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Faculty of Exact Sciences

Sciences of Matter Common Core

Courses for first-year undergraduate students in sciences of matter

Biotechnology

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Preface

Biotechnology is a field that uses biological systems, organisms or their derivatives to develop and improve products and processes in a variety of applications, from medicine to agriculture to environmental management. It harnesses the capabilities of living organisms, such as bacteria and enzymes, to make valuable advances in a wide range of industries.

In this course addressed to first-year undergraduates in sciences of matter, we will present the fundamental concepts and major technological advances in the field of biotechnology. The course is divided into three chapters. The first chapter serves as an introduction to biotechnology, where we will briefly explore its historical development, its various fields of application, and the tools and materials commonly used. The second chapter, entitled "chemical biotechnology", examines the role of biotechnology in the chemical sciences, focusing on biocatalysts, the regulation of DNA structure, biomanufacturing and the use of plant cells. The final chapter is devoted to environmental biology, focusing on the use of biotechnological processes in the context of bioremediation.

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Chapter 1: Introduction to Biotechnology

1.1. Introduction

Biotechnology refers to the creative and innovative use of biological processes, living organisms or their components to develop products, technologies, and solutions that benefit society. It encompasses a wide range of scientific and technical disciplines, from DNA manipulation and microbial fermentation to tissue culture and genetic engineering. By exploiting the natural mechanisms of life, biotechnology aims to solve complex problems in areas such as medicine, agriculture, the environment and industry, paving the way for significant and innovative advances.

The term "biotechnology" has its origins in two Greek words: "bios" meaning "life" and "technê" meaning "art" or "technique". Thus, the word "biotechnology" can be interpreted as the application of technical approaches to life-related processes. The modern concept of biotechnology emerged in the twentieth century as scientists began to understand more about biological mechanisms and to exploit them to solve various scientific and industrial problems. Since then, biotechnology has become an ever-evolving multidisciplinary field with a significant impact on many aspects of our daily lives.

1.2. History and development of biotechnology

Biotechnology is a field that has evolved over the millennia, from traditional practices such as selective breeding to deliberate genetic manipulation to improve crops and livestock. The Green Revolution* harnessed this genetic knowledge to increase crop productivity through improved natural selection. Today, genetic engineering directly modifies plants and animals to create new varieties adapted to unfavorable conditions. However, the environmental impact of genetically modified organisms remains controversial. Modern biotechnology combines genetics, molecular biology, computer science and nanotechnology to influence different areas such as agriculture, human health, and medicine. Despite its complexity, there are efforts to unify the approach to biotechnology while recognizing its expansion into other related human activities.

* The *Green Revolution* refers to a series of technological advancements and agricultural practices that took place during the mid-20th century, primarily between the 1940s and 1970s. Its main goal was to increase agricultural productivity and food production in order to address the growing global food demand, alleviate hunger, and reduce poverty in developing countries.

The history of biotechnology is marked by a number of important milestones that have led to the development and diversification of this field. Here are some of the most significant milestones (**Figure 1.1**):

i. *Domestication of plants and animals (10,000 BC - 4000 BC):* Agriculture and animal husbandry were crucial stages in the development of biotechnology. Humans began to select and cultivate plants for specific characteristics, such as fruit size or disease resistance. Similarly, animal breeding has made it possible to develop breeds of animals with desirable traits. These selective breeding processes form the basis of genetic improvement.

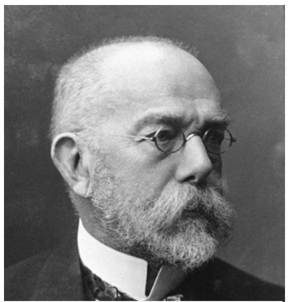
Gregor Mendel
1822-1884

ii. *The beginning of fermentation (6000 BC):* Fermentation, a biological process in which microorganisms convert substrates into useful products, was used to produce foods such as bread, beer, and wine. Fermentation was an early form of exploiting micro-organisms for industrial applications.

Louis Pasteur
1822-1895

iii. *Discovery of genetics (1865-1900):* Gregor Mendel's work on the heredity of peas laid the foundations of modern genetics. Mendel established the laws of heredity, showing how characteristics are transmitted from one generation to the next. This laid the foundations for understanding gene transmission and paved the way for molecular biology.

iv. *Development of microbiology (late 19th - early 20th century):* The discoveries of scientists such as Louis Pasteur and Robert Koch were fundamental to our understanding of micro-organisms and their role in disease and industrial production. Pasteur proved that micro-organisms are responsible for the fermentation and degradation of organic matter. Koch established the



Robert Koch
1843-1910

principles of medical microbiology and developed techniques for isolating and identifying specific pathogenic bacteria.

v. *The evolution of recombinant DNA (1970s):* The development of recombinant DNA technology enabled scientists to directly manipulate genes by inserting them into host organisms such as bacteria. This major advance paved the way for the large-scale and more precise production of recombinant proteins, such as human insulin.

v. *Sequencing of the human genome (2000):* The Human Genome Project* completed in 2003, resulted in the determination of the complete sequence of the human genome. This provided a detailed map of all human genes and laid the foundations for genetic research, personalized medicine, and the understanding of hereditary diseases.

vi. *Advances in personalized medicine† (21st century):* Personalized medicine has been made possible by a growing understanding of genomics. Genetic testing can help predict susceptibility to disease, personalize treatment, and avoid undesirable side effects.

vii. *Emergence of synthetic biology‡ (2000s):* Synthetic biology combines biology and engineering to design and build new biological systems with specific functions. This ranges from designing micro-organisms to produce biofuels to creating bacteria capable of breaking down plastics.

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* The *Human Genome Project* (HGP) was an international research initiative aimed at mapping and sequencing the entire human genome, which is the complete set of DNA in the human body. The project was initiated in 1990 and completed in 2003. It was a collaborative effort involving scientists, researchers, and institutions from around the world.

† *Personalized medicine*, also known as precision medicine, is an approach to medical care and treatment that takes into account individual variability in genes, environment, and lifestyle. It aims to tailor medical decisions, interventions, and therapies to the specific characteristics of each patient, with the goal of achieving better outcomes and minimizing adverse effects.

‡ *Synthetic biology* is an interdisciplinary field of science and engineering that focuses on designing, constructing, and modifying biological systems or organisms using engineering principles, genetic manipulation, and advanced molecular techniques. It seeks to create new biological functions, organisms, or systems that do not exist in nature, or to redesign existing biological systems for specific purposes. Synthetic biology combines elements of biology, engineering, chemistry, and computer science to create novel biological entities and applications.

These steps illustrate how successive discoveries and advances have shaped the field of biotechnology, from the earliest forms of exploitation of living organisms to the precise manipulation of genes and the design of innovative biological systems.

1.3. Areas of application of biotechnology

To make the complexity of biotechnologies more accessible and organized, a color-coded classification has been developed and adopted to order the diversity of existing biotechnologies. The classification is available in five distinct colors, each corresponding to a specific sector (**Figure 1.2**):

Red Biotechnologies (Health): This sector encompasses the biomedical and pharmaceutical applications of biotechnologies. It includes the production of drugs, vaccines, gene and cell therapies to treat various diseases. Red biotechnologies have revolutionized medicine by enabling more targeted and personalized treatments, such as gene therapies aimed at correcting genetic anomalies at source.

