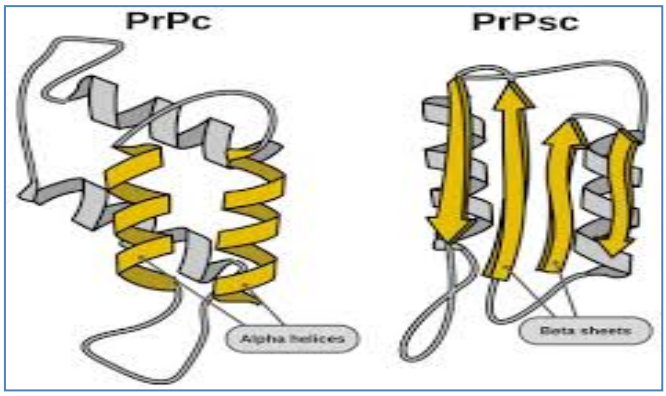


Figure 23: Structure of a prion (Vikrant, 2024)

A distinction is made between mammalian prions, which infect humans and various animal species, and prions found in fungi such as *Saccharomyces cerevisiae* (baker's yeast).

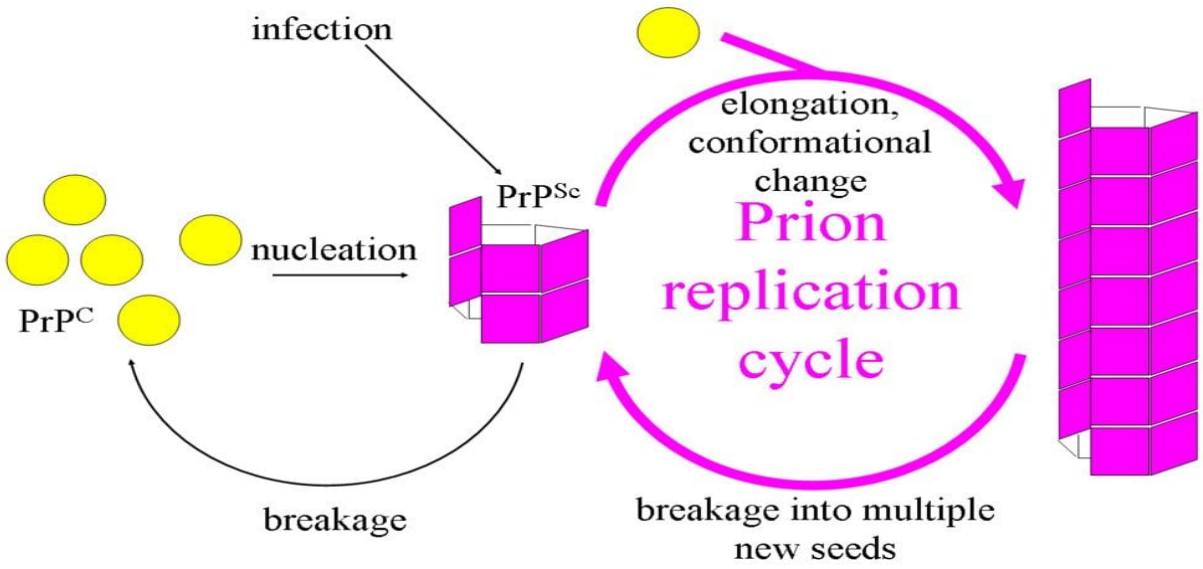


Figure 24: Cycle of prion replication (Libre texts biology, 2025).

IV.2.2. Prion diseases:

Mammalian prions are the pathogens responsible for TSE diseases. Variant TSE is a human neurological disease. First identified in 1996, it constitutes a new form of transmissible spongiform encephalopathy (TSE) or prion disease in humans. All of these diseases are characterized by degeneration of the central nervous system (brain and spinal cord) linked to the propagation or multiplication of prions in the infected host. In mammals affected by prion diseases, it is referred to as TSE disease. TSE are associated with the accumulation of an infectious protein agent in the central nervous system: the prion (Proteinaceous Infectious Only Particle).

The prion, in its normal form (PrP) (fig.24), plays a role in the animal's life processes, but this role is not clearly defined. In the pathological form (PrPc) (fig.24), this protein does not degrade and accumulates in the body, becoming toxic; it is then considered a pathogen.

- **The development of prion diseases**:

✓ Misfolding: The process begins with the introduction of an abnormal prion or a genetic mutation.

✓ Chain reaction: This abnormal protein transforms normal PrPsC into pathogenic prion.

✓ Accumulation of aggregates: Misfolded prion aggregate, forming fibers and plaques that accumulate in the brain.

✓ Neuronal death: The accumulation of these aggregates leads to the death of neurons, causing neurological disorders.

✓ Sponginess: Brain cells eventually die, and the empty spaces left behind give brain tissue a spongy appearance, hence the term "spongiform encephalopathy."

“TSE” human disease are characterized by asymptomatic incubation periods that can exceed 50 years in some acquired forms of the disease. It is now clearly established that TSE can be transmitted between individuals through blood transfusions.

- **Example of TSE disease :** Trembling in sheep and goats:

This is a very common disease in herds. It is a TSE disease (affecting the nervous system), the contamination occurs during feeding in pastures, and symptoms include herd weight loss and trembling.

IV.2.3. Detection of prion disease:

To detect prion disease in humans, a probable diagnosis is made using tests such as brain IRM, electroencephalogram (EEG), and lumbar puncture to analyze cerebrospinal fluid (CSF) with markers. However, a definitive diagnosis can only be made after death, during an autopsy that reveals the characteristic brain lesions and prion deposits.

Caracterics Tests (on a human scale):

Cerebral IRM: it can detect specific signs of brain damage, particularly in the basal ganglia and cortex.

Electroencephalogram (EEG): This test analyzes the brain's electrical activity and can reveal disturbances characteristic of prion diseases.

Lumbar Puncture: Cerebrospinal fluid sampling can be used to detect indirect markers, which indicate neuronal damage.

Genetic Analysis: A search for a genetic abnormality of the prion protein gene is also performed.

Warning Signs: Prion disease should be suspected when faced with rapidly progressive symptoms, such as cognitive decline (dementia), coordination problems, myoclonus (muscle twitching), balance problems, or behavioral changes.

To identify viruses, we have direct and indirect methods.

V.1. Direct detection:

V.1.1. Electron Microscope:

The electron microscope is a tool that allows the visualization of extremely small objects. For this reason, it has been used extensively to characterize and identify viruses. This technique allows the virus to be identified based on its structure and size. Its main advantage is its speed of performance.

