REPUBLIC ALGERIAN DEMOCRATIC AND POPULAR

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University of Abbes Laghrour-Khenchela

Faculty of Natural and Life Sciences

LECTURE HANDOUT :

Introduction To Biotechnology

Course Intended for Students:

2nd-Year Common Core – Biotechnology

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Matière: Introduction aux biotechnologies

Crédits : 6

Coefficients : 3

Objectifs de l'enseignement

Cette matière s'intéresse à donner un aperçu global sur les domaines d'application de la biotechnologie (environnement, agronomie, industrie et médicale).

Connaissances préalables recommandées (*descriptif succinct des connaissances* *requises pour pouvoir suivre cet enseignement – Maximum 2 lignes).*

Sans pré requis

Contenu de la matière

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1.2. Evolution des biotechnologies dans le temps
1.3. Les grands enjeux actuels des biotechnologies et bionanotechnologies
1.4. Définition des biotechnologies vertes, blanches, et rouges
1.5. Les produits types de biotechnologies
1.6. Domaines industriels concernés
1.7. Les défis d'innovation biotechnologiques

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Preface

Biotechnology is a highly multidisciplinary field that draws upon a wide range of scientific disciplines, including biochemistry, molecular biology, genetics, immunology, microbiology, pharmacology, fermentation technology, and agriculture, among others. Each of these contributing areas brings its own specialized terminology and nomenclature standards, which can create significant challenges in scientific communication.

This course is primarily intended for second-year undergraduate students (L2) in the field of Biotechnology. It is also designed for students and readers from other disciplines who have an interest in understanding the principles and applications of biotechnology.

Objective

This unit aims to introduce the fundamental concepts and the evolution of biotechnology by developing a solid understanding of its historical background and core definitions. It focuses on general principles, the integration of multiple scientific disciplines, the acquisition of specialized knowledge, and recent developments in specific areas of biotechnology.

Like any academic work, this material may contain limitations or unintentional omissions. Therefore, constructive feedback, corrections, and recommendations from fellow teachers and researchers actively working in the field are always welcome and highly appreciated.

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Chapter 01. Introduction

1.1. The Origins of Biotechnology

1.1.1. Etymological Origin of the Term "Biotechnology"

The word *biotechnology* is composed of two elements:

- "Bio" derives from the Greek *bios*, meaning *life*. This root later gave rise to the word *biology* at the beginning of the 19th century.
- "Technology" comes from the Greek *technologia*. The term first appeared in French texts in 1656 to designate *the study of techniques, tools, machines, and materials*.

1.1.2. Definition

Biotechnology refers to a set of methods or techniques that use living components (organisms, cells, subcellular, or molecular elements) to investigate, produce, or modify plant- or animal-derived elements or organisms (and sometimes others).

Thus, biotechnology encompasses procedures that contribute to the development of new products or services, as well as specific end products. It includes both traditional methods (such as bread, beer, and vinegar production) and modern biotechnologies based on molecular genetics and genetic engineering.

1.2. The Historical Development of Biotechnology

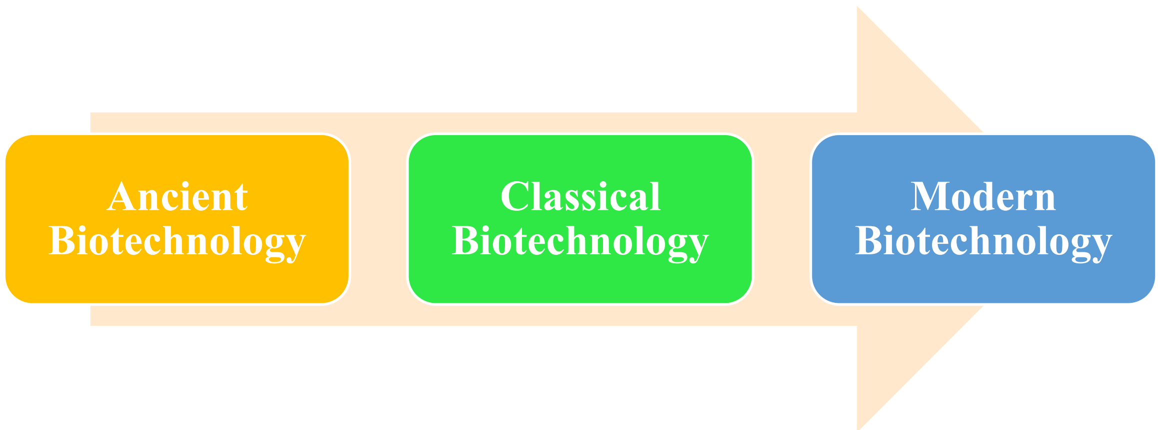


Figure 01. Schematic Representation of the Historical Evolution of Biotechnology.

1.2.1. Ancient Biotechnology (before 1800)

The history of biotechnology dates back to the period when humans began adopting a sedentary lifestyle, around 9000 B.C. This era was marked by the often-accidental discovery of food fermentation processes, which improved flavors and textures. The deliberate use of bacteria or fungi, such as molds, was a common practice. In 1866, Louis Pasteur published findings establishing the direct relationship between yeast and the fermentation of sugars. Later, in 1915, the industrial production of baker's yeast was initiated.

1.2.2. Classical Biotechnology

This period witnessed the emergence of various beverages (wine, cider) and the production of products such as vinegar, glycerol, acetone, butanol, lactic acid, antibiotics, and others. Chemical transformations were employed for the production of therapeutic products, in which the substrate reacted with a microbial enzyme to generate the final product.

1.2.3. Modern Biotechnology

In 1953, Watson and Crick unveiled the mysteries of DNA by proposing its structural model, known as the *double helix model of DNA*. This groundbreaking discovery marked the beginning of modern biotechnology.

1.2.4. Origin and Evolution of Biotechnology

1.2.4.1. Ancient Origins of Biotechnology

- **Early Applications (8000–2000 B.C.)**

The domestication of plants and animals marked the first steps toward biotechnology. Humans began using techniques to improve their food supply by domesticating plants such as wheat and potatoes, and by raising animals for milk, meat, and draft power.

- **Fermentation**

In ancient Egypt, yeasts were used in the production of bread and beer. This fermentation process represents one of the earliest forms of biotechnology, based on the observation that certain **microorganisms** can enhance or transform food.

- **Antiquity: Early Medicinal Practices (around 500 B.C.)**

In China, products such as moldy soybean curd (*fermented tofu*) were used to treat infections, anticipating modern discoveries of antibacterial substances. This empirical use of microorganisms illustrates that ancient societies had already recognized the therapeutic properties of certain biological processes.

1.2.4.2. Advances in Biotechnology in the Modern Era

- **1797: Discovery of the First Vaccine**

Edward Jenner developed the first vaccine by inoculating a young boy with cowpox to protect him against smallpox. This groundbreaking discovery marked the beginning of vaccination and laid the foundation for modern immunology.

- **1800–1833: Innovations in Proteins and Enzymes**

In 1833, Anselme Payen isolated the first enzyme, *diastase*, from barley. This discovery demonstrated the role of enzymes in catalyzing biological reactions and paved the way for the modern understanding of biochemical processes.

- **1857: Germ Theory and Fermentation**

Louis Pasteur proposed that microorganisms were responsible for fermentation and disease, a theory that contributed to the development of vaccines and the practice of pasteurization.

1.2.4.3. Evolution of Biotechnology in the Industrial and Contemporary Era

- **1919: Invention of the Term "Biotechnology"**

The word *biotechnology* was coined by Karl Ereky to describe the interaction between biology and technology, particularly the use of biological processes to produce industrial goods such as food and energy.

- **1950s–1960s: Development of Molecular Genetics**

The discovery of the DNA structure in 1953 by James Watson and Francis Crick opened the way for genetic manipulation. This breakthrough laid the foundation of modern biotechnology based on genome understanding. Early genetic engineering techniques were developed during this period, including gene isolation and recombination, which became the basis of today's biotechnologies.

- **1973: Recombinant DNA Technology**

Herbert Boyer and Stanley Cohen developed the first recombinant DNA technique, enabling the combination of genetic material from different organisms. This innovation marked the beginning of large-scale modern biotechnology.

- **1980–2000: Medical and Industrial Applications**

Biotechnology found major applications in medicine, such as the production of recombinant human insulin for diabetes treatment.

In agriculture, the development of genetically modified organisms (GMOs) had a considerable impact, increasing plant resistance to diseases and improving crop yields.

- **21st Century: Modern and Multidisciplinary Biotechnology**

Today, biotechnology integrates multiple disciplines such as genetic engineering, molecular biology, nanotechnology, computer science, and biochemistry.
It is applied across diverse fields, including precision medicine, gene therapy, biofuels, bioremediation, and biomaterials.

➢ **Application Areas of Biotechnology**

Biotechnology is inherently multidisciplinary, involving contributions from:

- Engineering
- Computer science
- Cell and molecular biology
- Microbiology
- Genetics
- Physiology
- Biochemistry
- Immunology
- Virology
- Recombinant DNA technology
- … and other related sciences

1.3. Major Current Challenges in Biotechnology and Bionanotechnology

1.3.1. Genetically Modified Organisms (GMOs)

GMOs are organisms (plants, animals, or microorganisms) whose genetic material has been deliberately modified in the laboratory using genetic engineering techniques. These modifications are designed to introduce new traits that the organism could not acquire naturally through crossbreeding or reproduction.

- **Applications of GMOs**

Agriculture: Widely used to create crops resistant to diseases, pests, and herbicides, or to enhance productivity (e.g., Bt corn or glyphosate-resistant soybeans).

Medicine: Employed in the production of insulin, vaccines, and gene therapies.

Industry: Used in microorganisms for biofuel production or wastewater treatment.

- **Production of GMOs (Genetically Modified Organisms)**
  - ✓ **Gene Selection (Transgene)**

The transgene is chosen according to the desired effects on the GMO.

This gene is cloned in order to be introduced into the target organism.

- **Construction of the Molecular Vector**

A vector is constructed *in vitro*.

This molecular vector allows the transport of the transgene into the cells to be transformed.

- **Cell Transformation Techniques**

Cells are transformed by introducing the transgene through different methods, including:

  - ✓ Micromanipulation
  - ✓ Intense electric fields (electroporation)
  - ✓ Use of plasmid or viral vectors

- **Reconstitution of the Organism**
  - ✓ The entire organism is regenerated from the transformed cells. If the transgene is present in germline cells, the GMO can transmit it to its offspring.

- ✓ For plants, a GMO can be regenerated from transformed cells using a *callus* (a mass of undifferentiated cells).

- **The Societal Debate**
  - ✓ **Perceived Risks**

Concerns about the spread of transgenes in the environment through gene transfer.

Potential impact on biodiversity and ecosystems.

Consumer concerns regarding the presence of GMOs in human or animal food.

- ✓ **Information Efforts**

Researchers, policymakers, industry stakeholders, and legislators work to inform the public and alleviate fears.

Nevertheless, the debate remains open, particularly regarding the safety and ethical aspects of GMOs.

1.3.2. Key Issues in Biotechnology

Biotechnology involves multiple issues both positive and negative that affect various sectors of society. The main issues include:

- **Ethical Issues**

Genetic Manipulation: The possibility of altering human, animal, and plant genes raises ethical questions. GMOs pose challenges regarding safety, unforeseen consequences, and the modification of genetic heritage.

Cloning: Whether reproductive or therapeutic, cloning sparks major ethical debates about the value of life and the creation of artificial forms of life.

- **Environmental Issues**

Impact of GMOs: The introduction of genetically modified plants into ecosystems may cause unpredictable effects, such as the emergence of herbicide-resistant *superweeds* or disruptions in food chains.

Bioremediation: Biotechnology enables the cleaning of polluted environments through microorganisms or enzymes. However, releasing these organisms into the environment may lead to unforeseen consequences.

Biodiversity: The extensive use of biotechnology in agriculture can reduce genetic diversity in crops and livestock, creating dependence on a limited number of genetically modified varieties.

- **Socio-Economic Issues**

Inequalities of Access: Medical biotechnologies, such as gene therapies or biopharmaceutical drugs, are often expensive, limiting access for poorer populations.

Resource Concentration: The biotechnology sector is frequently dominated by a small number of multinational corporations, which can exacerbate economic inequalities between developed and developing countries.