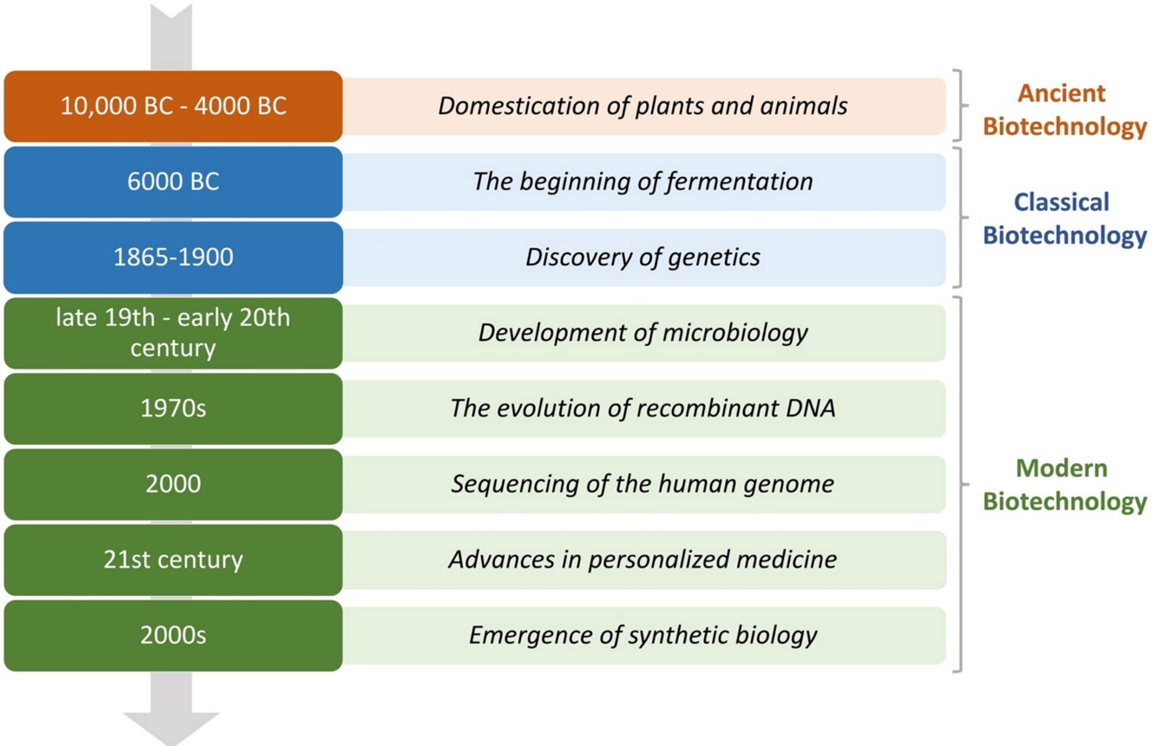


Figure 1.1. the important milestones in the evolution of biotechnology.

Green biotechnologies (Agriculture and Food): Green biotechnologies focus on the genetic manipulation of plants and crops to improve their agronomic and nutritional characteristics. This includes the creation of genetically modified organisms to increase resistance to diseases and pests, and to improve yields. However, this category also raises questions about food safety and the dissemination of modified genes.

White biotechnologies (Industry): White biotechnologies mainly concern the chemical industry and the production of materials. They use genetically modified organisms or enzymes to manufacture chemicals, biofuels, biodegradable plastics, and other industrial products. White biotechnology contributes to more ecological and sustainable production.

Gray biotechnologies (Environment): Gray biotechnologies focus on environmental solutions, including contaminant degradation and depollution. They include bioremediation methods to break down organic and inorganic pollutants present in water, soil, and air. Genetically modified micro-organisms can be used to break down toxic pollutants, helping to restore the environment.

Blue Biotechnologies (Marine Biodiversity): Blue biotechnologies exploit the biodiversity of marine ecosystems for a variety of applications. This includes the search for new drug molecules from marine organisms, the development of sustainable fishing and aquaculture techniques, and the use of marine micro-organisms for industrial and environmental applications.

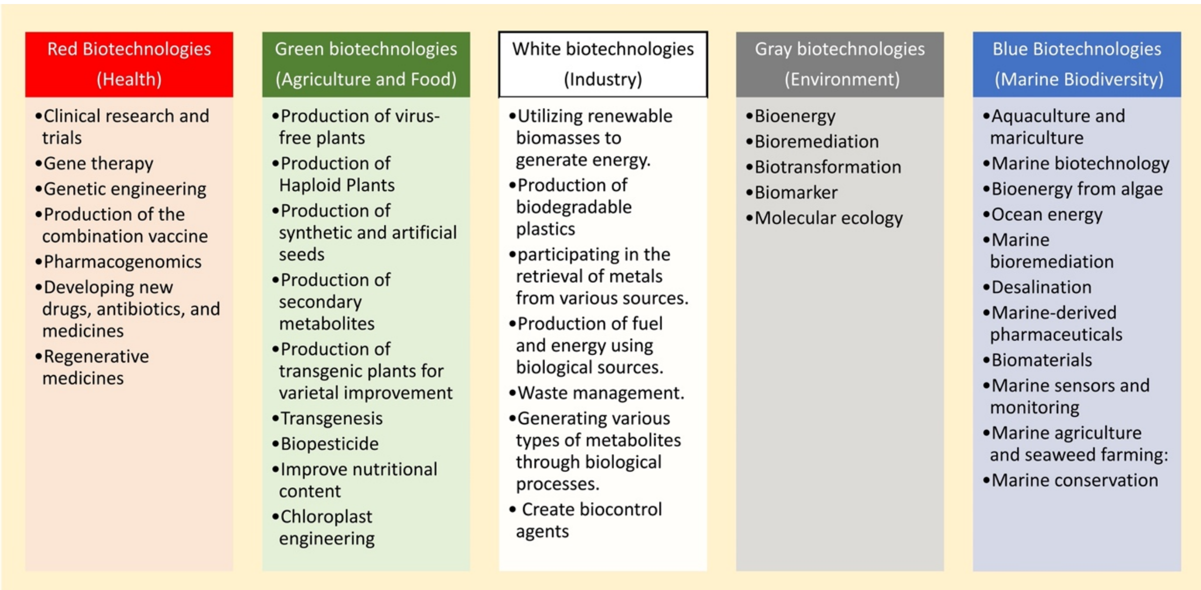


Figure 1.2. Areas of application of biotechnology by color.

1.4. Basic tools and techniques used in biotechnology

Biotechnology is a multidisciplinary field that involves the use of biological, chemical and physical processes to develop beneficial products and applications. Here are some of the basic tools and techniques used in biotechnology:

1.4.1. Recombinant DNA

The history of recombinant DNA dates back to the 1970s when researchers such as Herbert Boyer and Stanley Cohen used restriction enzymes to insert foreign DNA into bacterial plasmids, creating the basis for recombinant DNA technology.

Recombinant DNA is an artificial DNA molecule created in the laboratory by combining DNA sequences from two different organisms (**Figure 1.3**). This technique makes it possible to produce genetic sequences that are not naturally found together in a genome. Recombinant DNA is used to introduce new genetic sequences into organisms, which can modify existing characteristics or induce the expression of new characteristics.

The production of recombinant DNA begins with the identification of a DNA sequence of interest in one organism, then this sequence is introduced into another organism lacking this sequence. The steps include cloning the DNA, using polymerase chain reactions (PCR)* to amplify the sequences, using expression vectors, and creating recombinant proteins.

The applications of recombinant DNA are vast, ranging from drug production to agriculture. It is used to produce recombinant proteins, such as insulin and other drugs, using bacteria, yeast, insect cells, or mammalian cells. It can also be used to create transgenic plants that are resistant to disease and herbicides and to produce vaccines.

* The polymerase chain reaction (PCR) is a widely used molecular biology technique that allows the amplification of a specific segment of DNA. It was invented by Kary Mullis in 1983 and has since become a fundamental tool in a variety of fields, including genetics, forensics, medicine and biotechnology. PCR involves a cyclic process that replicates a targeted DNA sequence exponentially, generating millions of copies from a small initial amount of DNA. The process relies primarily on an enzyme, DNA polymerase, which synthesises new strands of DNA from a DNA template.