V.1.2. Viral Culture:

Among the direct methods: cell culture which is the most traditional diagnostic technique, but also the most time-consuming and expensive. It involves inoculating samples onto a cell layer and monitoring for the appearance of a cytopathic effect linked to viral multiplication. Cell culture allows cells to be kept growing under controlled conditions. This process has been widely used for the isolation and maintenance of viral strains. Although this approach is often slow and requires considerable skill, it has long been considered the standard for laboratories diagnosing human and animal viral diseases. This effect occurs several days or even weeks after inoculation and delays diagnosis accordingly.

The researcher will seek to detect, by microscopic examination of the cells in culture, signs of viral infection. We can cite the swelling or shrinkage of the cells. When the virus is cultivable, the use of direct detection can accelerate diagnosis and achieve faster detection. The techniques used for direct antigen detection are the same as those used for antibody detection, already described. We will therefore use agglutination, precipitation, and ELISA (Enzyme-Linked Immunosorbent Assay) techniques.

V.1.3. Direct detection of viral antigens:

Techniques for diagnosing infections can rely on the direct detection of viral proteins in samples. There are also methods that reveal the presence of viral antigens in cells by attaching an antibody labeled with a fluorescent substance to them. In the direct method, it is the antiviral antibody itself that carries the fluorescent label.

V.1.4. Nucleic acid detection:

Viral DNA or RNA can be detected by hybridization. The basic principle of this technique is simply the complementarity of double strands. If the nucleic acids are subjected to a high temperature (94°C), the strands dissociate. Upon cooling, complementary probes are added; the probes will then match the target strand. These probes are generally labeled with radioactive markers, which are easily identifiable.

- **Our example is PCR:**

The polymerase chain reaction (PCR) allows a known nucleotide sequence to be copied a very large number of times. This makes it easier to detect, using specific primers and a device called a thermal cycler (fig.25). This technique allows DNA fragments to be identified. Hybridization and amplification steps are repeated 30 to 40 times, resulting in the production of numerous copies of the target nucleotide segment. In a second step, the presence of PCR products is visualized, most often on a polyacrylamide gel. This technique makes it possible to copy precise DNA sequences in large numbers. This can be done from a small sample, such as a small drop of blood.

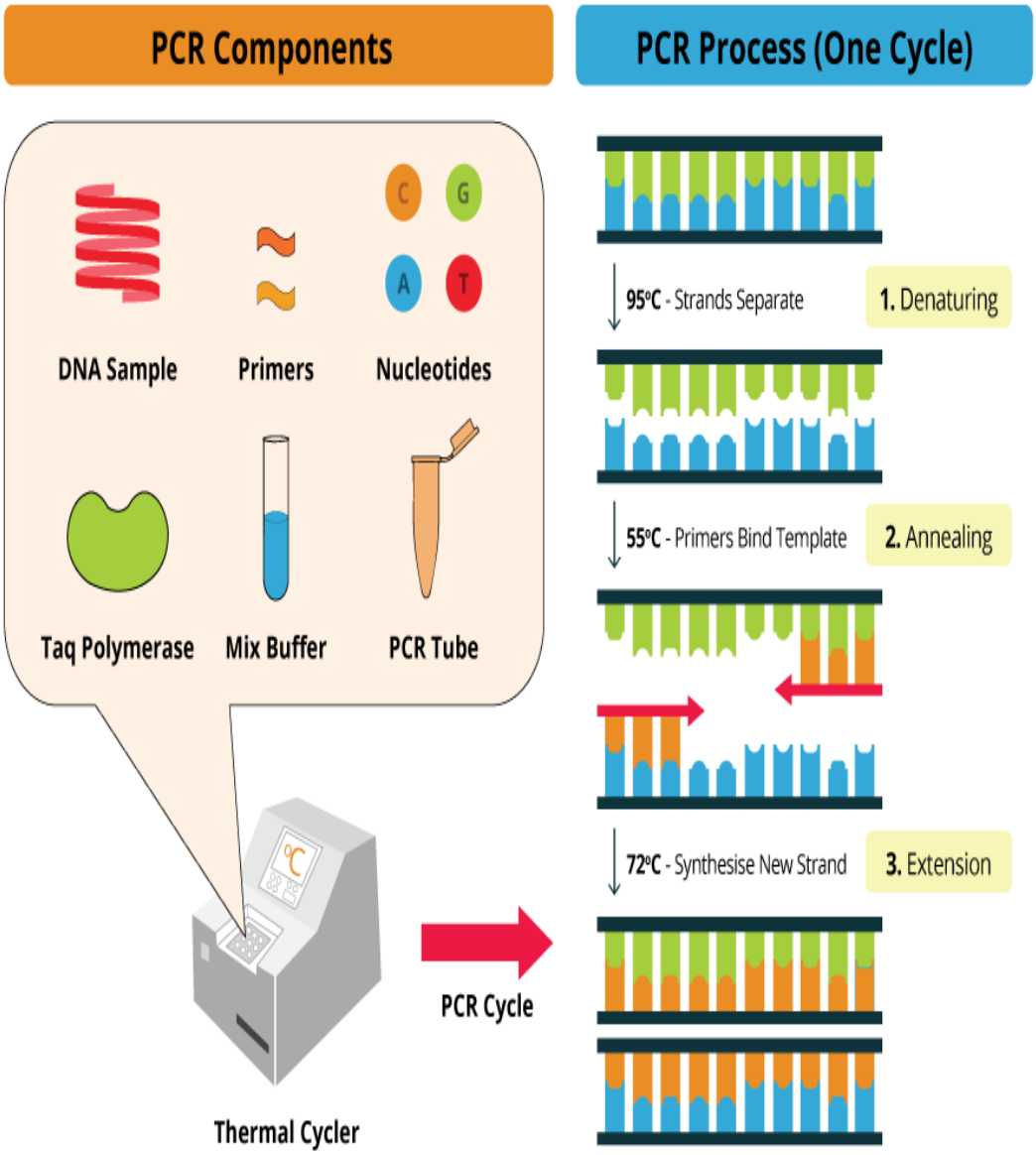


Figure 25: Diagrams to summarize the PCR technique (Facellitate, 2023).

V.2. Indirect detection:

It is the detection of antibodies, thus, the connection of Antibody/Antigen will be binding and labeling for identification (fig.26). These use microscopic particles (latex, gelatin, or red blood cells) coated with a viral antigen and its epitopes, which are mixed with a dilution of serum. Antibodies are capable of binding two epitopes and will therefore establish bridges between the particles, which will be visible as microscopic or macroscopic agglutination. For this type of test, the most used example is the ELISA (Enzyme Linked Immuno Sorbent Assay) test, and the western bloth which has the same principle.