Regulation and Safety: Legislation governing the safety of biotechnological products varies from country to country. The lack of harmonized regulation can create health and environmental risks.

- **Health Issues**

Safety of Food Products from Biotechnology: Concerns about the long-term safety of genetically modified foods and other biotech-derived products.

Antibiotic Resistance: The excessive use of antibiotics in livestock farming, a field linked to biotechnology, contributes to the emergence of antibiotic-resistant bacteria, representing a major threat to global public health.

1.3.3. Nanobiotechnologies

A nanometer is approximately:

- 500,000 times thinner than the line made by a ballpoint pen;
- 30,000 times thinner than the width of a human hair;
- 100 times smaller than a DNA molecule.

Nanotechnology refers to the set of techniques enabling the fabrication, observation, and measurement of objects, structures, and systems ranging from 1 to 100 nanometers in size, whose properties are specifically determined by this nanoscale dimension.

The development of nanotechnology began in the early 1980s with the invention of the scanning tunneling microscope, the first tool capable of observing objects at this scale.

Nanotechnologies are inherently transversal, combining physics, chemistry, and biology while dissolving the boundaries between traditional scientific and technological disciplines.

- **Application Fields of Nanotechnology**:

Information technologies: Increased computing power, miniaturization of electronic components, enhanced storage capacity.

Health: Development of new diagnostic tools, targeted treatment of diseased cells.

New materials and energy: Energy savings in transportation, new photovoltaic cells, and innovative materials.

- **Bionanotechnology**

Bionanotechnology is the application of nanotechnology principles to the life sciences. It involves manipulating materials at the nanoscale (1 nanometer = one billionth of a meter) in order to interact with biological systems.

➢ **Applications of Bionanotechnology**

Medicine: Development of nanoparticles for targeted drug delivery (e.g., cancer treatments), diagnostic devices, and smart medical implants.

Agriculture: Use of nanoparticles to improve the efficiency of fertilizers or pesticides, or to enable early detection of plant diseases.

Environment: Use of nanomaterials for bioremediation (soil or water decontamination).

➢ **Challenges of Bionanotechnology**

- **Ethical Issues**

Manipulation at the nanoscale: Intervention at the nanoscale on human cells or tissues raises questions about safety and the unforeseen risks of altering the human body.

Surveillance and control: Bionanotechnology devices, such as nanosensors or smart medical implants, may be used for monitoring purposes, raising concerns about privacy and individual freedoms.

- **Health Issues**

Toxicity of nanomaterials: Nanometric particles used in medicine or other industries may have toxic effects on human cells. Their extremely small size allows them to cross biological barriers such as the blood–brain barrier.

Targeted therapy: Bionanotechnology enables the development of more precise and less invasive treatments, such as targeted drug delivery within the body. However, the lack of understanding of the long-term effects of nanomaterials on health remains a major concern.

- **Environmental Issues**

Effects of nanomaterials: Nanomaterials used in biotechnological products may accumulate in the environment and food chains, with ecological effects that are still poorly understood.

Enhanced bioremediation: Conversely, bionanotechnology can be used to improve bioremediation methods by developing nanoparticles capable of cleaning contaminated sites more effectively.

- **Regulatory and Safety Issues**

Lack of specific regulation: Nanotechnology and bionanotechnology are relatively recent fields. Regulations on the safety and use of nanomaterials in biotechnology are still under development, creating areas of uncertainty regarding their long-term impact.

Transparency: Ensuring transparency in the use of bionanotechnologies, especially in the food and medical sectors, is essential to prevent unethical or hazardous applications.

1.4. Definition of Green, White, and Red Biotechnologies

Biotechnology applications are highly diverse and affect our daily lives. Many authors classify these applications by domain, assigning colors to each category **(Table 1).**

Table 01. Main Applications of Biotechnology by Color Code

Domain	**Applications**
Red Biotechnology / Medicine	Production of vaccines and antibiotics; molecular diagnostic techniques; pharmaceutical and cosmetic industries.
Green Biotechnology / Agriculture	Production of genetically modified plant varieties; production of genetically modified animal breeds; development of biofertilizers and biopesticides; agri-food applications.
Yellow Biotechnology / Environment	Conservation of biodiversity; pollution control and remediation.
White Biotechnology / Industry	Industrial processes (design and production of new everyday materials such as plastics, textiles, etc.) with low environmental impact; development of new sustainable energy sources such as biofuels.
Blue Biotechnology / Marine	Exploitation of marine resources to create new products; production of biomaterials and regenerative pharmacological agents.

1.4.1. Green Biotechnologies

The terms *green biotechnology (GB)* and *plant biotechnology* are synonymous and, in their broadest sense, refer to the use of modern methods such as tissue culture and marker-assisted selection (MAS) for plant improvement. In a narrower sense, these terms specifically refer to the use of biotechnology to alter the genetic makeup of a cultivated plant. It is precisely this domain the most controversial aspect of GB that is the focus of these guidelines.

Green biotechnologies are applied in the fields of agriculture and agri-food.

a) **Green biotechnology today and tomorrow in developing countries**

At present, the cultivation of genetically modified plants (GMPs) in developing countries is mainly limited to soybean, maize, canola, and cotton varieties that have been engineered with herbicide-tolerant and disease-resistant genes.

Many countries view genetic modification of crop plants as a means of achieving their agricultural development goals, provided that this technology contributes to reducing hunger and malnutrition while promoting local technical capacities.

b) **Advantages and disadvantages**

The advantages include the improvement of economically important plant species, enhanced agricultural productivity, and the production of novel products through plants, such as therapeutic molecules or renewable energy sources. Plant genetic engineering can provide crops with traits such as resistance to drought, pesticides, herbicides, or harmful insects, as well as improvements to their root systems.

Genetically modified organisms (GMOs) have become the focal point of a social, political, and scientific debate that raises numerous questions:

- With their increased resistance to diseases and environmental stress, will GMOs disrupt ecosystems?
- Will their presence be harmless or polluting?
- Will biodiversity be endangered and traditional varieties replaced by genetically modified ones?

1.4.2. Red Biotechnologies

Red biotechnologies encompass the fields of health (human and veterinary), medicine, diagnostics, tissue engineering, and the development of genetic or molecular processes for therapeutic purposes. This is the category that has received the most significant investment. By 2010, it was estimated that 80% of new drugs would originate, directly or indirectly, from modern biotechnologies. This explains the strong interest of industrial and financial sectors, particularly in the United States.

This category also includes biotechnologies involving embryonic stem cells, cloning techniques, and genetic diagnostic tools, all of which raise serious ethical questions and impose limitations on their use.

- **Tools of Red Biotechnology**

Red biotechnology brings together innovative tools and techniques for the medical and pharmaceutical fields, enabling the development of new treatments, diagnostics, and therapies. One of the key tools is recombinant DNA technology, or genetic engineering, which involves modifying DNA by inserting, deleting, or altering specific genes. This technology paved the way for the production of therapeutic proteins such as recombinant human insulin, which is essential for diabetic patients.

1.4.3. White Biotechnologies

White biotechnologies (also referred to as *industrial biotechnology*) form a distinct category within the field. They are applied to industrial processes and aim to sustainably produce biochemicals, biomaterials, and biofuels on an industrial scale using renewable resources.

- **White biotechnologies, an ancient practice**

As far back as Antiquity, yeasts were used to brew beer, and enzymes found in animal excrement were employed in the tanning of hides. More recently, biochemical engineering has enabled the use of microorganisms to produce chemical or biological compounds. Furthermore, advances in genetics have only accelerated these developments.

- **Examples of Industrial Biotechnologies: Biocatalysis**

Among the processes implemented in white biotechnologies is the use of enzymes—proteins produced by all living organisms—as catalysts for chemical reactions. Unlike chemical catalysts, enzymes are relatively energy-efficient and environmentally friendly. They are widely applied in industries such as paper manufacturing and detergent production.

- **White Biotechnologies and Fermentation**

Certain microorganisms, such as yeasts or bacteria, are capable of transforming organic substances (sugars, oils, etc.) into a wide range of products. For example, some bacteria use corn sugar as a fuel for their cellular processes, producing a polymer by-product: a bioplastic.

1.4.4. Blue Biotechnology (Marine Biotechnology)

Blue biotechnology harnesses marine resources to create products and applications of industrial interest. By exploiting marine biodiversity, it offers opportunities across various sectors.

Marine biotechnology uses marine raw materials such as gelling agents that are widely applied in the food industry, healthcare, and other fields. It also contributes to medicine and research through the use of molecules derived from marine organisms, while biomaterials and regenerative agents are being studied for their potential in these areas.

Other sectors, such as agriculture and cosmetics, are also exploring the potential of blue biotechnology for future development.

1.5. Typical Biotechnological Products

Biotechnology products encompass a wide range of applications across various sectors, including medicine, agriculture, industry, and the environment.

1.5.1. Medical and Pharmaceutical Products

In the medical and pharmaceutical sector, one of the most emblematic products is recombinant insulin, produced through biotechnology. This insulin, used to treat diabetes, is manufactured using genetically modified bacteria, providing a purer and more accessible version than that extracted from animals.

Additionally, monoclonal antibodies, produced by mammalian cells in laboratories, are widely used in the treatment of cancers and autoimmune diseases.

Gene therapy, another major breakthrough, aims to correct genetic abnormalities to treat diseases that were previously incurable.

Finally, cutting-edge technologies such as CRISPR-Cas9 open new perspectives for correcting genetic mutations with unprecedented precision.

1.5.2. Agricultural Products

In agriculture, biotechnology has led to the development of genetically modified crops (GMOs), such as Bt maize or herbicide-resistant soybean. These GMOs increase yields while reducing the need for chemical pesticides.

Furthermore, biofertilizers and biopesticides, derived from microorganisms, are used to improve soil fertility and protect crops in an environmentally friendly way.

In addition, nutrient-enriched crops, such as Golden Rice, have been developed to combat malnutrition by providing essential nutrients such as vitamin A.

1.5.3. Industrial Products

In the industrial sector, bioplastics represent an ecological alternative to traditional plastics, as they are produced from renewable raw materials such as corn. Biofuels, including ethanol and biodiesel, are also biotechnological products that provide a cleaner alternative to fossil fuels.

In addition, industrial enzymes are widely used in detergents and the food industry to enhance process efficiency while reducing energy and chemical consumption.

1.5.4. Environmental Products

Biotechnology also plays a key role in environmental preservation. For example, bioremediation microorganisms are used to degrade pollutants such as hydrocarbons and heavy metals in contaminated soils and waters, enabling a more ecological clean-up of polluted areas.

Furthermore, wastewater treatment systems integrate biotechnological processes to purify water and eliminate organic contaminants.

1.5.5. Food Products

In the food industry, fermented products such as yogurt, cheese, and beer are produced through biotechnological processes based on yeast and bacterial fermentation.

In addition, alternative proteins, produced from microorganisms such as algae or insects, are increasingly being used to meet the demand for sustainable nutrition.

Moreover, nutritional supplements, enriched with vitamins and minerals through biotechnology, play a crucial role in improving public health.

1.5.6. Veterinary Therapeutic Products

In veterinary medicine, animal vaccines and genetically modified animals are used to improve animal health and productivity in agriculture. These advances contribute to disease prevention in livestock and to strengthening global food security.

1.6. Relevant Industrial Sectors

Biotechnology applications extend to multiple industries, including:

- **Textile, starch, brewing, baking, winemaking, and fruit juice industries**: enzymatic degradation of starch into sugars for alcohol production.
- **Food industry**: additives to improve nutritional quality, lactose conversion in dairy products, cheese flavoring, biosynthetic food flavorings, and synthetic food colorants.
- **Animal feed industry**: protein hydrolysis for high-yield flour production.
- **Cosmetics industry**: production of cream bases and collagen.
- **Paper industry**: pulp processing, bleaching, starch viscosity control.
- **Leather tanning**: removal of hair and fats.
- **Fat processing**: hydrolysis of fats, production of solubilizing agents, bio-detergents, soaps, and saponification processes.
- **Fine chemistry**: pharmaceutical products.
- **Traditional fermentation processes**: alcoholic fermentation, organic acids (citric acid, acetic acid, etc.).
- **Antibiotic production, chemical derivatives, and biopolymers** via microbial cultures.
- **Molecular biology and recombinant DNA technology**: donor DNA, vector DNA, or host DNA for the synthesis of organic products (chemicals, bio-proteins such as synthetic hormones, antibodies, and blood factors).
- **Other associated industrial processes**: wastewater recycling systems; treatment, pre-filtration, and purification of drinking water; mineral extraction and purification; development of reactors without fossil fuels or polluting chemistry; isolation, concentration, and recovery of catalysts and organisms used in by-product manufacturing.