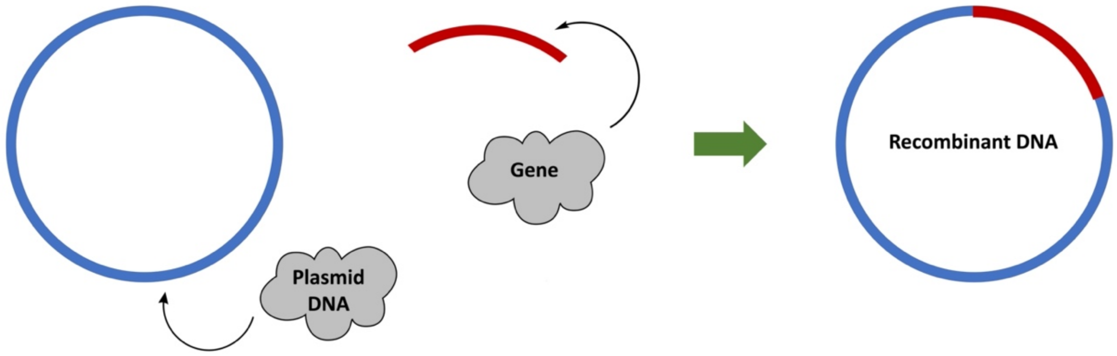
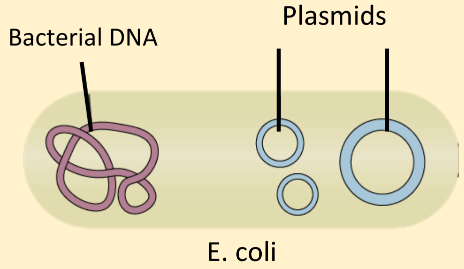


Figure 1.3. schematic diagram of recombinant DNA.

Plasmid-mediated bacterial competition and bacteriocins: Paving the way for biotechnological innovation

Bacteria compete closely for resources and space, resulting in the secretion of toxins known as bacteriocins. These substances, exemplified by nisin from Lactococcus lactis and colicins from E. coli, eliminate neighbouring bacteria. Colicins, produced by E. coli, include a range of types such as E1 and M, which use methods such as membrane perforation and nucleic acid degradation to disrupt neighbouring cells. It is essential that the producing cells escape the danger by producing immune proteins that neutralise the toxins. The ability to produce colicin is linked to a plasmid, a circular piece of DNA found in bacterial cells and certain eukaryotic cells. These plasmids contain the genes for colicin, immunity, replication, etc. By modifying these plasmids, scientists have opened the way to new advances in molecular biology, including the ability to express foreign proteins in bacteria for biotechnological applications.



The production of insulin using recombinant DNA is an emblematic example of the application of biotechnology in drug production. The general steps involved in producing insulin by this method are as follows (**Figure 1.4**):

i. *Selection of the insulin gene:* The first step is to identify and isolate the gene that codes for human insulin. This gene is then amplified by PCR to obtain sufficient DNA.
ii. *Construction of an expression vector:* An expression vector is a segment of DNA that can carry the gene of interest into a host cell. In this case, the vector will be designed to integrate the insulin gene and guarantee its expression. Regulatory sequences are added to ensure that the gene is expressed in a controlled manner.
iii. *Cellular transformation:* The expression vector containing the insulin gene is introduced into suitable host cells. *Escherichia coli* (E. coli) bacteria are often used because they reproduce rapidly and are easy to grow.
iv. *Expression and production:* Once the cells have been transformed, the insulin gene is expressed and the recombinant insulin protein is produced. However, the recombinant insulin produced in bacteria is generally in the form of an insoluble inclusion called an inclusion body.
v. *Recovery and purification:* The cells are harvested and the insulin-containing inclusions are extracted. The insoluble proteins are then solubilized and the recombinant insulin is purified using chromatographic and filtration methods.
vi. *Refolding:* Purified recombinant insulin is generally in the form of unfolded polypeptide chains. It must be folded correctly to be functional. This refolding process is carried out by controlling the pH, temperature, and reducing agent conditions.
vii. *Quality control:* Recombinant insulin samples are subjected to quality control tests to verify their identity, purity, biological activity, and safety.
viii. *Formulation and Packaging:* Once recombinant insulin is confirmed to be of high quality and safe, it is formulated into appropriate preparations (e.g. injectable solutions) and packaged for distribution.

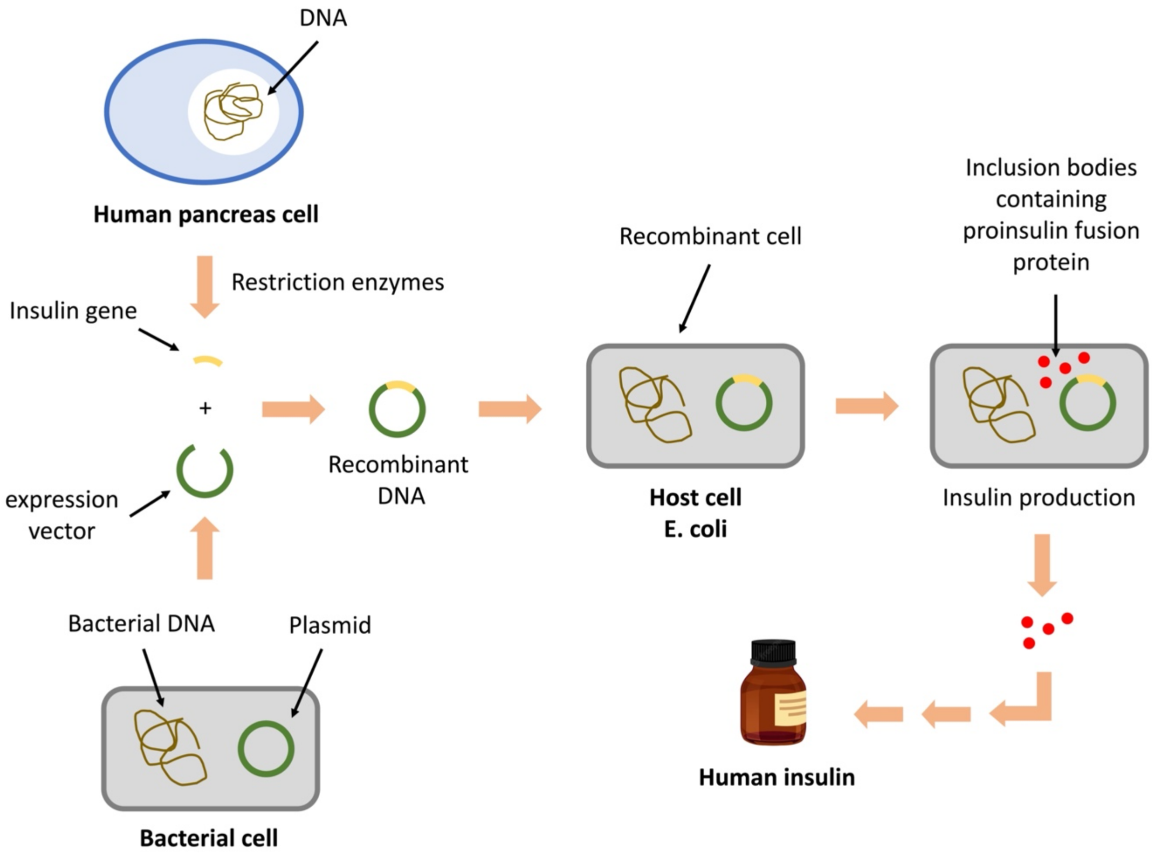


Figure 1.4*. production of human insulin by recombinant DNA.

1.4.2. Cloning

Cloning in biotechnology refers to the creation of genetically identical copies of an organism, a DNA molecule, or a part of DNA. The cloning process involves the precise reproduction of a DNA sequence, creating identical copies of the original genetic material. Cloning can be carried out at different levels, such as the cellular, molecular, or whole organism. As an example, **Figure 1.5** illustrates the process of animal cloning.

The applications of cloning in biotechnology are varied and have important implications in various fields. Here are some of these applications:

i. *Cloning whole organisms:* Animal and plant cloning is used to reproduce genetically identical individuals. This can be used for research purposes, for the

* Restriction enzymes are bacterial proteins that cut DNA at specific sequences. They are used in molecular biology for DNA manipulation, gene vector creation and other applications. Their ability to cut DNA at precise sites is essential for genetic research and biotechnology.

preservation of endangered species, and even for the production of high-quality meat from cloned animals.

ii. *Gene cloning (Recombinant DNA):* Gene cloning involves the production of identical copies of a particular gene. These cloned genes can then be studied in detail to understand their function, regulation, and involvement in disease.

iii. *Gene therapy:* Gene therapy involves the introduction of a specific gene into an individual's cells to treat or prevent a genetic disease. Gene cloning is essential to produce gene therapy vectors that carry the therapeutic gene.

iv. *Genetic engineering of plants:* Cloning genes in plants makes it possible to modify their DNA to introduce specific characteristics, such as disease resistance, drought tolerance, or improved nutritional quality.

v. *Improved agricultural yields:* The cloning of high-quality agricultural plants can be used to produce high-yielding crops of consistent quality.