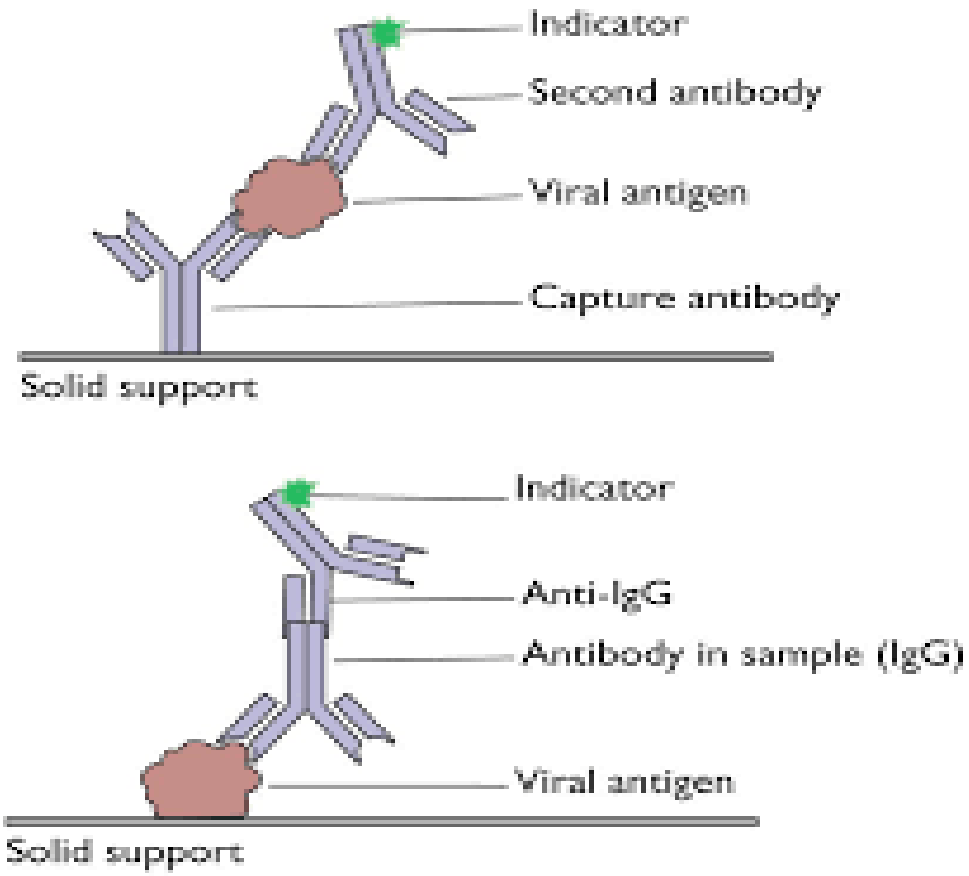


Figure 26: Diagram of antibody detection by Western blot (Facellitate, 2023).

A/ Western blot technique:

Western blotting is a molecular biology laboratory technique used to detect and quantify specific proteins in a complex sample. The procedure involves separating proteins by size during electrophoresis (Fig. 27), transferring them to a membrane (nitrocellulose), blocking the membrane, incubating them with specific antibodies to identify the target protein, and then detecting that protein using a conjugated secondary antibody.

- **Main steps of Western blotting**:

1. Gel electrophoresis (SDS-PAGE): Proteins in a sample are separated according to their molecular weight by passing through a polyacrylamide gel under the influence of an electric current.
2. Blotting: The separated proteins are transferred from the gel to a transfer membrane, usually nitrocellulose, using an electric field.
3. Blocking: The membrane is incubated with a blocking buffer to saturate free binding sites and prevent antibodies from binding nonspecifically.
4. Incubation with a primary antibody: A primary antibody, specific to the protein of interest, is added and binds to the target protein on the membrane.
5. Washing: Unbound primary antibodies are removed by repeated washing.
6. Incubation with a secondary antibody: A secondary antibody, conjugated to an enzyme or fluorescent substance, is added. This antibody recognizes and binds to the primary antibody.
7. Washing: Excess secondary antibodies are washed away.

8. Detection: The luminescent or fluorescent substrate corresponding to the enzyme in the secondary antibody is added, producing a signal (a bright band) that reveals the position of the target protein on the membrane.

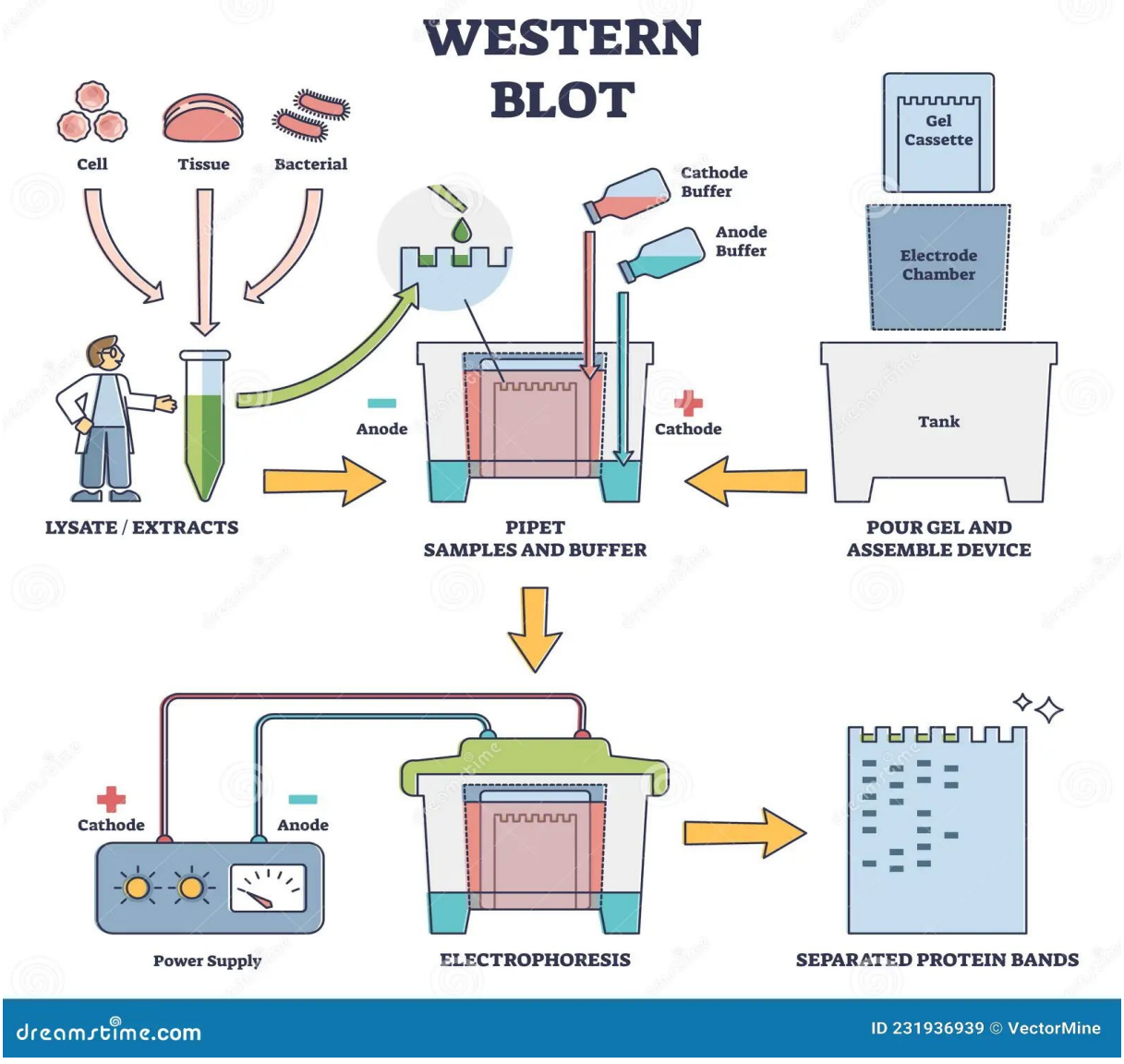


Figure 27: Diagram of the Western blot (Dreamstime, 2025).