1.7. Challenges of Biotechnological Innovation

Biotechnological innovation faces multiple challenges across strategic sectors, including health, environment, agriculture, and industry. Although biotechnological advances have opened up vast opportunities, they encounter scientific, ethical, and socio-economic barriers that must be overcome to fully exploit their potential.

1.7.1. Complexity of biological systems

Biological systems such as cells, tissues, and ecosystems are highly complex and sometimes unpredictable. Understanding and manipulating this complexity on a large scale remains a major challenge. Interdisciplinary approaches, combining biology, computer science, and engineering, are essential to decipher these systems and control their applications, particularly in personalized medicine and bioengineering.

1.7.2. Technology transfer and industrialization

Another major challenge lies in transforming laboratory discoveries into marketable products. Scaling up from research to industrial production requires overcoming technical, economic, and regulatory barriers. For example, large-scale production of biofuels or bioplastics requires advanced technologies, significant investments, and reliable industrial processes, while meeting sustainability and competitiveness standards.

1.7.3. Regulatory Challenges and Safety

Biotechnology is a highly regulated field, and safety requirements are critical, particularly for GMOs, gene therapies, and vaccines. Ensuring biosafety, protecting public health and the environment, while at the same time accelerating regulatory approval processes, represents a major challenge for both governments and companies. Regulations also vary from country to country, which further complicates the introduction of biotechnological innovations into global markets.

1.7.4. Ethics and Social Acceptability

Biotechnologies especially GMOs, gene therapies, and genome-editing technologies such as CRISPR raise significant ethical debates. Questions concerning the modification of living organisms, potential risks to biodiversity, and inequalities in access to cutting-edge technologies are major concerns. The challenge lies in promoting transparent dialogue with the public, policymakers, and the scientific community to ensure that these technologies are used responsibly.

1.7.5. Environmental Sustainability

In the context of climate change, biotechnologies must also address the challenge of sustainability. Producing biomaterials, bioenergy, or agricultural products from renewable resources while reducing environmental impact is essential for contributing to the ecological transition. Priorities include improving the efficiency of bioprocesses, ensuring the sustainable management of biological resources, and reducing the carbon footprint of these industries.

1.7.6. Accessibility and Cost of Innovations

Another challenge concerns the accessibility of biotechnological innovations, particularly in developing countries. Although advances such as biopharmaceuticals and GMO crops could potentially address health and food security issues, their high costs often limit adoption. Developing economic models that allow equitable distribution of these innovations while maintaining profitability for the companies that develop them is therefore necessary.

1.7.7. Cybersecurity and Biohacking

With the rise of digital biotechnologies—such as bioinformatics platforms and genetic editing cybersecurity risks are increasing. Controlling genetic data, preventing cyberattacks, and protecting against biohacking (the unauthorized use of biotechnology) are growing concerns in the field.

1.7.8. Continuous Innovation and Training

Biotechnology is rapidly evolving, with constant innovations in technologies such as CRISPR, synthetic biology, and bioprinting. One of the key challenges is maintaining an environment that fosters innovation while also developing a skilled workforce capable of mastering these new technologies. This requires strengthening collaboration between academic, industrial, and governmental sectors, as well as promoting education and lifelong training.

Chapter 02. Biotechnologies Applied to Environmental Issues

2.1. Climate Change and Ecosystem Evolution

2.1.1. Ecosystems

An ecosystem is the basic ecological unit composed of the environment (biotope) and the living organisms inhabiting it (biocenosis).

- **Biotope:** The physical environment characterized by specific features such as temperature, humidity, and climate.
- **Biocenosis:** The community of living organisms (animals, plants, and microorganisms) interacting and depending on one another within the biotope.

Ecosystems are in a state of continuous change. Some changes occur naturally, while others result from human intervention.

2.1.2. Climate Change

Also called *climatic disruption*, climate change refers to long-term modifications of the Earth's climate system.

These changes may result from:

- **Intrinsic Earth processes** (natural cycles and variations).
- **Human activities**, such as industrialization, intensive use of fossil fuels, and deforestation.

2.1.3. Greenhouse Effect and Global Warming

Greenhouse gases, such as carbon dioxide and methane, are naturally present in the atmosphere and play a crucial role in regulating the Earth's climate. They act as a protective layer around the planet, trapping solar heat and maintaining an average temperature of about 15°C.

Since the Industrial Revolution, human activities including the burning of fossil fuels (coal, oil, gas), deforestation, and intensive livestock farming have significantly increased the concentration of greenhouse gases. This accumulation in the atmosphere has intensified heat retention, leading to global warming.

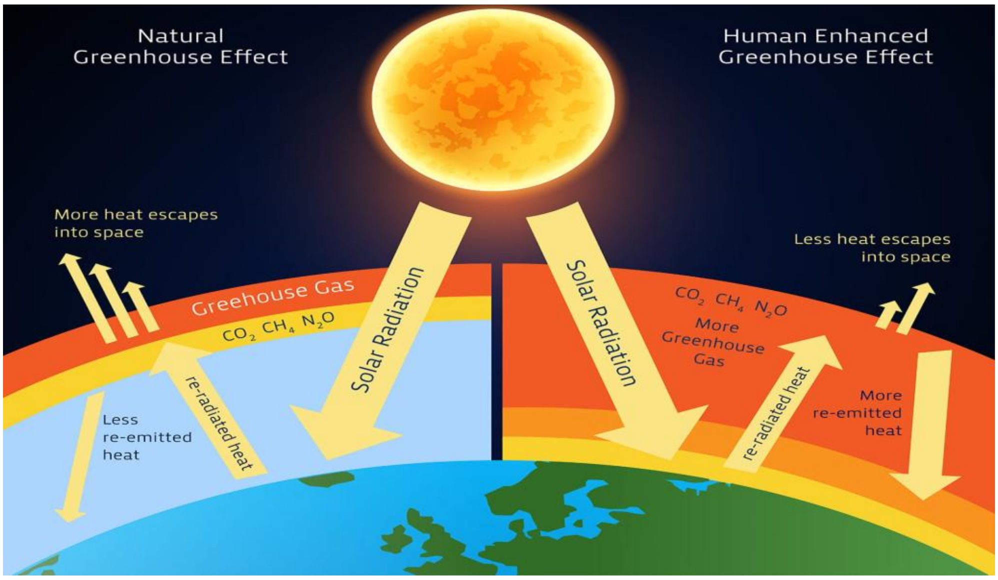


Figure 02. Illustration of the Natural and Human-Enhanced Greenhouse Effect. (**U.S. Environmental Protection Agency (EPA), 2016).**

2.1.4. The Main Greenhouse Gases

Greenhouse gases (GHGs) are naturally present in the atmosphere. They absorb part of the solar radiation and then re-emit it toward the Earth's surface, thus contributing to the greenhouse effect.

Under natural conditions, the concentration of GHGs in the atmosphere remains almost constant, regulated by exchanges of matter and gases between the atmosphere, the biosphere, and the oceans. For example, the water cycle and plant respiration help maintain a balance between emitted and absorbed gases.

However, the growing concentration of GHGs in the atmosphere, primarily due to human activities, is the main driver of global warming.

The principal greenhouse gases are:

1. **Water vapor (H_2O):** Mainly from evaporation processes.
2. **Carbon dioxide (CO_2):** Part of the natural carbon cycle, but significantly increased by fossil fuel combustion and deforestation.
3. **Methane (CH_4):** Produced through the fermentation of organic matter, including livestock digestion and rice cultivation.

4. **Nitrous oxide (N_2O):** Emitted naturally by soils and oceans, but amplified by fertilizer use in agriculture.
5. **Ozone (O_3):** Present in both the stratosphere (protective role) and the troposphere (as a pollutant and greenhouse gas).

It is important to note that although these gases occur naturally, human activities strongly influence their emissions and atmospheric concentrations, thereby intensifying the greenhouse effect.

2.1.5. The Anthropogenic Greenhouse Effect

The anthropogenic greenhouse effect results from greenhouse gases of human origin, which intensify the natural greenhouse effect and thereby drive global warming. This significant alteration of the climate has considerable impacts on humans and their environment.

The main anthropogenic greenhouse gases include:

- **Carbon dioxide (CO_2):** Emitted mainly from the combustion of fossil fuels and deforestation. It accounts for about 56% of the anthropogenic contribution to the greenhouse effect.
- **Methane (CH_4):** Produced by the anaerobic decomposition of organic matter. Anthropogenic emissions, largely from agriculture (about 40%), contribute to nearly 32% of the anthropogenic greenhouse effect.
- **Nitrous oxide (N_2O):** Released from the decomposition of nitrogen-based compounds (e.g., fertilizers). It represents slightly less than 6% of the anthropogenic contribution to the greenhouse effect.
- **Fluorinated gases:**
    - **Halocarbons:** Widely used as refrigerant gases.
    - **Sulfur hexafluoride (SF_6):** Employed in electrical insulation and transformations.

2.1.6. Evolution of Ecosystems

- A global average temperature increase of nearly 5°C.
- Reduction of snow cover and melting of glaciers.

- Rising sea levels leading to the disappearance of entire countries.
- Intensification of extreme climate events (heatwaves, floods, droughts).
- Increased acidity of oceans.
- Potential extinction of 20–30% of animal and plant species.
- Decline in agricultural production.
- Emerging health hazards.

2.2. Management of Microbiological, Plant, and Animal Resources

The management of microbiological, plant, and animal resources is a crucial aspect of environmental biotechnology, contributing to the optimization of biodiversity and the preservation of ecosystems. It relies on the use and improvement of living organisms in applications that promote environmental sustainability, agricultural productivity, and ecosystem health.

2.2.1. Management of Microbiological Resources

Microorganisms play a fundamental role in natural processes such as organic matter decomposition, pollutant degradation, and soil fertility. Managing these resources involves studying and selecting microorganisms capable of:

- Decomposing waste,
- Bioremediating contaminated soils,
- Enhancing plant and animal health through symbiotic interactions.

Examples of applications include:

- Nitrogen-fixing bacteria, which reduce the need for synthetic fertilizers.
- Fungi that degrade organic matter, used in the breakdown of pollutants.

2.2.2. Management of Plant Resources

Plant resources are essential for food production, biofuels, and biomaterials. Their management relies on crop selection and improvement techniques, including advanced technologies such as transgenesis, to develop plants resistant to climate stress, pests, and diseases.

Biotechnologies also optimize plant productivity by making more sustainable use of agricultural land and limiting chemical inputs. This is achieved through:

- Genetically modified crops (GMOs),
- Marker-assisted selection (MAS) for targeted genetic improvement.

2.2.3. Management of Animal Resources

In biotechnology, the management of animal resources aims to improve livestock productivity while ensuring animal welfare. Techniques include:

- Genetic selection to increase disease resistance,
- Production of transgenic animals for specific applications (e.g., milk enriched with proteins).

This management also extends to aquaculture, with innovations for the sustainable farming of aquatic species. In addition, biotechnologies are increasingly applied to reduce the environmental impact of intensive livestock production.

2.3. Agro-Environmental Pollution (Water, Air, Soil)

2.3.1. Definition of Pollution

Pollution refers to the introduction into the environment of natural, chemical, or radioactive substances, as well as domestic or industrial waste, that may cause undesirable or toxic effects on the biotope or the biocenosis.