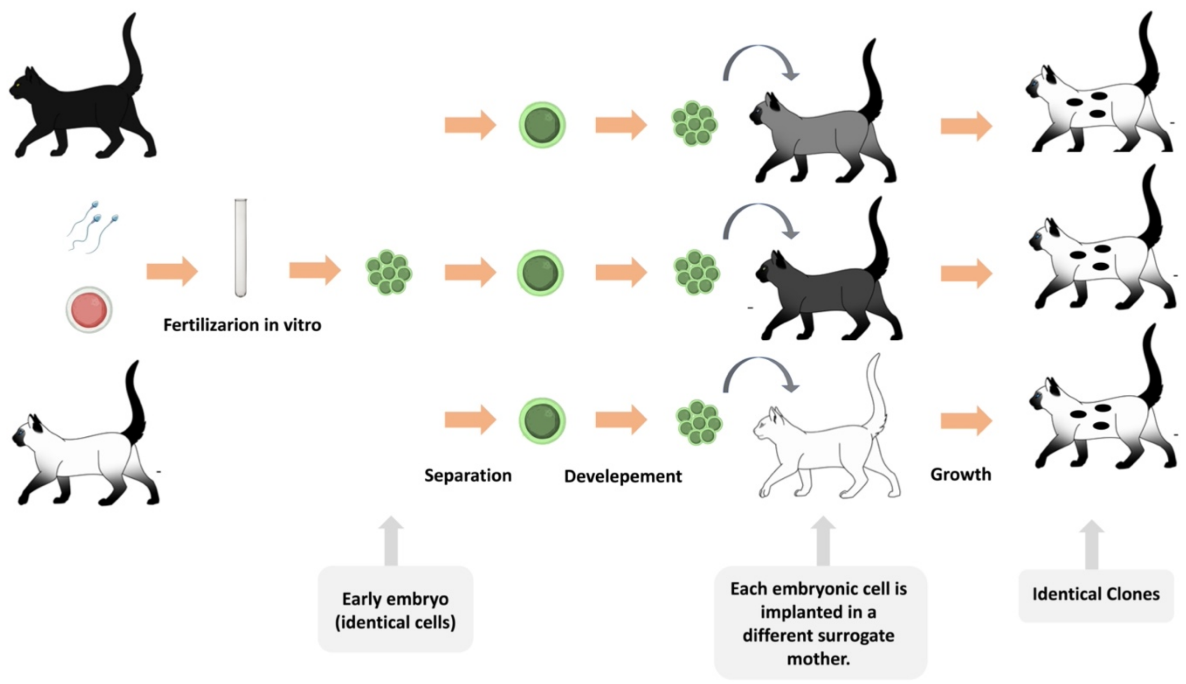


Figure 1.5. The process of animal cloning.

1.4.3. DNA sequencing

DNA sequencing, which consists in determining the order of the nucleotides that make up DNA, is a common procedure in biology and biotechnology laboratories. The technique is based on the advances of the last thirty years in our understanding of DNA replication mechanisms.

Sequencing makes use of specific enzymes called DNA polymerases. These enzymes have the ability to synthesize a complementary strand of DNA based on a template strand. The process involves the addition of deoxyribonucleotides (dNTP, for deoxyNucleotide TriPhosphate). Slightly different variants of dNTPs, known as dideoxyribonucleotides (ddNTPs), are used for sequencing. DdNTPs differ from dNTPs in the absence of a particular OH group (**Figure 1.6**). When a DNA polymerase uses a ddNTP instead of a dNTP, it can no longer add further nucleotides to the chain being synthesized, therefore stopping the reaction.

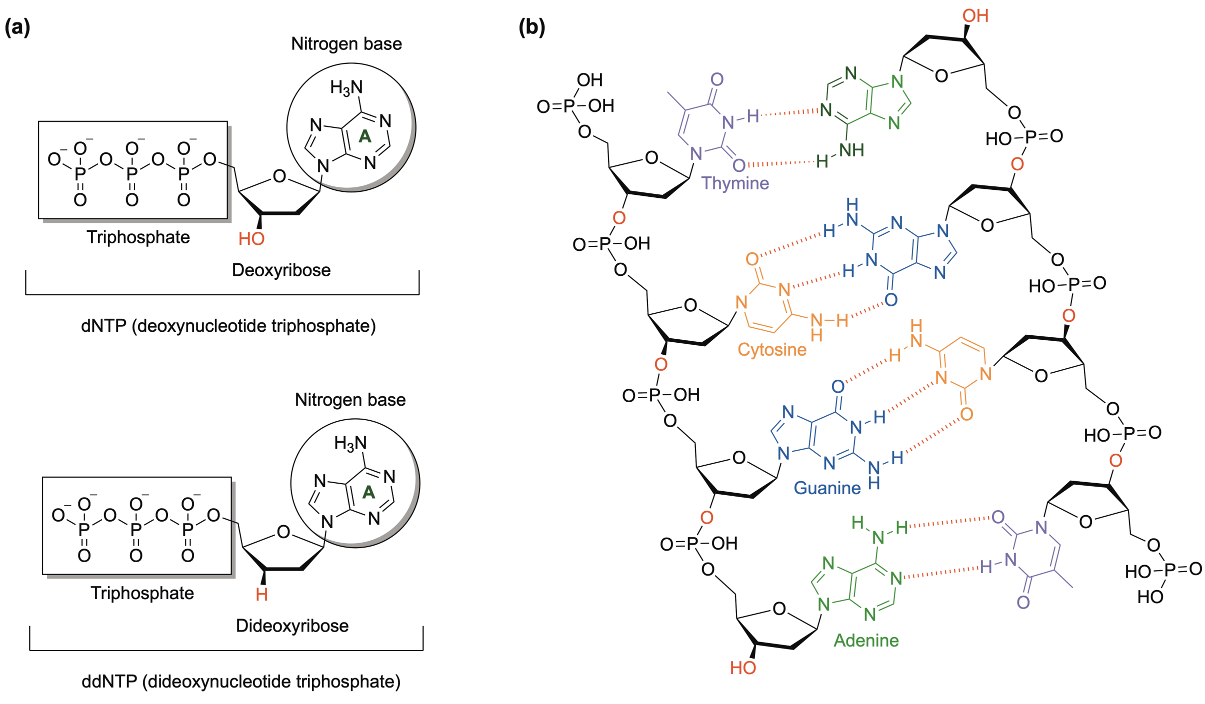


Figure 1.6. (a) Molecular structure of dNPT and ddNTP and (b) association of the nucleotides of two DNA strands by hydrogen bonding.

Sequencing techniques are based on these principles. Here's how it works: a DNA polymerase constructs the complementary strand of the DNA to be sequenced. The reaction medium contains a large quantity of dNTP and a small proportion of a specific ddNTP (ddATP, ddCTP, ddGTP or ddTTP). At a random moment, DNA polymerase will add a ddNTP to the chain being synthesized, causing it to stop.

For example, if the reaction medium contains a low proportion of Guanine dideoxyribonucleotide (ddGTP), at the end of the reaction a series of DNA strands of different lengths is obtained, each stop corresponding to the insertion of a ddGTP (meaning the presence of a Cytosine in the sequenced DNA strand, due to base complementarity). This process is repeated for ddATP, ddCTP and ddTTP.

The sequence is then read by migrating these fragments on a gel to separate them according to size. Each level of the gel corresponds to a specific size of DNA fragment. To read the four nucleotides of the DNA, the fragments from the four reactions (ddATP, ddCTP, ddGTP and ddTTP) are migrated separately.

It's important to note that a DNA polymerase needs a short fragment of DNA called a primer to initiate DNA strand synthesis. This primer is a 15-25 nucleotide oligonucleotide, complementary to a known DNA sequence, located just before the region to be sequenced.