- **Applications and Benefits**:

-Detection and Quantification: Western blotting identifies the presence and quantity of a protein of interest in a complex protein mixture.

-Protein Characterization: It determines the size of the target protein and compares protein expression levels between different samples.

-Application Areas: The technique is widely used in diagnostics, biotechnology, molecular biology, and proteomics

- **Disadvantages of the Western blot**:

-Cost and time: Compared to other tests such as ELISA, Western blotting can be more expensive and time-consuming.

-Complexity: The technique requires significant technical expertise and careful optimization of each step to obtain reliable results.

B/ ELISA (Enzyme-Linked Immunosorbent Assay):

It is a laboratory method for detecting and quantifying specific substances such as antigens or antibodies, using the immune response between an antigen and an antibody, coupled with an enzymatic reaction that produces a measurable colored signal. This technique, often performed on microplates, is widely used in research, medical diagnosis (such as for HIV), and food safety for its accuracy and speed.

- **Key Principles of the ELISA methods:**

-Antigen-Antibody Binding: The test relies on the ability of antibodies to bind specifically to their target antigen.

-Detection Enzyme: An antibody is coupled to an enzyme.

-Signal Production: The addition of a colorless substrate triggers a reaction catalyzed by the enzyme, transforming the substrate into a colored product.

- **How the ELISA method works:**

1. Immobilization: The substance to be detected (the antigen or antibody) is attached to the solid surface of a plate.
2. Washing: After each step, unbound reagents are washed away, leaving only the specific complexes.
3. Antibody addition: Specific antibodies, conjugated to an enzyme, are added.
4. Substrate addition: A substrate is added. The enzyme catalyzes a reaction that produces a colored signal.
5. Measurement: The intensity of the color is measured, which determines the presence and/or concentration of the target substance in the sample.

- Types of ELISA Tests:

There are four main types of ELISA (fig.28):

1. Direct: The antigen is directly attached to the plate.
2. Indirect: Used to detect or measure antibodies in the sample.
3. Competitive: Competition between a labeled and unlabeled antigen to bind to the antibodies.
4. Sandwich: The target antigen is trapped between two specific antibodies.

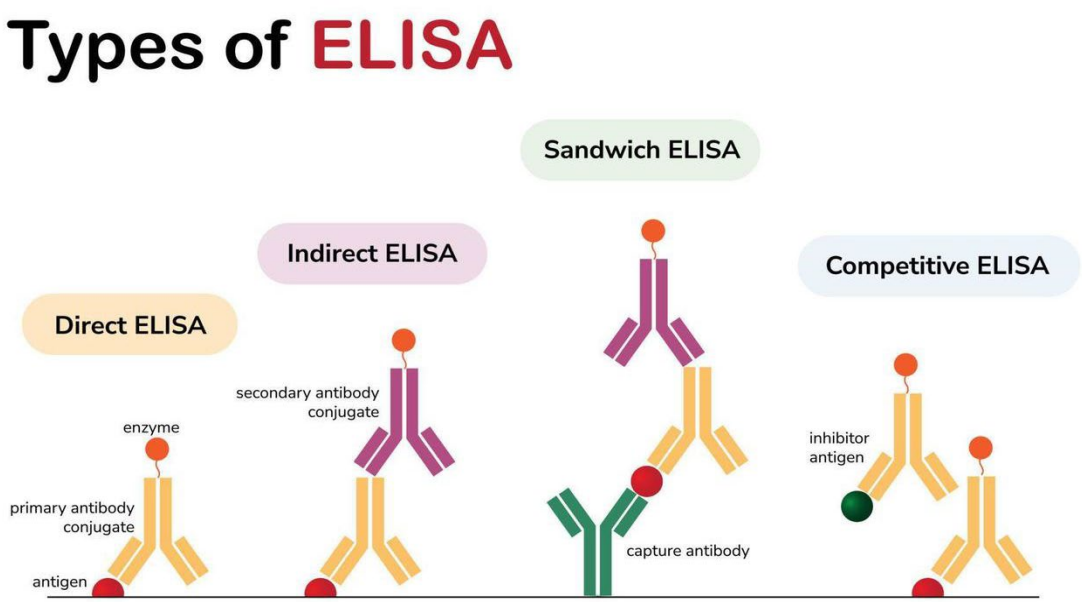


Figure 28 : Types of ELISA (Molecular Devices, 2025).

To summarize the main methods of virus detection, we have direct methods and indirect methods. Direct methods are performed by electron microscopy, viral culture, viral antigen detection, or PCR technique. In the other hand, indirect methods use antibodies and are carried out by agglutination tests such as the ELISA test.

I.V.3. Example of vaccine manufacturing:

Vaccine manufacturing requires a high level of expertise. This process is long and complex. The standards surrounding vaccine development are more stringent than those for drugs. The efficacy, quality, and safety of each batch must be assessed at every stage of manufacturing and even after marketing.

The development of a vaccine is divided into two parts: the manufacture of the active substance, followed by pharmaceutical production.

The goal is to produce an antigen (Ag) capable of stimulating the production of antibodies by our immune system. This Ag, which comes from the germ causing the disease, can be live and attenuated, or inactivated. Some vaccines, produced by genetic engineering from an animal cell or yeast, are called recombinant vaccines.

1. Production of the active substance:

Most of the active substance is produced in a sterile environment to avoid any contamination. The creation of a germ bank is the starting point of the process. This bank mainly includes bacteria and viruses, from which the Antigen will be developed. The germs must be well characterized and not present any mutations. In addition, they must maintain consistent properties to produce quality vaccines and reproducible batches.

1.1. Cultivation and amplification are specific to bacteria and viruses:

•In the case of bacteria, it is necessary to control the culture parameters (time, temperature, aeration, concentration, pressure, etc.);

•In the case of viruses, infected animal cells must be cultured. Viruses are not capable of multiplying independently. Controls must therefore be performed on these animal cells (quality, sterility, absence of contamination, etc.).