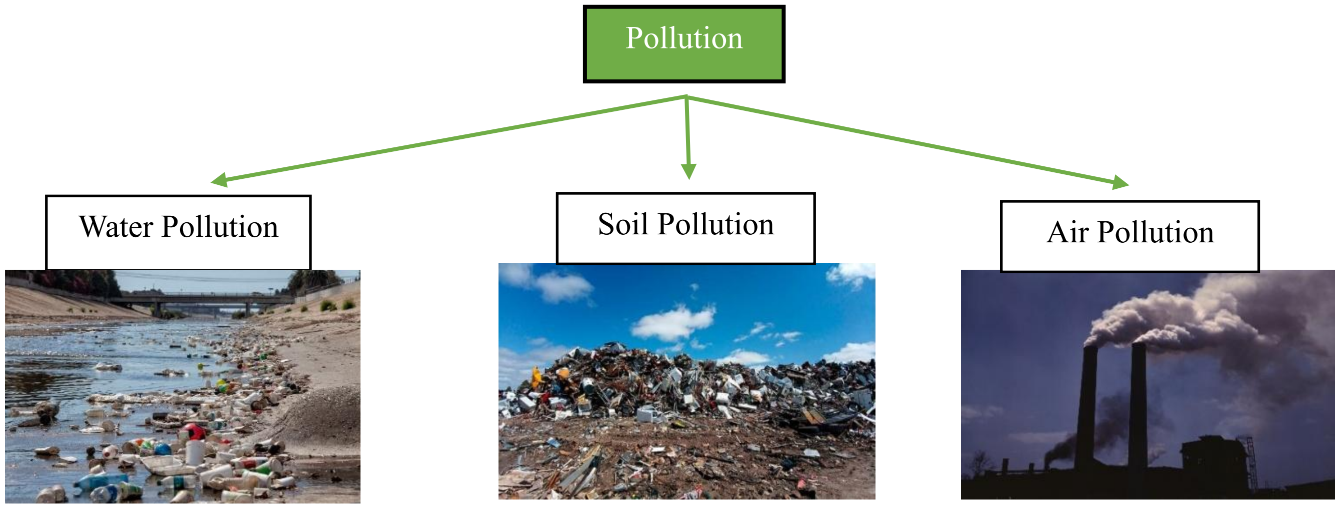


Figure 03. Main Types of Environmental Pollution **(Manahan, 2010).**

2.3.2. Soil Pollution

Soil pollution is primarily caused by the accumulation of toxic substances such as heavy metals (lead, mercury, cadmium), agricultural chemicals (pesticides, herbicides, fertilizers), industrial waste, hydrocarbons, and microplastics. These pollutants enter the soil through agricultural and industrial activities, as well as the discharge of untreated waste. They can alter soil structure and fertility, disrupt biodiversity, and contaminate the food chain by accumulating in plants and animals.

2.3.3. Air Pollution

The causes of air pollution are diverse and largely result from human activities:

- **Industrial Emissions**

  Chemical, petrochemical, metallurgical, and other industries release pollutants into the atmosphere, including gases such as sulfur dioxide (SO_2), nitrogen oxides (NO_x), and volatile organic compounds (VOCs). These substances are often responsible for acid rain and smog.

- **Transportation**

  Road vehicles, airplanes, and ships running on fossil fuels emit significant amounts of CO_2, carbon monoxide (CO), fine particulate matter, and NO_x. The transport sector contributes substantially to climate change and urban air pollution.

- **Energy Production**

  The combustion of coal, oil, and natural gas in thermal power plants releases large quantities of greenhouse gases and pollutants, notably SO_2 and mercury. Coal-fired power plants are particularly harmful to air quality and human health.

- **Agriculture and Intensive Livestock Farming**

  The massive use of nitrogen-based fertilizers in agriculture generates emissions of nitrous oxide (N_2O), a powerful greenhouse gas. Intensive livestock farming also produces methane (CH_4), mainly through animal digestion, and ammonia (NH_3) emissions, all of which contribute to air pollution.

- **Deforestation and Forest Fires**

  Deforestation releases the carbon stored in trees and reduces the capacity of ecosystems to absorb CO_2. Forest fires, whether natural or human-induced, release large quantities of pollutants and suspended particles, contributing to climate change and air pollution.

- **Waste and Incineration**

  The combustion of waste in incinerators, or open-air burning, generates toxic pollutants such as dioxins, which contaminate the air and pose significant health risks.

- **Domestic Activities and Heating**

  Heating with wood, coal, or fuel oil releases fine particulate matter, carbon monoxide, and volatile organic compounds. In urban areas, residential heating represents a major source of atmospheric pollution during winter.

2.3.4. Water Pollution

Water pollution can originate from various sources:

- **Industrial Pollution** Caused by the discharge of chemical products such as hydrocarbons or PCBs from industries, as well as wastewater released by factories.
- **Agricultural Pollution** Resulting from animal waste as well as phytosanitary products/pesticides (herbicides, insecticides, fungicides) and fertilizers used in agriculture. These substances infiltrate soils and reach groundwater, or run off into surface waters.
- **Domestic Pollution** Stemming from wastewater (toilets), as well as cosmetic and cleaning products (soaps, detergents, cleaning agents), paints, solvents, waste oils, and hydrocarbons.
- **Accidental Pollution** Occurs when toxic products are accidentally discharged into the natural environment, thereby disrupting ecosystems.

2.4. Environmental Decontamination through Biotechnology

Decontamination refers to the intentional elimination, inactivation, or transformation of pollutants (organic or inorganic, such as heavy metals) using chemical, physical, or biological agents the latter process being known as bioremediation**.**

2.4.1. Bioremediation

Bioremediation is a process that uses living organisms to degrade harmful contaminants present in polluted environments (such as soil or water) into non-toxic compounds. It is a technique that relies on biological agents to transform, destroy, or immobilize pollutants as part of environmental cleanup efforts.

It can be carried out directly in the polluted environment (in situ) or after transferring the pollutant to another location (ex situ).

2.4.2. Methods of Bioremediation

2.4.2.1. Soil Bioremediation

- **Land Farming (Surface Treatment)**

This method is mainly used to treat soils contaminated by petroleum waste. It relies on the pollutant-degrading activity of the soil microflora naturally present in the environment.

These microorganisms decompose and mineralize organic compounds, thereby recycling essential elements for plant production, which is based on the assimilation of atmospheric CO_2. They include protozoa, microscopic algae, fungi, bacteria, actinomycetes, cyanobacteria, and viruses.

- **Composting**

This process involves aerobic and thermophilic microorganisms traditionally used to degrade organic matter. Through fermentation, they transform organic waste into a smaller, carbon-depleted, and nutrient-rich product that can be used as fertilizer.

- **Phytoremediation**

Phytoremediation is an ecological and cost-effective technique that uses plants to remove, stabilize, or degrade pollutants present in the soil. This process relies on the natural abilities of certain plants, known as hyperaccumulator plants, to absorb, accumulate, or transform toxic substances such as heavy metals, pesticides, hydrocarbons, and other organic pollutants.

The main types of phytoremediation include:

- ✓ **Phytostabilization**
  Plants immobilize pollutants in the soil, reducing their mobility and minimizing the risk of contamination in surrounding environments.
- ✓ **Phytoextraction**
  Plants absorb pollutants through their roots and accumulate them in their aerial parts (leaves, stems). These plants can then be harvested and treated to permanently remove the pollutants.
- ✓ **Phytodegradation**
  Plants and their enzymes break down toxic chemical substances into less harmful compounds directly in the soil.
- ✓ **Phytovolatilization**
  In this process, plants absorb contaminants from the soil, transform them, and release them into the atmosphere as vapors, often in a less toxic form. This technique is particularly effective for volatile pollutants such as mercury and certain volatile organic compounds (VOCs).
- ✓ **Phytostimulation (or Rhizodegradation)**

  In this form of phytoremediation, plants release root exudates (sugars, organic acids, amino acids) that stimulate microbial activity in the rhizosphere. This microbial activity accelerates the breakdown of organic pollutants such as hydrocarbons and pesticides.

2.4.2.2. Bioremediation of Wastewater

Wastewater bioremediation is a treatment technique that uses microorganisms such as bacteria, fungi, and algae to degrade organic and inorganic pollutants in contaminated water. This natural process, based on biological activity, is commonly applied in wastewater treatment plants to reduce pollutant loads before discharge or recycling.

- **Aerobic Bioremediation**

This method uses aerobic bacteria, which require oxygen to break down organic matter present in wastewater. By injecting air or oxygen into treatment basins, microorganisms grow and consume pollutants, thereby reducing their concentration. Aerobic bioreactors are frequently used to treat both domestic and industrial wastewater.

- **Anaerobic Bioremediation**

In this technique, anaerobic bacteria degrade organic matter in the absence of oxygen, producing by-products such as methane (CH_4) and carbon dioxide (CO_2). This method is particularly suitable for wastewater with high concentrations of organic matter, such as effluents from the food industry. Anaerobic reactors are advantageous for treating high-strength effluents and also enable energy recovery in the form of biogas.

2.4.2.3. Bioremediation of Air

- **Biofiltration**

is a biological technique that has been widely applied in industry for the purification of gases and sometimes water. This process involves forcing the contaminated gas or effluent to pass through a granular material on which pollutant-degrading microorganisms are attached.

The biomass (microorganisms) is fixed onto a support and covered with a thin layer of water. As the polluted gas passes through the filter, a portion of the pollutants is absorbed and degraded by the microorganisms. This method is particularly effective for removing volatile organic compounds (VOCs), odorous substances, and other gaseous pollutants from industrial emissions.

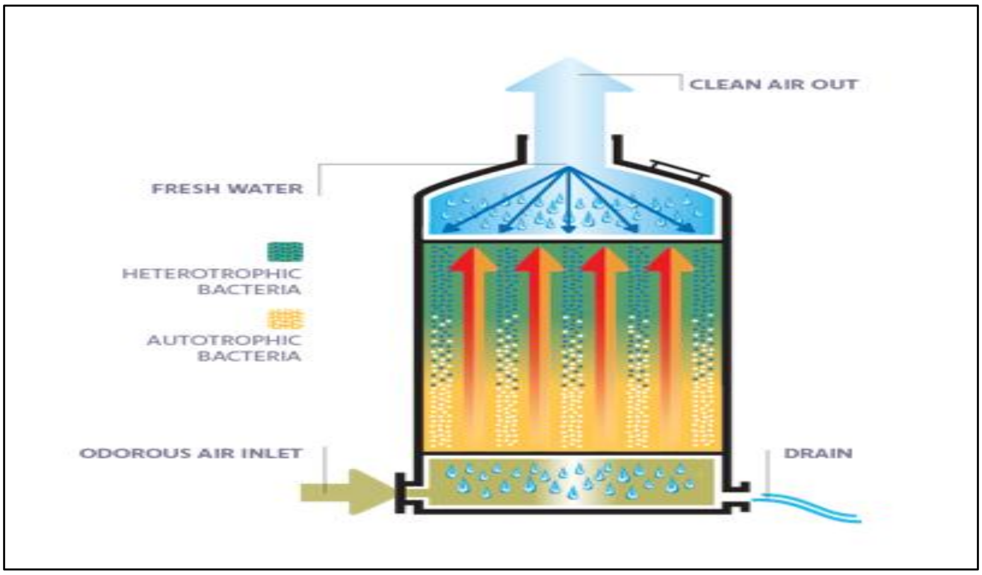


Figure 04. Schematic Illustration of the Air Biofiltration Process. (**Devinny *et al.*, 1999**)

2.4.2.4. Phycoremediation

Phycoremediation is a process based on the use of algal biomass as an agent for pollutant adsorption.
It is mainly employed for the treatment and purification of wastewater, where algae absorb and accumulate contaminants such as heavy metals, nutrients (nitrogen and phosphorus), and organic compounds, thereby helping to clean and restore water quality in an eco-friendly and sustainable manner.

2.5. Other Applications of Biotechnology for Environmental Protection

The applications of environmental biotechnology go beyond bioremediation processes. The clean production of new products (known as white biotechnology) represents an increasingly significant part of this field, sometimes referred to as yellow biotechnology.

2.5.1. Bioenergy

Traditional fuels such as coal, oil, and gas are fossil-based, non-renewable, and major contributors to greenhouse gas emissions.

Biotechnology seeks to develop new, cleaner sources of energy, such as biofuels, which are produced from renewable biological materials (e.g., plants, algae, and agricultural waste) and help reduce environmental pollution.

2.5.2. Bio-based and Biodegradable Plastics

These plastics are manufactured from renewable plant-based resources, such as wheat, corn, sugar beet, or potatoes. Using these renewable sources reduces dependence on petroleum and lowers greenhouse gas emissions.

Their biodegradability (ability to be decomposed by microorganisms) also helps reduce waste volume and limit environmental accumulation of plastics.

2.5.3. Biofertilizers

A biofertilizer is a biologically active product containing living microorganisms that enhance plant growth by increasing the availability of nutrients in forms that are more easily absorbed by plants.