The traditional method, still used in some manual sequencing, involves electrophoresis on acrylamide gel, which takes two to four hours. Then, to visualize the DNA fragments, they can be marked with a fluorescent molecule (on the primer) or with radioactive nucleotides. The sequence is read by observing the fluorescence or by exposing a photographic film to the gel (where dark bands appear where DNA was present).

Most of today's sequencing is carried out using automated sequencers. These machines perform the sequence reactions and read them by marking the DNA fragments with fluorescent markers. After the reaction, fragment size is determined chromatographically, by detecting the fluorescence emitted. The latest systems can even read all four nucleotides using a single chromatography column.

The result is presented in the form of curves showing the fluorescence detected and its interpretation in terms of nucleotides. Automatic sequencers offer many advantages, including automation, speed and low cost once the initial investment in the machine has been amortized. They can also read several hundred nucleotides with great accuracy, unlike manual methods which are limited to around 200-300 nucleotides.

Figure 1.7 shows a general diagram for DNA sequencing based on fluorescent ddNTPs.

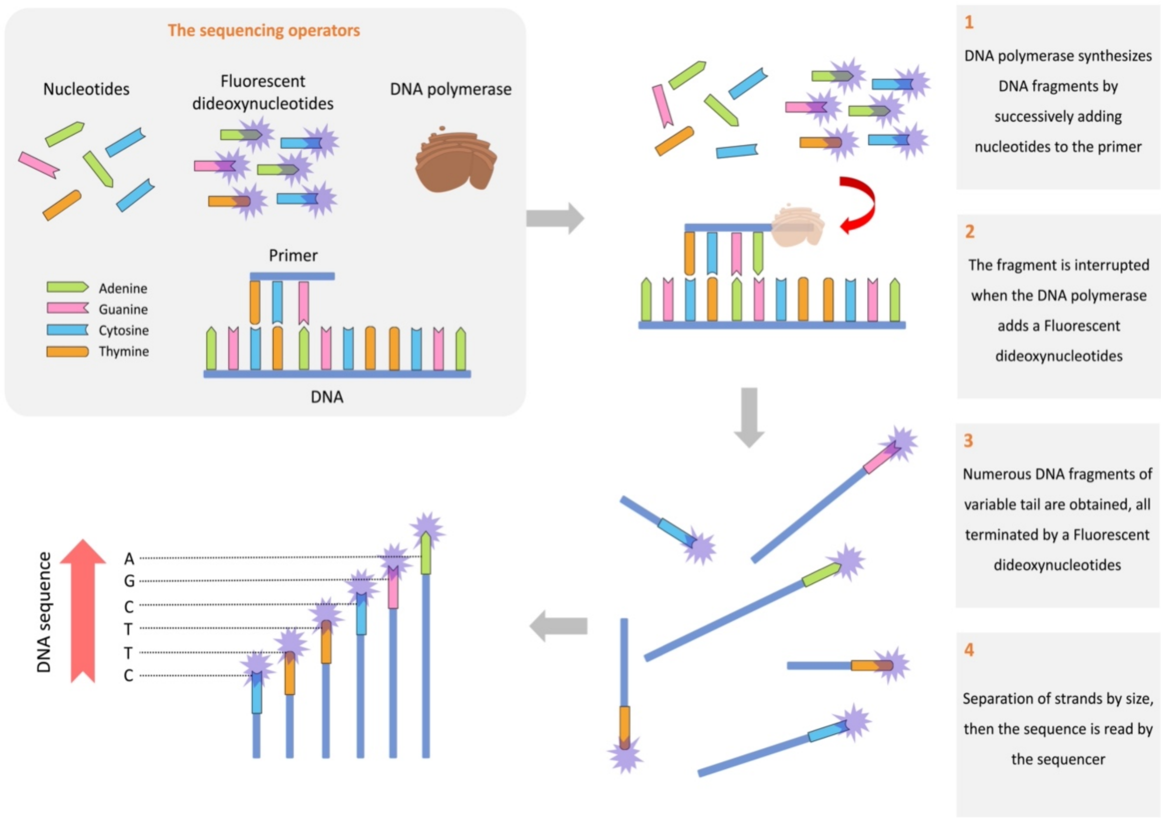


Figure 1.7. General diagram of DNA sequencing using fluorescence.

1.4.4. Cellular culture

Cell culture is an essential part of biotechnology. The origins of this discipline can be traced back to Roux's pioneering work in 1880. Today, we can isolate, proliferate, and control virtually any class of cell in cultured environments. At the same time, we are able to obtain various products from these cultured cells, and even implant them to treat certain pathologies.

Cell culture involves the reproduction and survival of cells in an artificial environment. It has a wide range of applications, such as the production of monoclonal antibodies, vaccines, enzymes, hormones, and growth factors. Progress in this field has paved the way for regenerative medicine, with its emphasis on cell differentiation. This depends on the cell's internal program and environmental conditions. Understanding these processes makes it possible to manipulate cells in culture.

Cell culture requires sterile conditions in a dedicated laboratory to avoid contamination. Environmental monitoring, both macroscopic and microscopic, is crucial. Limited space in the culture vessel and diminishing nutrients make cell passage* essential. Passage criteria depend on cell concentration, pH, and time since the last passage.

Sometimes, it is necessary to freeze and store cells, which requires post-trauma cell viability tests. Cells lose their ability to proliferate and survive over time due to physiological or pathological changes, similar to apoptosis and cellular aging. Identifying apoptosis and measuring cell viability are crucial in cell culture studies.

Maintaining purity in cell cultures is crucial. The various origins of contamination, such as unsanitary environments, non-sterile equipment, airborne microorganism-carrying particles, and neglected incubators, can lead to unwanted alterations. Specially developed aseptic technique establishes a protective barrier between external micro-organisms and the sterile cell medium. Here are a few tips to help prevent contamination:

i. Before and after handling cell cultures, wash hands with an odorless soap. For added protection, latex gloves are highly recommended.
ii. Work surfaces must be meticulously cleaned with 70% ethanol to remove potential contaminants.
iii. Glassware intended for use must undergo prior sterilization, ensuring an environment free from undesirable factors.
iv. All operations relating to culture media, such as preparation and adjustment, must be carried out in a laminar flow cabinet, to prevent any unwanted intrusion.
v. It is essential to maintain strict separation between sterile solutions and cultures in the work area, avoiding any risk of contact and cross-contamination.

* *Cell passage* refers to the process of subculturing or transferring cells from one culture vessel to another. In cell culture, living cells are grown in controlled environments outside of their natural setting, often in laboratories. As cells grow and multiply, they can become too crowded in their initial culture vessel, which can lead to a decrease in cell viability and overall health. To prevent this, researchers perform cell passage to transfer a portion of the cells into a new culture vessel with fresh growth medium. This helps maintain an optimal cell density, provides sufficient nutrients and space for the cells to grow, and allows for the continuation of experiments or the production of desired cellular products

vi. Medium and culture vials require careful handling, avoiding any contact with the neck area which could potentially lead to contamination.

vii. Distinguishing between clean and contaminated material is crucial, requiring separate storage to avoid confusion or unwanted transmission.

viii. Regular maintenance and disinfection of tools and equipment is a fundamental measure in maintaining a contaminant-free environment.

ix. Once experiments have been completed, appropriate disposal of laboratory waste is necessary. As a further precaution, final sterilization may be undertaken using a UV lamp.

Cell culture is a complex process involving the growth and multiplication of cells in a controlled artificial environment. Here is a general procedure for culturing cells in the laboratory (**Figure 1.8**):

i. Select the appropriate cells for your study. Different cell types require specific culture conditions.

ii. Culture medium: Prepare a suitable culture medium. This medium contains essential nutrients such as salts, sugars, amino acids, and vitamins required for cell growth.

iii. Choose a suitable culture surface, such as Petri dishes, flasks, or culture plates. These surfaces can be treated to promote cell adhesion.

iv. Inoculation: Transfer cells from a previous culture to the new medium. This can be done by adding a small number of cells to the medium, often called "seeding".

v. Place culture surfaces in an incubator at a constant temperature appropriate to the cell type (usually around 37°C for human cells). The incubator also provides a humidified atmosphere containing 5% CO_2 to maintain optimal conditions.

vi. Medium change: Periodically, the culture medium needs to be changed to provide fresh nutrients to the cells. This may vary according to cell type and growth rate.

vii. Monitoring: Regularly examine cultures under the microscope to assess cell growth, morphology, and health.

viii. Passage: When cells reach an appropriate density, they should be detached from the culture surface and reseeded in new containers with fresh medium. This process is called passage and helps prevent overcrowding and cell stress.

ix. Contamination tests: Regularly check cultures for bacterial, fungal, or viral contamination. In the event of contamination, the culture must be discarded and restarted from an uncontaminated source.

x. Experiments and applications: Use cell cultures to perform specific experiments according to your research objectives, such as drug testing, cell biology studies, etc.