Harvesting consists of extracting the Antigen produced from the culture medium. During the purification and concentration phase, impurities are removed and the substance is concentrated using physical processes such as centrifugation.

The use of Messenger RNA vaccines.

With ribonucleic acid (mRNA) vaccines, the protein antigen is produced by the body receiving the vaccine from RNA, which codes for the pathogen's proteins, triggering an

immune response. The RNA, which is very fragile, is protected in lipid nanocapsules which, once injected, release it to allow protein synthesis.

2. Pharmaceutical Production:

Pharmaceutical production allows to obtain the final product.

- ✓ During formulation, all ingredients are mixed: adjuvants, stabilizers, or preservatives may be added if necessary.
- ✓ Filling is the step during which the vaccine is sterilely introduced into a syringe or vial.
- ✓ Lyophilization extracts the water contained in the preparation to transform it into a powder and this operation ensures greater stability and therefore better preservation.
- ✓ During packaging, the vaccine is labeled in accordance with regulatory requirements and packaged in batches (a homogeneous manufacturing unit for doses).
- ✓ Batch release is authorized after quality assurance has confirmed that the product has been manufactured and tested in accordance with applicable procedures. It is up to each country's regulatory authority to issue or deny final authorization for vaccine distribution within its territory.
- ✓ Storage: a guarantee of vaccine efficacy and safety and the cold chain must be scrupulously maintained, from vaccine production to administration. Storage conditions are checked at every stage of the process. Vaccines are generally stored between + 2°C and + 8°C, although the ARNm vaccine against COVID-19 can be stored at lower temperatures (-70°C to -80° C). Most vaccines are sensitive to heat and freezing.
- ✓ Controls: Quality and safety controls account for 70% of vaccine manufacturing time. Dual control is necessary: testing is the responsibility of both the manufacturer and an independent national authority.

The manufacturer carries out tests relating to the quality, efficacy and safety of the vaccine at each stage of its manufacture. Their results determine the release of vaccine batches, the phase which precedes marketing.

Regulations impose stricter marketing conditions on vaccine manufacturers than for other drugs. Quality controls cover all batches before marketing. A batch release certificate, which can then be circulated throughout the market.

In conclusion, the pharmacy team is responsible for ensuring the quality and safety of the vaccines they dispense, by respecting storage conditions and expiration dates. They must sometimes manage batch recalls, triggered by the manufacturer as a precautionary measure.

Pharmacists are authorized to vaccinate adults against seasonal influenza, as defined by current vaccination recommendations, with the exception of individuals with a history of severe allergic reaction to ovalbumin or to a previous vaccination. As part of the pandemic response strategy, they have been authorized to prescribe and administer COVID-19 vaccines.

To be able to handle microorganisms in the laboratory and understand certain basic and practical notions in industrial microbiology and general microbiology, I designed some exercises concerning the counting of microorganisms and the analysis of pathogenic flora for certain food products, just as I am attempting to make artisanal yogurt and visiting a dairy manufacturing plant. All this with the aim of getting a good understanding of industrial microbiology. Therefore, I am proposing the following practical exercises.

Exercise 1:

1. Define industrial microbiology.

2. What are the benefits of using microorganisms in industry?

3. List the microbial products that can be obtained and used in industry?

4. What is the aim of microbiological control?

Exercise 2:

Provide a diagram for carrying out the following dilutions:

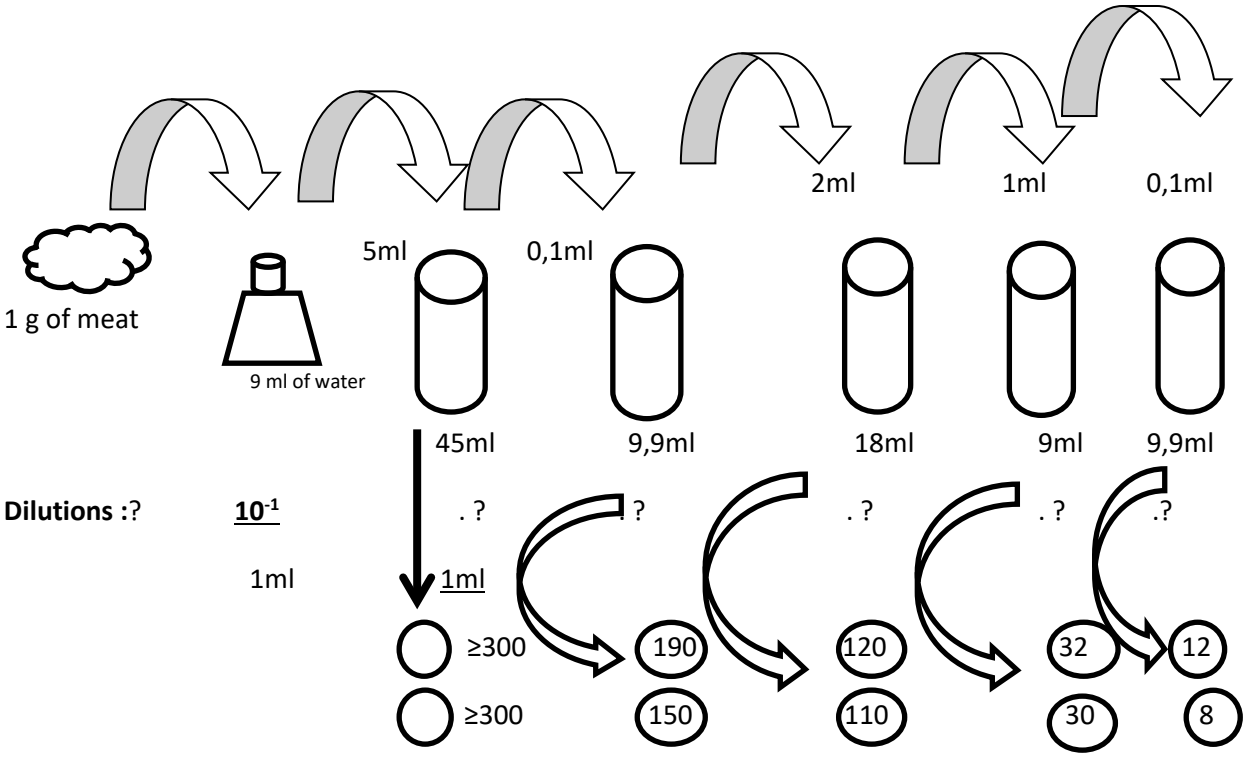
10^{-1}	10^{-2}	10^{-4}	10^{-6}	10^{-7}

Exercise 3:

We want to perform a microbiological analysis of a meat sample.

1) How do we perform the sampling and its preparation?