They represent a natural and eco-friendly alternative to chemical fertilizers, helping reduce soil and water pollution while promoting sustainable agriculture.

Chapter 03. Agricultural Biotechnologies for Food Purposes

3.1. Biotransformation and Conservation

3.1.1. General Concepts

Biotransformation and bioconversion refer to biotechnological processes used to transform organic raw materials (carbohydrates, lipids, and proteins) into new molecules with specific properties through reactions such as hydrolysis, decarboxylation, isomerization, and oxidation.

3.1.2. Biotransformation

Definition

Biotransformation involves the use of whole living organisms, such as bacteria or fungi, to carry out chemical reactions. These organisms catalyze a series of transformations (oxidation, reduction, hydrolysis, etc.) in order to produce specific compounds.

3.1.2.1. Characteristics

- Biotransformation allows the realization of reactions that are difficult or impossible to achieve using conventional chemistry.
- The organism provides not only the enzymes but also a favorable environment for the reactions to occur.
- Microorganisms continuously produce enzymes during the process, which supports the chemical transformations.

3.1.2.2. Applications

- **Pharmaceutical production:** *Streptomyces* spp. converting precursors into corticosteroids such as cortisone
- **Organic acid production:**
  - Lactic acid by *Lactobacillus* spp.
  - Citric acid by *Aspergillus niger*

3.1.2.4. Advantages and Disadvantages

Advantages

- Eco-friendly and cost-effective
- Produces biodegradable and natural-like compounds

Disadvantages

- Sensitivity to pH and temperature
- Possible accumulation of inhibitory by-products

3.1.3. Bioconversion

Definition

Unlike biotransformation, bioconversion relies on the use of *isolated enzymes* (without whole organisms). It is often used to convert one molecule into another through one or two simple reaction steps.

3.1.3.1. Characteristics

- High reaction specificity
- Easier control of operating conditions
- Faster and simpler than biotransformation

3.1.3.2. Applications

- **Sugar production:** Conversion of starch into glucose by amylase
- **Biofuel production:** Conversion of fats into biodiesel by lipase

3.1.3.3. Advantages and Disadvantages

Advantages

- Simplified and well-controlled processes
- Reduced formation of unwanted by-products

Disadvantages

- High cost of isolated enzymes

- Limited suitability for complex reactions

Table 02. Comparison Between Biotransformation and Bioconversion

Criteria	Biotransformation	Bioconversion
Biological system used	Uses whole living organisms (bacteria, fungi, etc.)	Uses isolated or purified enzymes
Process complexity	Often involves several complex steps	Generally limited to one or two simple steps
Enzyme production	Enzymes are produced continuously by the microorganism during the process	Enzymes are already isolated and ready to use
Reaction control	More difficult to control due to the involvement of living organisms	Easier to control under defined conditions
Examples	Transformation of precursors into corticosteroids by *Streptomyces*	Conversion of starch into glucose using amylase

3.1.4. Biotransformation by Microorganisms

This type of biotransformation, also known as microbial fermentation or microbial bioconversion, involves:

- Using microorganisms (bacteria or fungi) grown under specific conditions to convert a precursor into a desired product.
- Microorganisms are selected for their metabolic and biochemical diversity, their ability to produce a wide range of molecules, and their adaptability to different environments.

Advantages of Microorganisms

- **High metabolic diversity:** Microorganisms exist in all types of environments and possess varied metabolic pathways.
- **Economic advantage:** They enable the production of complex molecules in a more cost-effective and eco-friendly way than chemical processes.

3.1.6. Fermentation Processes

They are processes caused by anaerobic microorganisms capable of decomposing organic matter (carbohydrates, lipids, proteins) into organic acids and alcohols, producing different end products depending on the type of fermentation.

The term fermentation comes from the Latin *fervere*, meaning "to boil," because a fermenting liquid such as one undergoing alcoholic fermentation produces a strong release of gas and appears as if it were boiling.

3.1.6.1.Types of Fermentation

3.1.6.1.1. Alcoholic Fermentation

This process is carried out by yeasts of the *Saccharomyces cerevisiae* genus under anaerobic conditions and leads to the production of ethanol.

Reaction: Sugar → CO_2 + Alcohol + Energy

Examples of products:

Bread, beer, and wine.

3.1.6.1.2. Lactic Fermentation

This process is carried out by lactic acid bacteria of the genera *Streptococcus*, *Lactobacillus*, and *Lactococcus* under anaerobic conditions and leads to the production of lactic acid.

Reaction: $glucose \rightarrow \ lactic\ acid +$ energy

Products: yogurt, cheese, fermented milk, etc.

3.1.6.1.3. Acetic Fermentation

This process is carried out by yeasts of the genus *Saccharomyces cerevisiae*, which first produce alcohol (ethanol). Then, under aerobic conditions, bacteria of the genus *Acetobacter aceti* transform the ethanol into acetic acid.

Product: vinegar

3.1.6.1.4. Propionic Fermentation

This process is carried out by bacteria of the genus *Propionibacterium*, leading to the production of acetic acid, propionic acid, and carbon dioxide (CO_2) from lactic acid.

Reaction: lactic acid → propionic acid + acetic acid + CO_2 + H_2O

Products: hard cheeses

3.1.6. Food Conservation and Preservation

Food preservation is an essential process that aims to extend shelf life, maintain nutritional and sensory qualities (taste, texture, aroma), and prevent the growth of pathogenic or spoilage microorganisms.

3.1.7.1. Cold Preservation

- **Refrigeration**
  - ✓ Maintains food at a temperature between 0 and 4°C.
  - ✓ Slows down or temporarily halts enzymatic activity and microbial growth.

Examples: Fresh vegetables, meat, dairy products.

- **Freezing**
  - ✓ Lowers the temperature below 0°C, typically to –18°C or lower.
  - ✓ Completely inhibits microbial growth by solidifying the water in food.

Examples: Frozen meats, fruits, and vegetables.

3.1.6.2. Heat Preservation

- **Pasteurization**
  - ✓ Heating between 60°C and 100°C, followed by rapid cooling.
  - ✓ Eliminates heat-sensitive microorganisms while preserving most nutrients and flavor.

Examples: Pasteurized milk, honey, fruit juices.

- **Sterilization**
  - ✓ Heating to above 100°C to destroy all microorganisms, including spores.
  - ✓ Often combined with airtight packaging to prevent recontamination.

Examples: Canned foods, ready-to-eat meals in jars.

- **UHT Treatment (Ultra-High Temperature)**
  - ✓ Rapid heating between 135°C and 150°C for 1 to 5 seconds.
  - ✓ The product is aseptically packaged, allowing long shelf life at room temperature.

Examples: UHT milk, industrial soups.

3.1.6.3. Other Preservation Methods

- **Dehydration**
  - ✓ Removes water from food to inhibit microbial growth.
  - ✓ Allows long-term storage with reduced weight and volume.

Example: Dried fruits, beans.

- **Salting and sugaring**
  - ✓ Salting or candying reduces the water activity in food, preventing microbial development.

Example: Salted cod, jams, candied fruits.

- **Alcohol and vinegar preservation**
  - ✓ Acidity (vinegar) or high alcohol concentration inhibits microbial growth.

Example: Pickles (vinegar), wine (alcohol).

- **Fermentation**
  - ✓ Natural production of acids or alcohol by microorganisms (lactic acid bacteria or yeasts).
  - ✓ Ensures preservation while developing unique flavors.

Example: Yogurt.

- **Irradiation**
  - ✓ Use of ionizing radiation to kill microorganisms or slow their growth.
  - ✓ May slightly alter vitamins or nutrients.

Example: Spices, exotic fruits.

- **Modified Atmospheres packaging**
  - ✓ Replacement of air by a gas mixture low in oxygen and rich in nitrogen or carbon dioxide.
  - ✓ Slows down oxidation and inhibits aerobic microorganisms.

Example: Packaged salad mixes.

3.1.6.4. Biopreservation (Bioconservation)

Definition

Biopreservation uses microorganisms or their metabolites to inhibit spoilage and pathogenic microorganisms.

Principles

- pH reduction
- Production of bacteriocins

Applications and Advantages

- Use of lactic acid bacteria
- Natural antimicrobials (e.g., nisin E234)
- Preservation of nutritional and sensory quality

3.2. Production of Food Matrices in Bioreactors

3.2.1. Bioreactors (Fermenters or Cytocultors)

A bioreactor is a device used for enzymatic reactions (bioconversion/biotransformation, fermentation) or for the cultivation of microorganisms (yeasts, bacteria, microscopic fungi, algae, animal or plant cells) in order to produce biomass or metabolites, under controlled conditions (pH, temperature, aeration, etc.).

The term biofermenter is used for the culture of microorganisms, while bioreactor or cytocultor is used for the culture of animal cells.

A computer-controlled monitoring system allows the recording and regulation of all operational parameters.

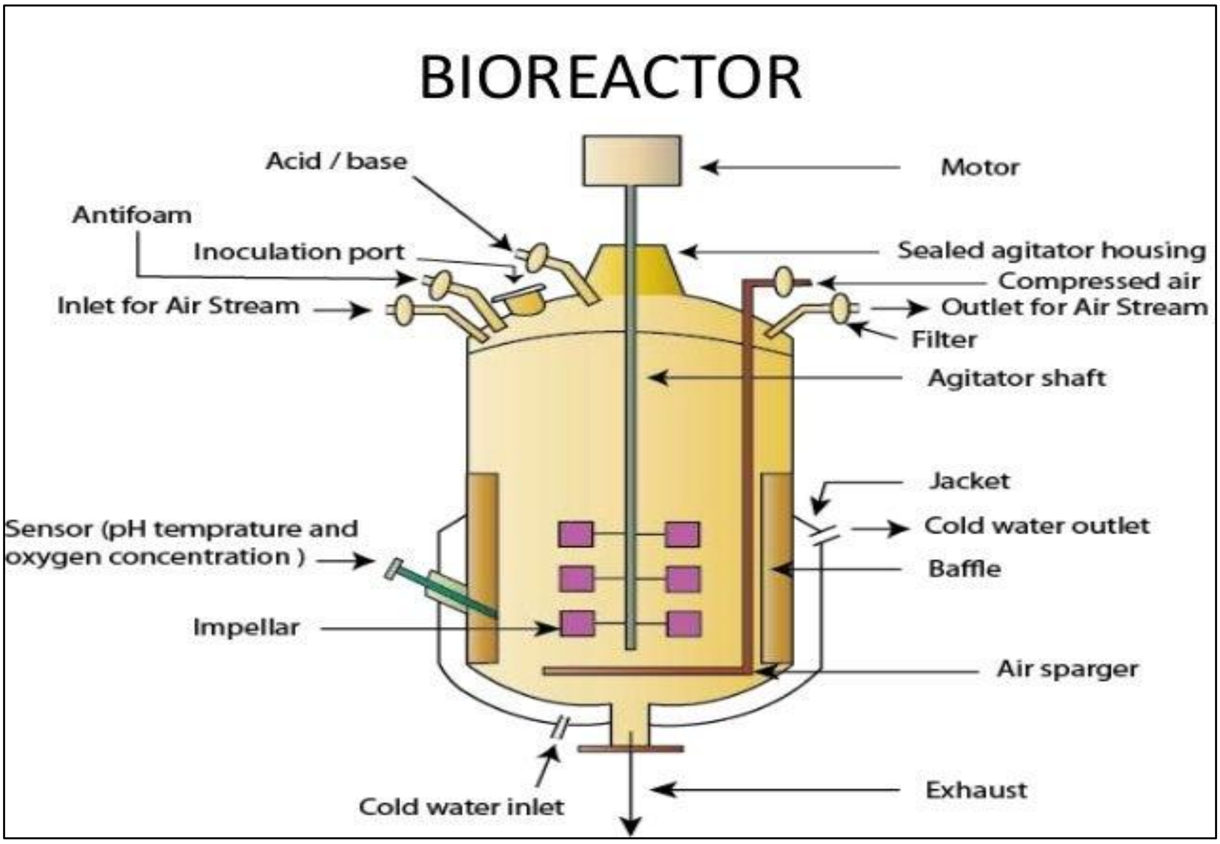
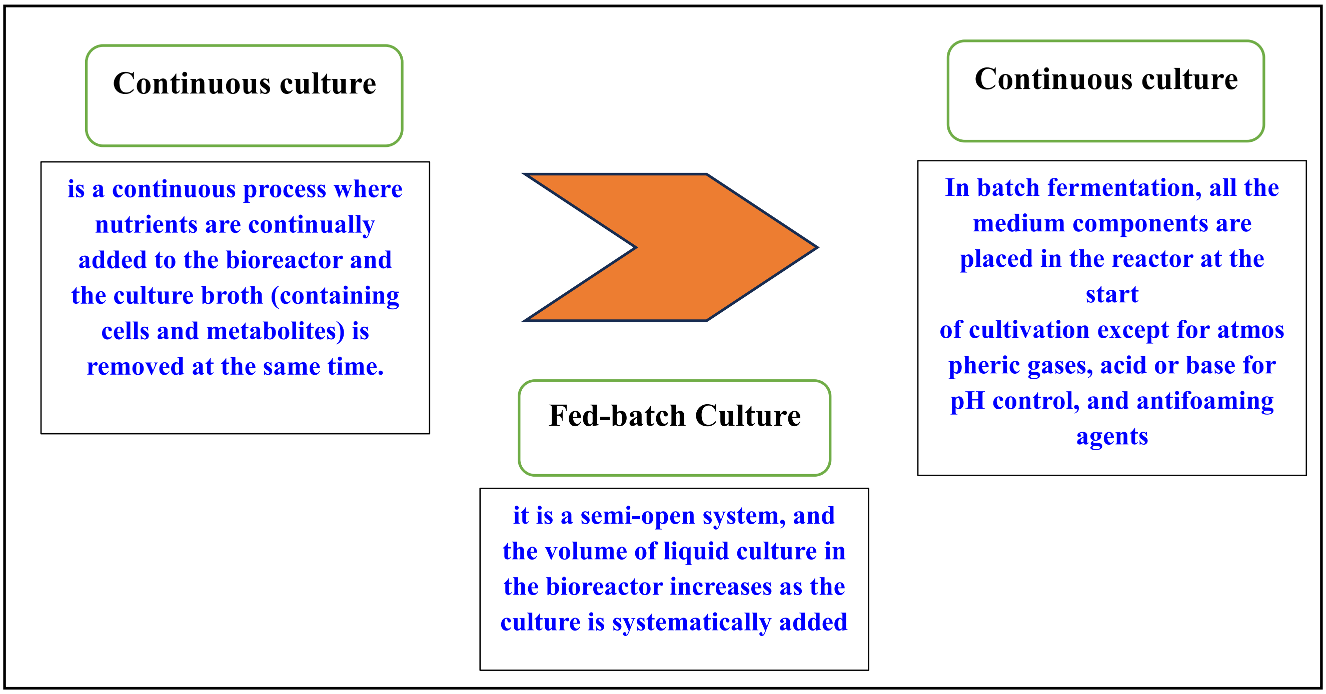