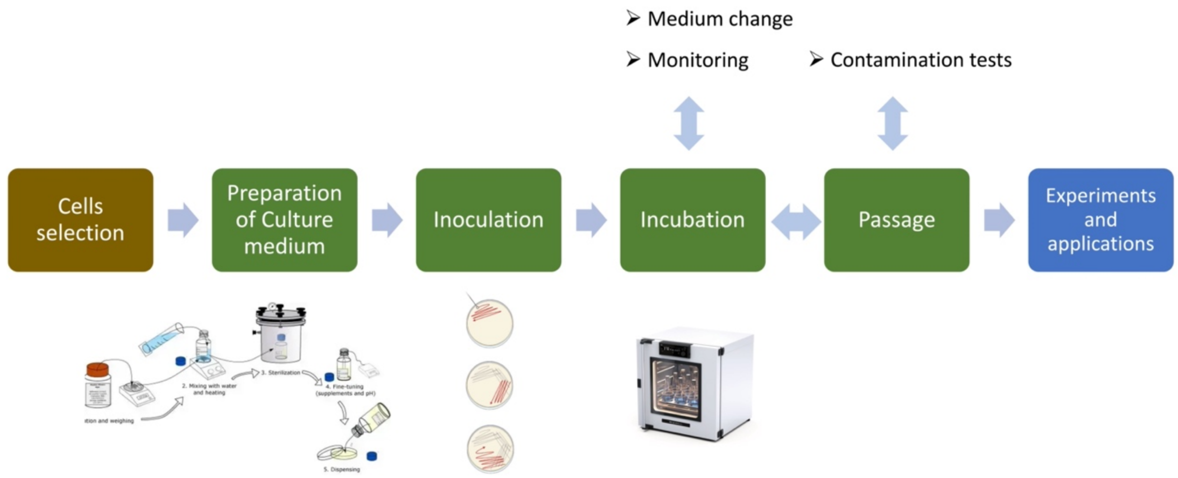


Figure 1.8. General procedure for culturing cells in the laboratory.

Cell culture is fundamental to cellular and molecular biology, and offers a wide range of applications. It provides model systems for examining normal cell physiology, exploring interactions with pathogens, probing drug and toxic effects, studying mutagenesis and carcinogenesis, and facilitating drug screening and development. Cell culture enables the transformation of normal cells into cancer cells, the replication of viruses for vaccine production, research into the toxicity of products, the production of vaccines, the generation of genetically modified proteins such as monoclonal antibodies and insulin, and the exploration of gene therapies by modifying cells before their introduction into the patient. It is also used for genetic research, the detection of chromosomal abnormalities in fetuses, and the evaluation of drug dosage and efficacy.

Cell cultures, performed in multi-well plates, play a central role in the pharmaceutical industry, demonstrating their versatility and importance within scientific and medical research.

1.4.5. Fermentation

Fermentation is a natural process that transforms sugars into valuable products. Its roots go back to around 10,000 BC, with the emergence of the first civilizations in the Middle East. Despite the absence of modern scientific knowledge, ancient man exploited fermentation for food preservation, creating products such as bread, wine, and beer. Later, Louis Pasteur established a link between fermentation and micro-organisms, paving the way for biotechnological applications. Today, fermentation remains vital to a variety of industries, meeting the food demands of our growing global population and illustrating one of the earliest examples of mankind's use of biotechnology.

Fermentation is a remarkable process that enables certain micro-organisms to produce energy without depending on oxygen, highlighting an alternative way of obtaining vitality. This anaerobic pathway, known as fermentation, relies on the use of sugars to fuel living cells, thus producing energy without the need for oxygen. The type of fermentation that ensues is determined by the micro-organisms involved and the by-products that result. There are two main categories: lactic fermentation and alcoholic fermentation. In lactic acid fermentation, lactose is used as the starting sugar, and lactic acid bacteria such as Lactobacillus derive energy from it. This process produces lactic acid, ATP, and water, and therefore plays an essential role in food preservation due to lactic acid's inhibitory effect on undesirable micro-organisms. Alcoholic fermentation uses glucose as the initial sugar and is carried out by yeasts, fungi, and certain bacteria. This form produces ATP, ethanol, carbon dioxide (CO_2), and water, the gas CO_2 giving the process its name due to its resemblance to boiling. These fermentation processes, exemplified by lactic acid fermentation and alcoholic fermentation, are not only fascinating, but also essential for a variety of valuable applications in industry and beyond.