2) Calculate the microbiological flora of the following diagram:



- **For the solution of the exercise above:**

Count number of micro-organisms in solid medium:

To carry out a count of microorganisms in solid medium, we must first carry out dilutions in order to reduce the microbial load, then establish an average (**Microorganism / ml**, and its unit is : "**CFU**" : **C**olony **F**orming **U**nit) using the following law:

$$N = \sum nb_{\text{in plates}} / \sum (F_d \times Nb_{\text{used plates}}).$$

With:

N: number of microorganisms.

Nb in plates: number of colonies in plates.

Fd: Factor of dilution.

Nb used plates: number of pates which were counted.

Exercise 4:

The analysis of a food product gave the following results:

Dilution Factor	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	Standards
Flora C	200 210	134 154 121	Not Analyzed	32 28 24	21 18 13	$2{,}1X\ 10^{3}$ CFU/ml

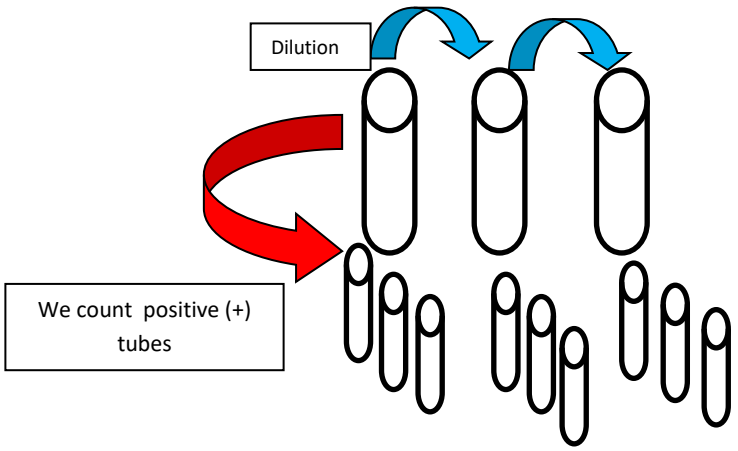
❖ What can you conclude about this food?

➢ *Count the number of microorganisms/ml in a liquid medium*:

1) First we choose the **"N":** (number of positives tubes):

• When counting the number of positive tubes, we always start with the maximum number "3" for example, then we take the two numbers that follow it: **Example:** 3 2 1 1, in this case, **N** will be: **321.**

• If we have two numbers of the number 3, we start with the number from the second dilution, then we take the two numbers that follow it. **Example:** 3 3 2 1 ➡ **N:** 321.



2) Next, you go to **the Mc Grady table (Table 2)** and choose the "**MPN**": (**M**ost **P**robable **N**umber), the **MPN** is used to count the number of **microorganisms /ml : "Nm",**
Knowing that:

$N_m = MPN / Fd_{median}$ **With**

MPN: Most **P**robable **N**umber

F_d: factor of median dilution.

Table 2 : Mc Grady Table :

N : number of positive tubes	MPN : Most probable number
320	**90**
321	**150**
322	**210**
323	**290**
210	**15**
211	**20**
110	**7**
111	**11**

Application Exercise :

The analysis of a food product gave the following results:

/	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	**Standards**
Flora A	3	2	1	1	0	10^5 CFU /ml
Flora B	3	2	2	1	0	2x 10^4 CFU /ml

- What conclusions can we draw about this food? Mc Grady's table is given above.

Exercise 5:

A sample of milk is taken and tested for certain strains of pathogenic flora. The results obtained are shown in the following table.

Dilution	10^{-1}	10^{-2}	10^{-4}	10^{-6}	10^{-7}
Flora A	300 Contaminates 265	190 170 165	80 95 Contaminates	60 Condensed 55	24 18 15
Flora B	3	3	2	2	1
Flora C	2	2	1	1	0

1/ How is the sampling done?

2/ Calculate the number of flora: A, B, C?

3/ Say if this milk is consumable or not? Knowing that the standard is: ***4 x 10^6 CFU/ml.*** The McGrady table is given above.

Exercise 6:

Answer the following questions:

1/ Define fermentation?

2/List kinds of fermentation?

3/ Give two (2) uses for a fermenter?

4/List the steps of cheese manufacturing?

5/ How do you classify viruses?

6/ List the main steps of PCR?

7/ Which disease is caused by prion?

8/ Are antigen detections virus classifications? (**True or false**).

9/Can prion be classified according to their form and life stage? (**True or false**).

10/ To sample milk, are dilutions carried out directly? (**True or false**)

Exercise 7:

1/ Calculate the number of microorganisms, according to the following table:

Dilution	**10^{-1}**	**10^{-3}**	**10^{-4}**	**10^{-5}**	**10^{-7}**	**Standards**
Flora A	270 266 /	130 / 134	100 90 86	78 56 60	24 15 20	10^{6} cfu/ml
Flora B	3	3	2	1	1	$2x10^{5}$ cfu/ml
Flora C	2	2	1	1	0	$1,5x10^{5}$ cfu/ml

2/ what can we conclude about the food?

Practical Exercises in Industrial Microbiology:

In order to learn about the production of food products using microorganisms and to understand the importance of fermentation in industry, we produced artisanal yogurt at the pedagogical laboratory.

We make yogurt in the laboratory, using milk, lactic ferments or a yogurt box containing lactic ferments. We make our preparation by electric heating and the incubation is done at a temperature of 45°C for 6 hours.

We also make soap with vegetable oil and NaoH at the pedagogical laboratory. Finaly, we organize a pedagogical outing to a factory of dairy products, to see and discover manufacturing of cheese and yogurts

1. Industrial microbiology:

Industrial microbiology, or microbial biotechnology, uses microorganisms for practical applications. It covers the identification, selection, and optimization of microorganisms to produce various products through fermentation, such as foods, pharmaceuticals (antibiotics, insulin), biofuels, and chemical biomolecules. Processes include submerged or solid-state fermentations in bioreactors, with growth parameters controlled to maximize production.

- Main Objectives :

1. Production of Products of Interest: Use of microorganisms to manufacture useful substances such as acids, enzymes, alcohols, antibiotics, fermented foods, and biofuels.

2. Biotechnology: Application of microorganisms as tools for bioconversion and biosynthesis processes.

- **Microorganisms Used:**

-Identification and Selection: Microbes are isolated from natural sources and selected based on their properties and ability to perform a specific industrial task.

-Genetic Engineering: The creation of genetically modified strains (such as yeast or *E. coli*) is used to produce specific molecules, such as human insulin.

- **Fermentation Processes:**

-Types of Fermentation: Submerged fermentation, where microorganisms are in contact with the liquid medium, is common. Solid-state fermentation, which requires less sterility, is also used.

-Bioreactors: Large-capacity equipment, called bioreactors, is used to cultivate microorganisms under controlled conditions for mass production.

-Optimization: The growth conditions of the microorganisms are optimized (temperature, pH, oxygenation) to maximize the yield of the desired product.