Figure 05. Description of a Bioreactor. (**Stanbury *et al*., 2017**).

3.2.2. Culture Techniques in Bioreactors

In bioreactors, microbial and cell cultures can be grown using different cultivation strategies depending on the production objectives and process control requirements. The main culture techniques include batch, fed-batch, and continuous cultures. These approaches differ in nutrient supply, system openness, and culture duration, which directly influence cell growth, metabolite production, and process efficiency.



3.3. Food Safety, Traceability, and Quality

3.3.1. Food Security

According to the FAO (1996), food security exists when *all people, at all times, have physical, social, and economic access to sufficient, safe, and nutritious food to meet their dietary needs and food preferences for an active and healthy life.*

Food security encompasses not only the quantity of food available but also its quality, safety, and accessibility.

3.3.1.2. The Four Pillars of Food Security

- **Availability**

Refers to a country's ability to produce or import sufficient quantities of food to meet the needs of its population. Ensuring adequate food production is essential to respond to the demands of a growing population. For example, countries should diversify their crops to reduce dependence on a limited number of staple foods.

- **Accessibility**

Food must not only be available but also accessible to all individuals. This depends on economic and physical access, meaning that populations need adequate income, stable economies, and reliable infrastructure for food distribution even in remote areas.

- **Utilization (Quality)**

Food must be safe for human consumption and provide adequate nutritional value. This includes:

  - **Sanitary aspects:** absence of contaminants or pathogens.
  - **Nutritional aspects:** appropriate caloric and micronutrient content.
  - **Organoleptic aspects:** taste, texture, and appearance that make food desirable.

- **Stability**

Access to food must be consistent over time, even during crises such as conflicts, natural disasters, or economic instability. For example, international food aid programs help stabilize food access during emergencies or shortages.

3.3.2. Food Traceability

Traceability is the process of tracking food or its ingredients at every stage of production and distribution, from origin to the final consumer. It is essential to ensure the quality and safety of food products.

3.3.2.1. Objectives

- **Upstream traceability** Identify the origin of a product or ingredient in case of a problem (for example, contamination).
- **Downstream traceability** Enables the rapid withdrawal of a defective product from the market, thereby limiting health risks.

Example In the case of a contaminated batch of milk, traceability makes it possible to trace back to the originating farm, verify production conditions, and withdraw only the affected products.

3.3.3. Food Quality

Food quality is not limited to the absence of contamination. It encompasses a set of characteristics that make foods suitable for consumer needs whether nutritional, sanitary, or sociocultural.

3.3.3.1. Food Quality Criteria

- **Sanitary:** Foods must be free from harmful substances such as pesticides, heavy metals, or pathogenic microorganisms.
- **Nutritional:** They must provide the necessary elements for maintaining health, such as proteins, vitamins, minerals, and fibers.
- **Sensory:** Consumers often prefer foods that are visually appealing, have a pleasant taste, and a satisfying texture.
- **Sociocultural:** Certain foods must meet religious requirements (halal, kosher) or respect local traditions.

3.3.3.2. Quality Standards and Certification

To guarantee this quality, several organizations have established international standards:

- **ISO 22000:** An international standard defining the requirements of a food safety management system to ensure food quality and safety throughout the supply chain.
- **IFS (International Food Standard):** A standard used by agri-food companies to ensure that their products meet the expectations of consumers and retailers.

3.3.3.3. Role of Biotechnology in Food Safety and Quality

Biotechnologies play a key role in improving food safety and quality through techniques such as

- **Genetically Modified Crops (GMOs):** Increase agricultural yields while reducing the need for pesticides.
- **Biopesticides and Biofertilizers:** Enable more environmentally friendly agriculture.
- **Biopreservation:** Uses microorganisms to extend the shelf life of foods without relying on chemical additives.

Example: Lactic acid bacteria used in the production of yogurt or cheese not only improve preservation but also provide nutritional benefits (probiotics).

Chapter 04. Biotechnologies and Industry for Non-Food Purposes

Introduction

Biotechnologies for non-food purposes involve the use of living organisms or biological systems to produce industrial goods that are not intended for human consumption. These applications contribute to sustainable development, the energy transition, and industrial innovation.

4.1. Bioenergy

4.1.1. Definition of Industrial (White) Biotechnology

Industrial biotechnologies, also known as “white biotechnologies”, aim to use renewable resources, enzymes, microorganisms, or biological processes to replace traditional chemical processes. They differ from “green” biotechnologies (agriculture) and “red” biotechnologies (health).

4.1.2. Biomass and Bioenergy

Bioenergy is based on the use of biomass, a renewable biological raw material, to generate various forms of energy such as heat, electricity, and fuels. It represents a promising alternative to fossil energy sources in a context marked by the depletion of natural resources and the fight against greenhouse gas emissions.

- **Biomass:** biological materials such as energy crops (maize, sugar beet, sugarcane), agricultural and forest residues, and organic waste.
- **Bioenergy:** includes fuels such as biogas and bioethanol, obtained through processes such as fermentation or methanization.

4.1.3. Interest and Advantages of Bioenergy

- Reduction of CO_2 emissions and other greenhouse gases.
- Search for alternatives to fossil fuels.
- Strategic energy independence.
- Biotechnological innovations (for example, cellulosic ethanol produced through genetic engineering).

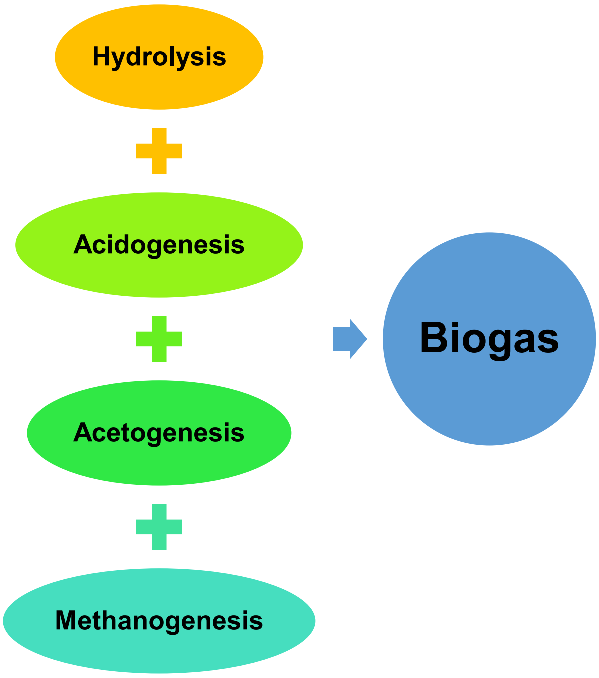
4.1.4. Types and Applications of Bioenergy

4.1.4.1. Biogas

Definition

Biogas is a renewable gas produced by the decomposition of organic matter in the absence of oxygen through a process called methanization (or anaerobic digestion). It is composed mainly of methane (CH_4) and carbon dioxide (CO_2), with small amounts of other gases such as hydrogen sulfide (H_2S).

❖ **Production process**



➢ **Stages of Methanization**

1. **Hydrolysis:** Complex molecules (proteins, carbohydrates, lipids) are broken down into simpler molecules (amino acids, sugars, fatty acids).
2. **Acidogenesis:** Simple molecules are converted into organic acids, CO_2, and hydrogen (H_2) by bacteria.
3. **Acetogenesis:** Organic acids are transformed into acetate, CO_2, and H_2.
4. **Methanogenesis:** Methanogenic bacteria convert acetate, CO_2, and H_2 into methane (CH_4).

Applications

- **Energy production:** Biogas can be burned to generate electricity and heat (cogeneration).
- **Fuel:** After purification (removal of CO_2 and hydrogen sulfide H_2S), biogas becomes biomethane, which is used as a fuel for vehicles (bio-CNG).

4.1.4.2. Biofuels

Definition

Biofuels are liquid or gaseous fuels derived from biomass (organic plant or animal materials). They partially or fully replace fossil fuels such as gasoline and diesel while reducing greenhouse gas emissions.

4.1.4.2.1. Generations of Biofuels

Biofuels are classified into several generations based on the origin of the raw materials:

1.First-generation biofuels

Produced from food crops rich in sugars (sugarcane, beet) or oils (rapeseed, soybean, palm).

Techniques:

- **Bioethanol:** Fermentation of sugars into ethyl alcohol by yeasts (*Saccharomyces cerevisiae*).
- **Biodiesel:** Transesterification of vegetable oils with an alcohol, most often methanol.

2. Second-generation biofuels

Produced from agricultural waste (straw), forestry residues, or non-food crops.

3. Third-generation biofuels

Produced from algae, which generate oils or hydrocarbons through photosynthesis.

Examples

- **Bioethanol:** Mixed with gasoline to reduce CO_2 emissions. *Example:* E85 fuel, which contains 85 percent bioethanol.
- **Biodiesel:** Used in diesel engines. *Example:* Biodiesel produced from rapeseed in Europe.
- **Biogas as fuel:** Used in natural gas vehicles. *Example:* Buses operating on biomethane in France.

4.1.4.3. Wood Energy

Definition

Wood energy refers to the use of wood and its derivatives (wood chips, pellets, charcoal) as an energy source to produce heat, electricity, or as a direct fuel.

Advantages

- Renewable and widely available energy source.
- Relative carbon neutrality: the CO_2 emitted during combustion is compensated by the absorption carried out by trees during their growth.

Limitations

- Air pollution: emission of fine particulate matter (PM10, PM2.5).
- Deforestation: excessive use can lead to overexploitation of forests.