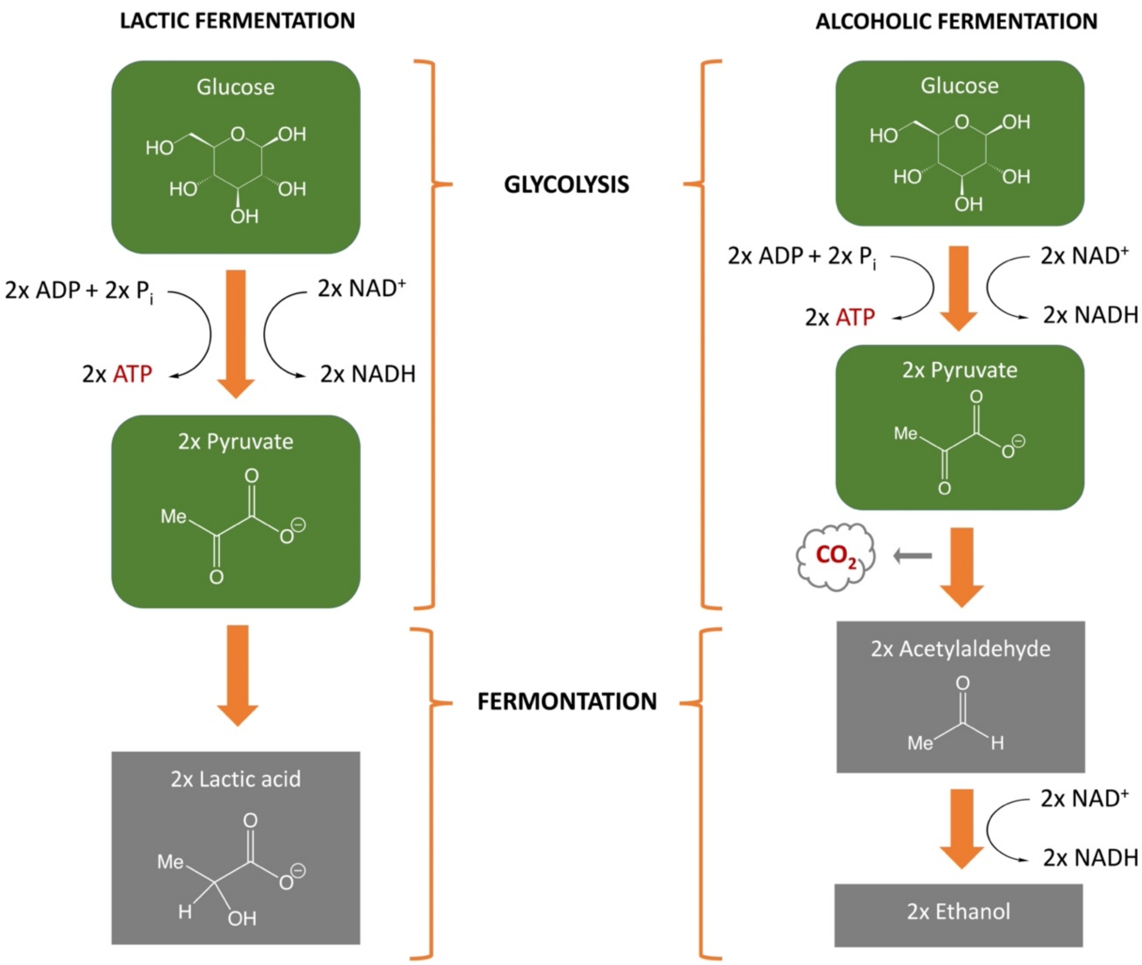


Figure 1.9. General scheme of lactic fermentation and alcoholic fermentation

Fermentation underpins many aspects of our food experiences, producing staples such as soft bread, flavorful yogurt, and tasty cheeses. Fermentation extends to health-oriented creations such as probiotics, and micro-organisms that promote intestinal well-being and inhibit the growth of pathogens by fermenting food in our digestive system. In addition, fermentation plays a transformative role in converting raw materials such as flour into desirable products, as demonstrated by the vital role of CO_2 gas in giving bread its characteristic texture and flavor. Beyond the food sector, the biotechnological potential of fermentation is remarkable. It forms the basis for the production of vital drugs such as antibiotics and antivirals, with pioneering examples such as penicillin being derived from microbial fermentation. Surprisingly, microbes also contribute to energy production, ethanol being one of the main results of microbial fermentation.

Derived from plants such as corn and sugar cane, these sugars fuel the fermentation process, ultimately producing ethanol, a renewable alternative to gasoline.

The manufacture of penicillin is an important example of the use of fermentation in biotechnology. Here are the main steps in this method:

i. Isolating the penicillin-producing bacterium: The first step is to isolate the bacterium that naturally produces penicillin. This bacterium generally belongs to the genus Penicillium, such as *Penicillium chrysogenum*.
ii. Preparing the culture environment: Penicillin-producing bacteria require a specific environment in which to grow. A suitable culture medium, rich in nutrients such as glucose, minerals, and vitamins, is prepared to support their growth.
iii. Culture medium inoculation: Isolated bacteria are introduced into sterile culture medium. This allows the bacteria to start growing and multiplying.
iv. Fermentation: The bacteria grow and multiply in the culture medium. As they grow, they naturally produce penicillin as a secondary metabolic product.
v. Penicillin extraction: Once the bacteria have produced a sufficient quantity of penicillin, the extraction process begins. The culture medium is treated to separate the bacteria from the penicillin-containing liquid.

1.5. Biomaterials

Biomaterials, which encompass a range of non-living materials, are attracting great research interest, with promising applications in a variety of industrial sectors. Their use is aimed at improving or partially or totally replacing tissues, organs, or corporal functions, while promoting quality of life, without negatively impacting the organism. They are used in fields such as prosthetics, diagnostics, therapy, and storage, based on their biocompatibility, mechanical integration with tissues, non-toxicity, and affordability. In tissue engineering, a biomaterial matrix facilitates cell adhesion, growth, and differentiation, while degrading harmoniously. Biomaterials have become mainstays of modern medicine, with applications ranging from organ implants and dental fillers to antimicrobial agents and drug carriers, paving the way for exciting innovations in the future.

The desired properties of biomaterials in medical applications encompass various factors. The integration of material properties, system nature, device architecture, and physiological specifications aims to treat, enhance, or replace tissue organs and bodily functions. Achieving these goals requires the combination of biological system features and material characteristics to attain the desired functional outcomes. Biocompatibility is a crucial aspect, ensuring that materials are acceptable within living organisms without causing harm or inducing immune responses. Host response, toxicity, mechanical properties, corrosion, wear, fatigue properties, and manufacturability also play significant roles. Ultimately, a biomaterial should exhibit the appropriate physical, chemical, and mechanical traits, stability, and ease of processing, sterilization, and accessibility while adhering to biocompatibility standards.

Biomaterials can be classified into several types according to their properties and applications. Some of the main types of biomaterials are listed below:

1.5.1. Metals and metal alloys

Metals and metal alloys play the role of precursors to biomaterials, as pioneering elements in the manufacture of implants. Among them, stainless steel occupies a dominant position in terms of volume and is still widely used in orthopedic surgery, due to its advantageous mechanical properties. Titanium is of particular importance, being widely used in orthopedic surgery and for the design of dental implants, as well as in devices such as pacemakers and implantable pumps. Its remarkable biocompatibility, which encourages spontaneous bone adhesion, is one of its major advantages. Shape memory alloys are an intriguing variant in this category, alongside other alloys such as cobalt, chromium, molybdenum, and tantalum.

Metals and metal alloys are used as biomaterials because of their unique properties, but they also have certain limitations. Here are the advantages and disadvantages of using metals and metal alloys as biomaterials:

Advantages:

i. Mechanical strength: Metals and metal alloys generally have excellent mechanical strength, making them suitable for withstanding loads and stresses in the human body, particularly in orthopedic implants.

ii. Durability: Metals and metal alloys have a long service life, which reduces the need for frequent replacement of implants.

iii. Electrical conductivity: Some metals, such as titanium, are electrically conductive, making them suitable for applications such as pacemakers and neural implants.

iv. Biocompatibility: Some metals, such as titanium, have good biocompatibility, which means that they are well tolerated by body tissues and minimize immune or inflammatory reactions.

v. Osseointegration: Some metals, such as titanium, promote bone adhesion, allowing the bone to bond directly to the implant, enhancing implant stability and fixation.

Disadvantages:

i. Weight: Some metals can be relatively heavy, which can cause problems in certain applications where lightness is essential.

ii. Chemical reactivity: Some metals, such as iron, can be susceptible to corrosion in body environments, which can compromise the durability of the implant.

iii. Allergies: Some metals, such as nickel, can cause allergic reactions in some people, which can lead to implant complications and rejection.

iv. Difficulty of shaping: The manufacture and shaping of certain metals and alloys can be complex, which can increase the cost and complexity of medical procedures.

v. Lack of resorption: Unlike some natural biomaterials, metals do not resorb into the body, meaning that they remain in place indefinitely, which can be a disadvantage in some applications.

Table 1.1. shows some metallic biomaterials and their use and **Figure 1.10** shows some images of metallic biomaterials.

Figure 1.10. Some metallic biomaterials.

Table 1.1. Some metallic biomaterials and their use.

Biomaterials	Use	Benefits
Titanium and titanium alloys	Orthopedic surgery (hip and knee replacements), dental implants, pacemakers, and implantable pumps.	Good biocompatibility, promotes bone adhesion, corrosion-resistant.
Stainless steel	Orthopedic surgery, fasteners (screws, plates), vascular prostheses.	High mechanical strength, durability.
Cobalt-chromium	Hip and knee replacements, dental implants, artificial heart valves.	Wear resistance, durability, high mechanical strength.
Zirconium	Dental implants, joint replacements, vascular components	Low chemical reactivity, biocompatibility.
Platinum	Cancer treatment equipment (catheters, coils), brain implants.	Chemical inertness, electrical conductivity.
Magnesium	Vascular stents, bone fixers, nails.	Light weight, potential for degradation in the body.

1.5.2. Ceramics

Ceramics are an important class of biomaterials widely used in medicine, dentistry, and biomedical research. They are characterized by their crystalline structure and unique properties that make them suitable for a variety of biomedical applications.

Ceramics have many advantages as biomaterials, including biocompatibility, mechanical strength, and durability. However, their fragility and difficulty of shaping are important considerations when using them in biomedical applications. The specific advantages and disadvantages will depend on the type of ceramic, the intended application, and the needs of the patient.

Here are some of the main advantages and disadvantages associated with biomedical ceramics:

Advantages

i. *Biocompatibility:* Many ceramics are biocompatible, which means that they are well tolerated by body tissues and generally do not cause allergic or inflammatory reactions.
ii. *Resemblance to human anatomy:* Some types of ceramics, such as hydroxyapatite, have a composition similar to that of natural bone tissue, making them easier to integrate and regenerate.
iii. *Corrosion resistance:* Ceramics are generally resistant to corrosion, making them suitable for use in moist body environments.
iv. *Mechanical strength:* Ceramics have high compressive strength, making them suitable for applications where mechanical strength is essential, such as bone implants.
v. *Durability:* Some ceramics, such as zirconia, are extremely durable and wear resistant, making them suitable for dental and orthopedic applications.
vi. *Thermal stability:* Ceramics retain their stability at high temperatures, which can be important when sterilizing biomedical implants.