- **Industrial Applications:**

-Food: Production of fermented foods such as cheese, alcoholic beverages, and breadmaking.

-Pharmaceuticals: Manufacture of antibiotics and growth hormones.

-Chemistry and Energy: Production of organic acids and biofuels (such as ethanol).

-Pharmaceuticals: Production of enzymes and biomolecules.

-Environment: Detoxification of industrial and municipal effluents.

- **Advantages of Industrial Microbiology:**

-Cost: Microorganisms are often more economical and easier to handle than other production methods.

-Independence: Production is not subject to seasonal or geographical constraints, unlike the extraction of enzymes from animals.

-Safety: The use of microorganisms, particularly recombinant organisms, offers increased safety by avoiding the transmission of viruses or prions.

2. Microbiological control:

It consists of systematically evaluating the presence of microorganisms (bacteria, viruses, yeasts, molds) in a product, a raw material, or a production environment, with the aim of ensuring the safety of food and products, their hygienic quality, and controlling critical points in a production chain. It allows the detection, identification, and counting of microorganisms using techniques such as culture on nutrient media, microscopy, or PCR, and ensuring compliance with standards via qualitative or quantitative criteria.

- **Objectives and Scope of Microbiological Control:**

-Ensure food safety: Prevent products from containing pathogens or toxins that causes illness.

-Assess hygienic quality: Verify that finished products or raw materials established microbiological quality standards.

-Control processes: Monitor critical production steps (personnel, equipment) to prevent contamination.

-Detect spoilage: Identify microorganisms that can affect product quality.

- **Steps and Methods of Microbiological Control:**

1. Sampling: Samples are taken from the product to be analyzed, from surfaces, or from the environment.

2. Laboratory Analysis: Samples are cultured on specific nutrient media to allow the growth of microorganisms.

3. Identification and Enumeration:

- Microscopic Observation: Allows microorganisms to be visualized.
- Biochemical Techniques: Used to identify different types of germs.
- PCR (Polymerase Chain Reaction): A modern and sensitive technique for identifying microorganisms via their DNA.

4.Interpretation of results: The results are compared to microbiological criteria, which can be:

-Qualitative: Determine the presence or absence of a microorganism.

-Quantitative: Establish levels of contamination that should not be exceeded.

- **Types of Controls:**

1. Preventive Control: Performed before manufacturing, on raw materials, additives, and the production environment.

2. In-Process Control: Performed during the production process on the product, personnel, equipment.
3. Finished Product Control: Performed on the final product to ensure its compliance with standards.

3.The Bioreactor:

A bioreactor is a device or container that provides a controlled environment for the growth of living cells, microorganisms, or tissues, in order to carry out specific biological reactions or processes. It works by maintaining optimal conditions (temperature, pH, oxygen, nutrients) to cultivate organisms and obtain products of interest, whether it is the production of biomolecules (fermentation), the degradation of pollutants in soils, or the treatment of wastewater.

- **Operating Principle:**

-Controlled Environment: The bioreactor maintains stable and optimal conditions for the cultivation of living organisms.

-Substrates and Nutrients: It provides the necessary nutrients (gases such as oxygen, nutrients) for the growth and activity of cells or microorganisms.

-Production or Transformation: Biological processes (fermentation, cell culture, biological degradation) transform substrates to produce specific compounds or purify substances.

-Parameter Control: Sensors and control systems continuously monitor and adjust key parameters such as pH, temperature, oxygenation, and agitation to ensure optimal and reproducible conditions.

- Applications :

-Food and Biotechnology: Production of products such as milk, antibiotics, enzymes, proteins, and other compounds of interest through fermentation.

-Water and Soil Treatment: Removal of pollutants in wastewater treatment plants (membrane bioreactors) and remediation of soils contaminated by biological degradation.

-Research and Development: Cell and bacterial culture for the discovery of new molecules or the study of fundamental biological processes.

- **Key Components :**

✓ The tank: Often made of stainless steel, glass, or polymer, it contains the culture medium.

✓ Agitation System: Allows for mixing the liquid, distributing oxygen, and diffusing nutrients for cellular activity.

✓ Access Ports: For adding culture media, inserting sensors, and collecting samples.

✓ Aeration Systems: Diffusion of gases such as oxygen, air, or carbon dioxide for metabolic activity and pH regulation.

✓ Sensors and Control Systems: For measuring and adjusting temperature, pH, oxygenation, etc.

- **Fermentation Process:**

The fermenter uses the fermentation process in industry. The fermentation process is a biochemical reaction, often anaerobic (without oxygen), in which microorganisms (bacteria, yeasts, molds) transform organic compounds, such as carbohydrates, into other substances such as acids, alcohols, or gases, producing energy and changing the taste and texture of food. Glycolysis is the first step, transforming glucose into pyruvic acid, which is then converted into various end products depending on the type of fermentation.

- **Key Steps in the Process:**

1/ Glucolysis: Glucose (a carbohydrate) is converted into pyruvic acid. This step produces a small amount of energy (ATP).

2/Coenzyme Reoxidation: Pyruvic acid is used as the final electron acceptor to reoxidize reduced coenzymes (such as NADH). This reoxidation regenerates the coenzymes needed to continue glycolysis.

3/Final Production: Pyruvate is then reduced to form various end products:

-Alcoholic Fermentation: Pyruvate is converted into ethanol and carbon dioxide (CO2), producing energy, typical of yeasts.

-Lactic Acid Fermentation: Pyruvate is converted into lactic acid, as in yogurt production.

-Other Fermentations: Bacteria or molds can also produce other acids or gases.

- **Factors Influencing Fermentation :**

-Microorganisms: The type of microorganism (yeast, bacteria, mold) determines the type of fermentation and the resulting products.

-Temperature: Temperature must be controlled because it influences the rate of fermentation and the survival of microorganisms.

-Absence of Oxygen: Fermentation is an anaerobic process, meaning it occurs in the absence of oxygen.

- Examples of products derived from fermentation

- Alcohols: fermentation of grape juice by yeast.
- Dairy products: Yogurt, cheese (lactic acid fermentation by bacteria).
- Bakery products: Bread (fermentation by yeast to develop the dough).

4. Viruses:

A virus is an infectious agent, that is, a microorganism capable of causing an infection. Viruses are necessarily intracellular agents, which means they need to invade a living cell (the host cell) to replicate by using its metabolism. They consist of a nucleic acid (DNA or RNA) surrounded by a protein capsid and, in some cases, an envelope.

- **Key Characteristics of Viruses as Infectious Agents:**

-Obligate Parasitism: A virus can only reproduce within a host cell, using its own mechanisms to multiply.

-Simple Structure: A virus is a minimal infectious particle, containing genetic material (DNA or RNA) encapsulated in a capsid.

-Variability: Viruses can mutate, changing their shape and virulence.