Example In developing countries, wood remains the main energy source for cooking, but it causes indoor pollution and health problems.

4.2. Biomaterials and Agro-Polymers

Definition

Biomaterials represent an essential class of non-living materials specifically designed to interact with living biological tissues. These materials play a key role in numerous medical and biotechnological applications, including the manufacture of implants, prostheses, and functional devices capable of replacing or enhancing defective biological tissues.

Agro-polymers, on the other hand, include materials of biological origin, often used in agricultural and environmental fields to promote sustainable practices.
Together, biomaterials and agro-polymers pave the way for technological innovations that respect the environment while improving quality of life.

4.2.1. Properties of Biomaterials

Biomaterials are designed to meet specific criteria that ensure their effectiveness and integration in various biological contexts. These properties include:

- **Biocompatibility**
  Biocompatibility is the ability of a biomaterial to perform its function while avoiding any undesirable reaction in the human body, such as inflammation or immune rejection. This property is particularly critical for medical implants such as joint prostheses or heart valves.
- **Biodegradability**
  Biodegradable biomaterials break down under the action of enzymes or microorganisms, generating simple molecules such as carbon dioxide (CO_2), methane (CH_4), or water (H_2O). These characteristics make them essential for temporary medical devices and environmentally friendly packaging.
- **Bioresorbability**
  Certain biomaterials, such as biopolymers, have the ability to degrade naturally inside the human body. These materials are gradually replaced by living tissue, a key property for sutures and tissue regeneration scaffolds.

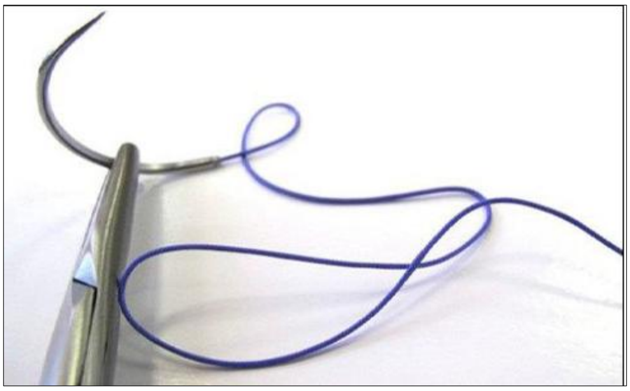


Figure 06. Needle and Suture Material Used in Medical Procedures. (**Townsend *et al.*, 2021**).

4.2.3. Sectors and Applications

Biomaterials are used in various sectors:

1. **Medical Sector:** Biomaterials are widely used in bone implants, artificial heart valves, contact lenses, dental prostheses, and orthopedic implants. These devices must be biocompatible and durable to ensure long-term performance.

Figure 07. Protheses Used in Dental Roots. (**Misch, 2015**).

2. **Pharmaceutical Sector** Biomaterials are used to develop controlled drug delivery systems. For example, biodegradable capsules or gels allow targeted and prolonged release of therapeutic agents.
3. **Agriculture**
   Biodegradable agro-polymers, such as mulching films, reduce plastic waste in fields while preserving soil moisture and limiting weed growth.

Figure 08. Mulching Films Used in Agricultural Fields. (**Kasirajan & Ngouajio, 2012**).

4. **Automotive and Packaging Industries** Bioplastics, produced from renewable sources, are gradually replacing traditional plastics to reduce environmental impact. They are used particularly in car interior panels and food packaging.

4.2.4. Polymers

Definition

Polymers, whether natural or synthetic, are macromolecules composed of repeated chains of small units called *monomers*. These units are linked together by covalent bonds. Depending on their origin, polymers are divided into two main categories:

4.2.4.1. Types of Polymers

- **Natural Polymers**

Examples: DNA, proteins, polysaccharides (starch, cellulose).

These polymers, found in biological systems, are biodegradable and produced through natural biological processes.

- **Synthetic Polymers**

Examples Polyethylene, polystyrene, PVC (polyvinyl chloride).

Manufactured through chemical processes, they are often non-biodegradable and have customized properties designed for various industrial applications.

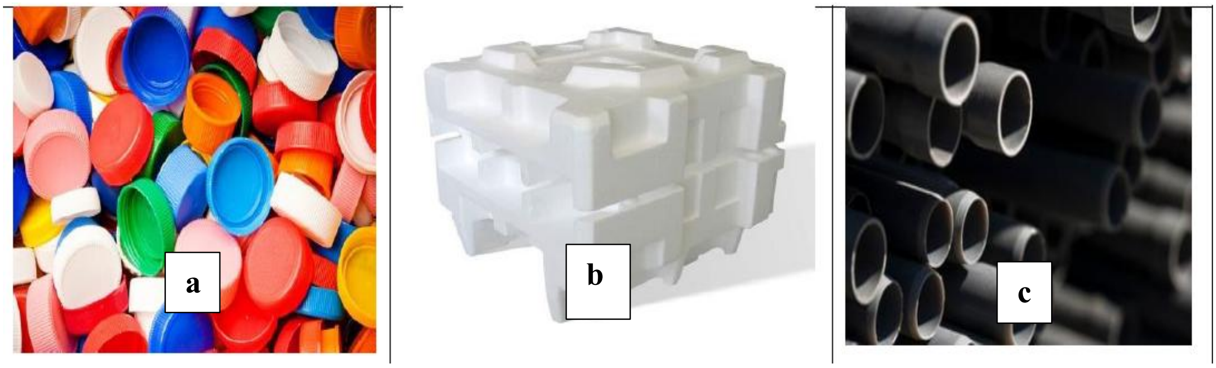


Figure 09. Examples of Synthetic Polymers: (a) Polyethylene, (b) Polystyrene, and (c) Polyvinyl Chloride (PVC). (**Callister & Rethwisch, 2018**).

Table 03. Comparison Between Natural and Synthetic Polymers

Criteria	Natural Polymers	Synthetic Polymers
Origin	Produced by living organisms (plants, animals, microorganisms)	Produced by chemical synthesis in industry
Biodegradability	Generally biodegradable	Often non-biodegradable
Environmental Impact	Environmentally friendly, minimal pollution	Can cause long-term pollution, especially plastics
Structure	Complex, highly organized	Simpler or customizable structures
Cost	Often more expensive to extract or purify	Cheaper to mass-produce
Applications	Medicine, food industry, biotechnology	Packaging, construction, textiles, automotive
Mechanical Properties	Sometimes limited (sensitive to heat, moisture)	Highly adjustable and optimized for performance

4.2.6. Polymerization Mechanisms

Polymerization refers to the chemical process through which monomers join together to form polymers. There are two main types of polymerization:

4.2.6.1. Condensation Polymerization

This mechanism involves the formation of polymers through the bonding of monomers accompanied by the elimination of small molecules, such as water or methanol. Examples of polymers produced by condensation: polyesters, nylon, polyamides.

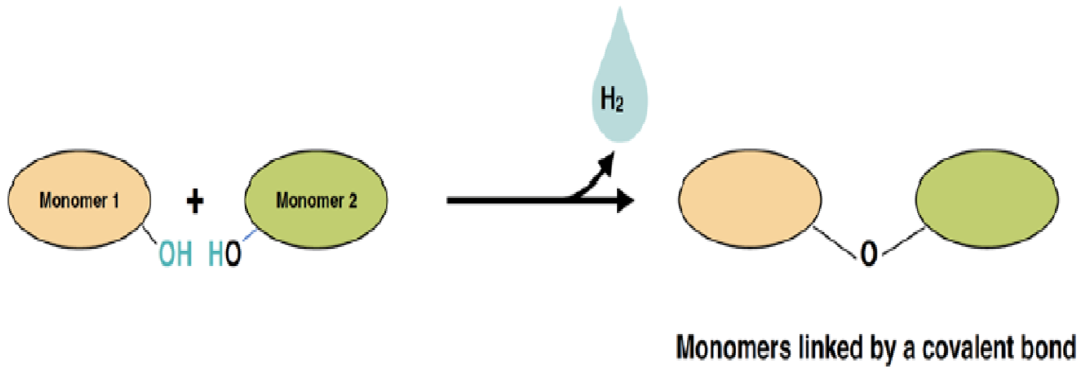


Figure 10. Mechanism of a Condensation Reaction. (**Nelson *et al.*, 2017**).

4.2.6.2. Addition Polymerization

In this mechanism, unsaturated monomers (containing double bonds) bind together without any loss of atoms. This type of polymerization is commonly used to produce polymers such as polypropylene, polyethylene, and PVC (polyvinyl chloride).

4.3. Biomolecules and Cellular Activities

4.3.1. Biomolecules

Crude plant extracts are a valuable natural source of bioactive molecules. Biomolecules are the fundamental chemical compounds of living organisms, including proteins, lipids, carbohydrates, and nucleic acids.

They play essential roles in various cellular processes, such as:

- Structural support
- Energy storage and metabolism
- Enzymatic catalysis
- Genetic information storage and transmission

Many plant extracts exhibit antibacterial, antioxidant, and anti-inflammatory activities. These properties make them important in pharmaceutical, cosmetic, and biotechnological applications.

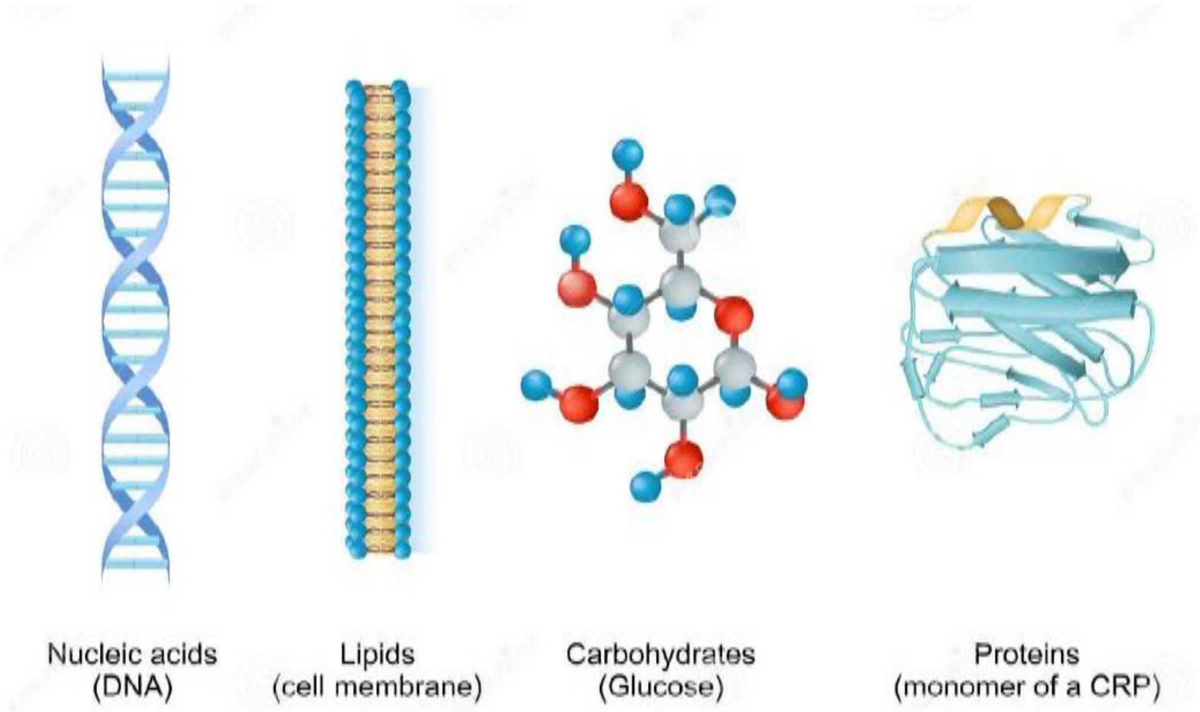


Figure 11. Structure of Major Biological Molecules (DNA, Lipids, Carbohydrates, and Proteins). (**Nelson *et al*., 2017**).

4.3.2. Biological Activities of Plant Extracts

4.3.2.1. Antibacterial Activity

Certain essential oils, such as that of *Thymus vulgaris* (thyme), exhibit strong antibacterial properties against both Gram-positive and Gram-negative bacteria.

4.3.2.2. Antioxidant Activity

Antioxidants such as those found in extracts of *Uncaria tomentosa* (cat's claw) or in curcumin neutralize free radicals and reduce cellular damage associated with oxidative stress.