-Cell Penetration: To infect a cell, the viral particle must first attach to it via specific receptors and then enter the cell.

The term "virus" can refer to a pathogen to be combated in industrial processes or, on the contrary, a sophisticated biotechnological tool for targeted medical and industrial applications.

- **Viruses as Industrial and Biotechnological Tools:**

-Gene Therapy and Vaccine Production: Retroviruses, lenti-viruses, and adenoviruses are used to introduce genetic material into cells and thus create gene therapies and vaccines, such as those developed for vaccination against SARS-CoV-19.

-Nanotechnology: Viruses are used to manufacture nanomaterials for applications in electronics, pharmacology, and other sectors.

-Food Applications: Bacteriophages are used as "probiotics" to target and eliminate specific pathogenic bacteria without affecting beneficial bacteria, offering an alternative to antibiotics in the food industry.

-Research and Development: Viruses are also being used as "muses of innovation," paving the way for the discovery of new molecules and drugs, note CNRS researchers.

- **Vaccine Manufacturing :**

Live-attenuated vaccines contain live pathogens derived from a virus that have been "attenuated," or weakened. According to Dr. Scully, live-attenuated vaccines are produced by selecting viral strains that still produce a sufficiently robust immune response, but do not cause disease.

Vaccines are composed of fragments of microbes called "vaccine antigens," which are derived from viruses. The goal is to produce an antigen capable of stimulating the immune system to produce antibodies

- **Process:**

1. ***Preclinical Research and Development*** :

-Vaccine Design: Scientists identify the pathogen and develop a strategy to stimulate the immune system without causing disease.

-Preclinical Trials: The vaccine is tested in cell cultures and animals to assess its effectiveness and safety.

2. ***Clinical Trials:***

Phase 1: The vaccine is tested on a small group of volunteers to verify its safety and ability to induce an immune response.

Phase 2: The vaccine is tested on a larger group of people to refine the dosage and confirm its effectiveness.

Phase 3: Thousands of people participate to assess the vaccine's effectiveness and safety on a large scale.

3. Manufacturing and Quality Controls

-Active Ingredient Production: The vaccine is manufactured in large quantities using standardized processes.

-Formulation and Packaging: The active ingredient is formulated into a final product and packaged in vials.

-Quality Control Testing: Rigorous testing is conducted to ensure the purity, potency, and safety of each vaccine batch before it is released to the market.

4. Authorization and Monitoring

-Regulatory Approval: Health authorities review clinical trial and quality control data to approve the vaccine for marketing.

-Post-Marketing Surveillance: Even after approval, the vaccine's safety and effectiveness are continuously monitored.

5. Virus detection methods:

Virus detection methods include PCR and nucleic acid amplification tests to identify the virus's genetic material, viral antigen detection (immune-assays) to identify viral proteins, serology, which detects antibodies produced by the body, viral isolation by cell culture, which grows the virus in the laboratory, and electron microscopy to visualize the virus itself. The choice of method depends on the desired detection speed, the specificity of the virus, and the state of the infection

1. **Direct Methods**:

- ✓ Nucleic Acid Amplification Tests: PCR is a highly sensitive method that detects the presence of viral RNA or DNA.
- ✓ Viral culture: A biological sample is cultured in a laboratory to grow the virus.
- ✓ Electron microscopy: Allows direct observation of viral particles using high magnification.
- ✓ Tests based on viral proteins.
- Antigen tests: These tests directly identify proteins present on the surface of the virus.

- Direct immunofluorescence tests: These use fluorescent antibodies to detect viral antigens in cells.

2. Indirect Tests (based on the body's immune response):

Serological tests: These look for antibodies (such as IgM or IgG) produced by the body in response to a viral infection. The presence of IgM antibodies may indicate a recent infection, while IgG antibodies may signal a past infection or vaccination.

➢ Choice of Method :

The type of test used depends on the suspected pathogen and the patient's characteristics. For example, PCR is often the gold standard for rapid and accurate diagnosis. Rapid antigen tests can be used at home for more immediate detection.

6. Prions:

Prions are infectious agents composed of an abnormal protein capable of transforming normal proteins into their own abnormal form, leading to accumulation and degeneration of cells, particularly in the brain. They cause neurodegenerative diseases called prion diseases or transmissible spongiform encephalopathies, such as Creutzfeldt-Jakob disease in humans or bovine spongiform encephalopathy, and are extremely resistant to conventional decontamination methods.

➢ Key Features :

-Infectious Nature: Prions are contagious, capable of spreading from cell to cell and from one individual to another.

-Resistance: They are highly resistant to heat, radiation, and chemical agents, making their inactivation very difficult.

-Associated Diseases: They cause serious neurodegenerative diseases, including Creutzfeldt-Jakob disease in humans, mad cow disease in cattle, scrapie in sheep, and chronic wasting disease in deer.

-Interspecies Transmission: A prion protein from one species can be transmitted to another species, as demonstrated by the case of variant Creutzfeldt-Jakob in humans.

- **Transmission Mechanisms** :

Prions are transmitted through contact with infected tissues, either through food (such as animal meal) or through contaminated medical instruments.

- **Mechanism of Action :**

- Normal Protein (PrPC): The body naturally possesses a prion protein (PrPC) that has a normal structure, primarily alpha helices.
- Abnormal Transformation (PrPsC): A pathogenic prion, the PrPsC protein, has the same amino acid sequence as PrPC, but a different spatial structure, rich in beta sheets.
- Domino Effect: Abnormal PrPqC interacts with normal PrPC, transforming it into its own pathological state.
- Aggregation and Degeneration: PrPsC can not be degraded by normal proteases and aggregates to form amyloid plaques that accumulate in the brain.
- Neuronal Death: These plaque accumulations lead to the death of neurons, causing neurological disorders and spongy brain function.

Conclusion:

Industrial microbiology involves the use of microorganisms in industry, its first use dates back to ancient centuries, and its development has improved and continues to improve to offer us a variety of products to consume. The study of industrial microbiology involves knowing it's basics, microbiological control and its importance, the bioreactor and its operating principles through fermentation, because this process is the basis for transforming raw materials. Then we discuss viruses and prions as well as virus detection methods in order to learn about vaccine production, which is done by the pharmaceutical industry.

We conclude that the transformation of raw materials by microorganisms dominates the industry in different fields. It is done in a natural and feasible way, costs less compared to a typically chemical transformation, provides us with non-harmful products, it can also be improved by selecting traits of interest by carrying out genetic modifications of microbial strains. Finally, we can say that good production control leads to the improvement of the industry.

The outlook for industrial microbiology is vast and growing, driven by innovation and demand in the healthcare, food, environmental, and energy sectors. Trends include the production of biofuels and metabolites through fermentation, the use of microorganisms for bioremediation, and the development of innovative therapies. The expertise of microbiologists is highly sought after to address environmental, health, and large-scale production challenges.

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