4.3.2.3. Anti-inflammatory Activity

Ginger, known for centuries for its anti-inflammatory effects, acts by inhibiting the production of molecules responsible for inflammatory processes.

Chapter 05. Microbial Biotechnologies and Infectious Diseases

Introduction

Biotechnology refers to the set of techniques that exploit microorganisms, animal and plant cells, or their biological components for practical applications. Although the term itself is relatively recent, biotechnological practices date back to the origins of human civilization. Early applications were mainly empirical and focused on health-related issues, notably through the pioneering work of Louis Pasteur at the end of the nineteenth century.

The development of microbiology and immunology led, during the first half of the twentieth century, to the production of several vaccines against viral and bacterial diseases. These vaccines were based on inactivated microorganisms, detoxified toxins combined with immune adjuvants, or attenuated pathogens with reduced virulence. Such advances were made possible by parallel progress in tissue and animal cell culture techniques.

More recently, major breakthroughs resulting from advances in biochemistry, molecular biology, genetics, and immunology have profoundly transformed the diagnosis, prevention, and treatment of infectious and parasitic diseases. Among the most significant discoveries are:

- the determination of the structure of deoxyribonucleic acid (DNA) as the carrier of genetic information,
- the understanding of protein structure and biosynthesis,
- the development of recombinant DNA technology,
- and cell fusion techniques leading to the production of monoclonal antibodies.

Together, these discoveries form the foundation of modern medical and microbial biotechnology.

5.1. Diagnostics

In general, the diagnosis of infectious diseases whether viral, bacterial, or parasitic relies on either the detection of the pathogen itself or some of its components (direct diagnosis), or on the detection of elements of the immune response induced by the antigens carried by that pathogen (indirect diagnosis).

5.1.1. Direct Diagnosis

For direct diagnosis, the techniques most commonly used such as macroscopic or microscopic detection of the agent, staining methods including the use of fluorescent markers, culture, isolation, and characterization are gradually being replaced by new methods that are more specific and more sensitive.

Among these are immuno-enzymatic techniques, which are based on detecting antigenic determinants carried by the target agent using specific antibodies labeled with enzymes that reveal the formation of antigen–antibody complexes. These complexes are visualized through the enzymatic activity: the enzyme acts on an appropriate substrate, transforming it into a colored product that is easy to observe.

The specificity and sensitivity of these techniques can be greatly enhanced by the use of monoclonal antibodies, obtained by fusing a single antibody-producing cell (B-plasma cell) with a cell providing immortality and secretion ability (myeloma). Similarly, sensitivity can be increased by using a chemiluminescent marker instead of an enzymatic one.

More recently, advances in nucleic acid knowledge have enabled the development of direct diagnostic techniques based not on detecting the infectious or parasitic agent itself or its antigenic components, but on detecting its genetic material, DNA or RNA.

A decisive breakthrough in detecting genetic material from infectious agents is the technique known as Polymerase Chain Reaction (PCR). This method is extremely sensitive and can also be highly specific, depending mainly on the primers used.

All of these techniques some of which (hybridization, gene amplification) are still restricted to specialized laboratories and individual diagnostics are now available in the form of commercial kits, providing all necessary components for the reaction.

In the near future, their automation will make them suitable for large-scale diagnostics, and their simplification will make them accessible to non-specialized laboratories and even general practitioners.

5.1.2. Indirect Diagnosis

For indirect diagnosis, the techniques traditionally used to detect antibodies such as rapid and slow agglutination, complement fixation, hemagglutination inhibition, neutralization, etc. although still widely used, including as reference methods, are gradually being replaced by immuno-enzymatic techniques, which are often more specific and, above all, more sensitive.

Indirect diagnostic techniques are based, like direct diagnostic techniques, on detecting antigen–antibody complexes.

However, in indirect diagnosis, the complexes are formed between known antigens added to the reaction (viruses, bacteria, parasites, or their extracts) and antibodies potentially present in the sample being analyzed.

Once formed, these antigen–antibody complexes can be detected using different techniques, all based on enzyme-labeled reagents that participate in enzyme–substrate reactions producing visible colored products.

5.1.3. Contribution of Biotechnology to Indirect Diagnosis

Biotechnology has also improved indirect diagnosis by enabling the detection of cellular components of the immune response:

5.1.3.1. In vivo tests

These measure delayed-type hypersensitivity (intradermal reactions) and are useful for diagnosing many viral or bacterial infections and parasitic infestations. Example: Tuberculin skin test (IDR).

5.1.3.2. In vitro tests

These target different cellular elements of the immune system to detect their level of sensitization to specific antigens.

Example: Lymphocyte cytotoxicity tests.

5.2. New Therapeutic Approaches

First, biotechnology has enabled the production of medicines that could not be obtained or no longer could be obtained using traditional industrial methods (such as extraction from living organisms, often animals, which poses purification challenges and risks of contamination, especially viral contamination).

Examples include:

- Growth hormone
- Interferons

Their production became possible thanks to genetic engineering techniques, particularly gene cloning and the synthesis of therapeutic proteins.

Additionally, monoclonal antibodies, specifically designed to block the action of certain agents or receptors, are another major innovation produced through biotechnologies.

Furthermore, the availability of pure proteins in large quantities has enabled the development of many diagnostic kits that are simple to use, highly sensitive, and highly specific, thereby transforming the diagnosis and management of many diseases.

With the sequencing of the genomes of numerous living organisms especially the human genome the identification of new therapeutic targets has become possible. Likewise, the use of increasingly sophisticated biochips (microarrays), as well as the discovery of new molecular mechanisms, has greatly facilitated the search and development of entirely new classes of medicines.

5.2.3. Beyond Conventional Medicines: Emerging Therapies

Tested since the early 1990s in numerous trials on both animals and humans, gene therapy has given rise to the concept of *"DNA as a drug."*

This concept was validated by the first successful human treatments in the year 2000, with the landmark therapy of "bubble children" suffering from X-linked severe combined immunodeficiency (SCID-X1), conducted by Prof. Alain Fischer and his team at Necker Hospital in Paris.

Despite the challenges associated with ex vivo gene therapy such as the reinsertion of a normal gene using retroviral vectors into bone marrow cells and the potential risk of inserting the transgene near an oncogene, additional successful trials in three related diseases confirmed the potential of this therapeutic approach.

New techniques, such as gene surgery or exon skipping, have also emerged. These methods allow for the deletion of mutated exons and the restoration of the gene's reading frame. They have been successfully tested in animal models (mice and dogs) targeting the dystrophin gene in Duchenne and Becker muscular dystrophies, and are currently being evaluated in humans.

5.2.4. Cell Therapy and Regenerative Medicine

Cell therapies benefit from rapid advances in the study of stem cells, which can multiply indefinitely allowing large-scale production and differentiate into specialized cell types. These approaches pave the way for regenerative medicine, which aims to restore organ function by replacing damaged or destroyed cells.

However, clinical demonstration of these concepts on a large scale is still ongoing.

5.2.5. Predictive Medicine

Predictive medicine aims to prevent or even cure certain diseases using early detection of predisposition genes. These genetic markers are being identified thanks to large-scale analyses of thousands of genotypes across extensive patient cohorts, including individuals suffering from various forms of cancer.

5.3. Fight Against Doping and the Use of Narcotics

The use of doping substances has been part of the history of sports for decades. The increasing misuse of these substances in the 1960s led to the establishment of systematic controls and the implementation of strict regulations and sanctions. Today, monitoring the number of positive doping cases by sport provides an estimate of the extent of substance use across different athletic disciplines.

Three major classes of substances account for the majority of positive doping cases:

1. Anabolic agents (substances that promote protein synthesis and muscle growth)
2. Stimulants
3. Narcotics / recreational drugs

The type of substance used is closely linked to the athlete's objectives:

- Anabolic steroids are mainly found in strength sports.
- Stimulants are commonly used in speed-based sports.
- Endurance sports are targeted by substances that improve oxygen transport.

In many cases, the actual improvement in performance is not scientifically proven, yet athletes still use these products, exposing themselves to serious health risks.

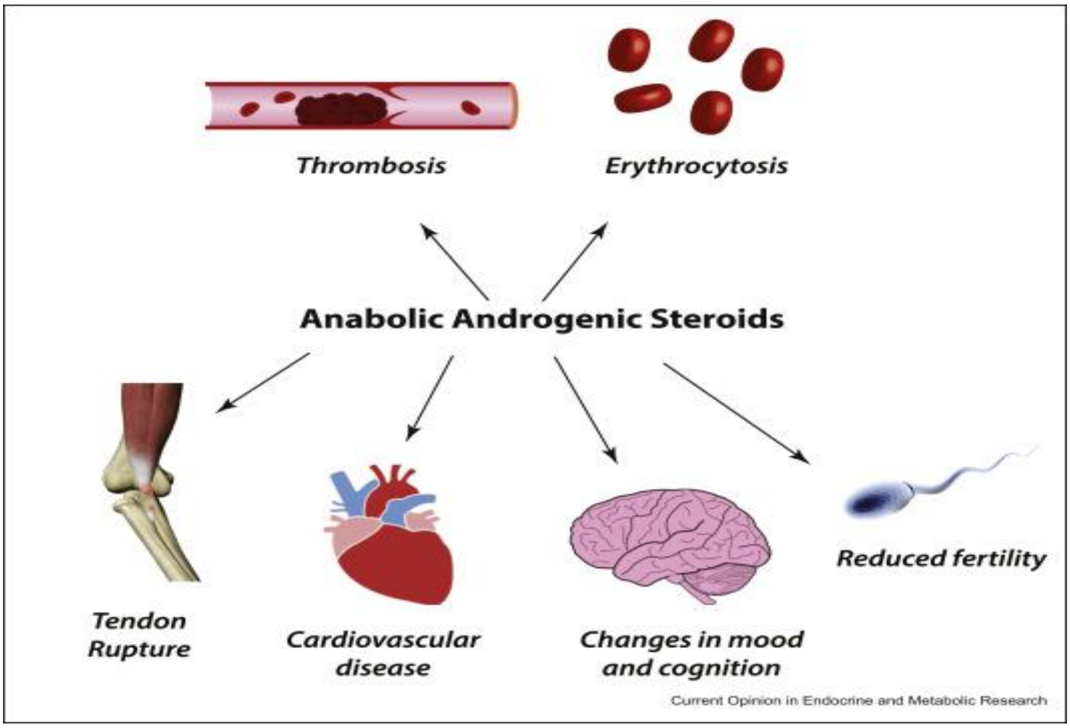


Figure 12. Physiological Effects of Anabolic Steroids in Athletes. (**Grant *et al.*, 2024**).

5.3.1. Doping Substances

1. Substances increasing muscle strength

- **Anabolic steroids**

These are usually derivatives of testosterone, such as nandrolone. They stimulate protein synthesis and lead to significant muscle mass increase.

- **Growth hormone (GH)**

Another category of performance-enhancing molecules that increases overall body strength and muscle development.

2. Substances improving oxygen transport

- **Erythropoietin (EPO)**

This natural hormone stimulates the production of red blood cells, thereby enhancing oxygen supply to the muscles.

Risks include:

- Increased blood viscosity
- Higher risk of heart attack, stroke, or sudden death

3. Substances increasing alertness or reducing fatigue

These include psychotropic and psychostimulant substances, such as:

- **Psychostimulants**

Amphetamines Act on mental processes (desire, focus, reaction speed), increase vigilance, reduce fatigue, and suppress appetite.

- **Narcotics and recreational drugs**
  - Heroin

- Morphine
- Methadone
- Cocaine
- Cannabis
- Caffeine

These act on the brain and the central nervous system, altering perception, mood, and behavior. Some are euphoric agents.

5.3.2. Methods for Combating Doping

To fight against doping effectively, it is necessary to detect even the smallest traces of prohibited substances in athletes. This requires:

- Highly sensitive and specific analytical techniques
- The ability to detect molecules present in extremely low quantities (sometimes less than one nanogram, i.e., one billionth of a gram)

Modern anti-doping laboratories continuously develop more advanced technologies to stay ahead of new doping strategies used by athletes and trainers.

However, detection remains challenging because:

- Substances may be used in micro-doses
- Some molecules disappear quickly from the body
- Athletes may use masking agents or sophisticated "doping cycles"

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