Disadvantages

i. Fragility: Ceramics are generally brittle and have low toughness, which means they tend to fracture when subjected to high mechanical stress.

ii. Difficulty of shaping: The manufacture of complex ceramics can be difficult due to their fragility and the need for special manufacturing methods, such as sintering.
iii. Limited electrical conductivity: Some ceramics have poor electrical conductivity, which can limit their use in applications requiring electrical conduction, such as implantable electronic devices.
iv. Contact reaction: Although many ceramics are biocompatible, some people may experience allergic or hypersensitivity reactions on contact with specific ceramics.
v. Cost of manufacture: Ceramic manufacturing methods can be expensive due to the special processes required to obtain high-quality ceramic parts.

Ceramics used in biomedicine can be classified into several categories according to their composition and properties. **Figure 1.11** shows some examples of ceramics and their use.

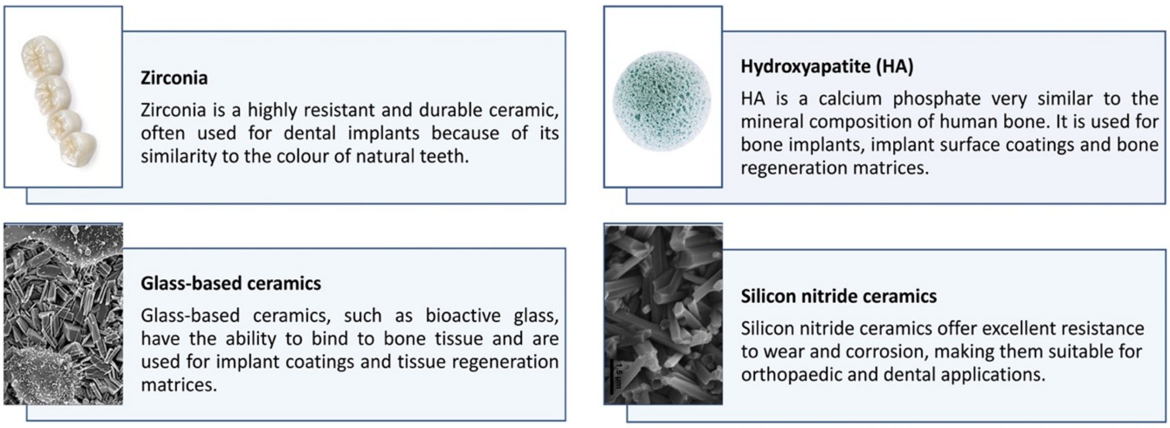


Figure 1.11. Some examples of ceramics and their use.

1.5.3. Polymers

A polymer is a substance or material made up of large molecules, called macromolecules, composed of multiple repeating subunits. Polymers, whether synthetic or natural, play a fundamental role in everyday life because of their wide range of properties. They range from commonly used synthetic plastics such as polystyrene to natural biopolymers such as DNA and proteins, which are essential to biological structure and function.

The formation of polymers, whether natural or synthetic, involves the polymerization of numerous small molecules called monomers. Because of their high molecular weight compared with small-molecule compounds, polymers have distinctive physical characteristics. These include increased strength, high elasticity, viscoelastic properties, and a propensity to form both amorphous and semi-crystalline structures rather than crystals.

Polymers play a crucial role in biotechnology, but there are also advantages and disadvantages to consider. Here is a list of the advantages and disadvantages of polymers in biotechnology:

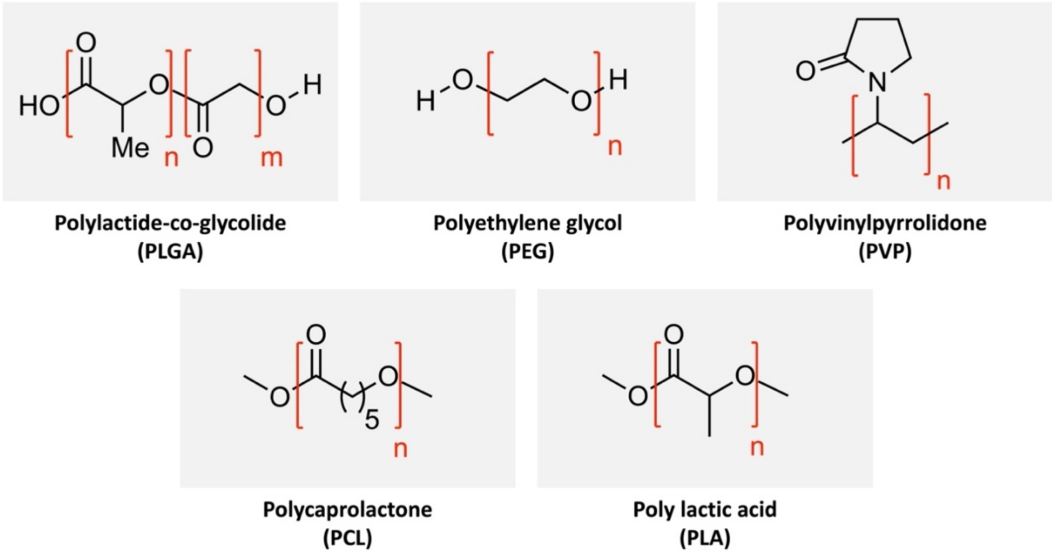
Advantages

i. Polymers are versatile and can be adapted to meet various biotechnological needs by modifying their composition, structure, and physical properties.
ii. Many polymers are compatible with biological tissues, making them suitable for use in medical applications such as implants and medical devices.
iii. Polymers are flexible and their properties can be adjusted to create materials with specific characteristics, such as stiffness, elasticity, and permeability.
iv. Some biodegradable polymers degrade naturally over time, avoiding the need for surgery to remove implants or devices.

Disadvantages

i. The degradation of certain polymers can also be considered a disadvantage because it can lead to a reduction in their mechanical properties and a premature release of active substances (toxic).
ii. Some patients may have immune or inflammatory responses to polymers, which may compromise their biocompatibility.
iii. Certain additives or manufacturing residues in polymers may have toxic effects on tissues and cells.
iv. The development of specific polymers for biotechnology applications can be complex and requires a thorough understanding of material properties.

Figure 1.12. Shows the chemical structure of some polymers and their use in biotechnology.



PLGA	A biodegradable polymer used to manufacture long-term drug delivery systems, such as microparticles and implants.
PEG	Used to create biocompatible surfaces, reduce immune reactions and promote drug solubility.
PVP	Used to stabilise medicines, formulate ophthalmic solutions and manufacture contact lenses.
PCL	A biodegradable polymer used for sutures, implants and tissue regeneration matrices.
PLA	Used for sutures, bone fixings and biodegradable implants.

Figure 1.12. Chemical structure of some polymers and their use.

1.5.4. Naturally-derived biomaterials

The exploration of natural biomaterials in the field of biotechnology has gained considerable interest due to their ability to reproduce the biological and mechanical functions of native extracellular matrices in various tissues. These biomaterials, particularly those based on proteins, polysaccharides, and tissues/organs, have biocompatibility, biodegradability, and non-toxicity characteristics that make them promising candidates. However, their application to the generation of solid organs has its limitations: restricted mechanical strength, manufacturing variability, and potential impurities leading to immunogenicity. Despite these limitations, *in vitro* bioengineering of various tissues has been successfully carried out over the last two decades, focusing particularly on thin cell sheets for the replacement of tissues such as skin, intestine, oesophagus, bladder, bone, and arteries.

Naturally occurring biomaterials can be divided into distinct categories, including protein-based biomaterials such as gelatin, collagen, and silk, polysaccharide-based biomaterials such as chitin/chitosan, cellulose, and glucose, and decellularized extracellular matrix scaffolds such as decellularized heart valves, blood vessels and livers [6]. In the following, we will present a brief overview and some applications of the main biomaterials of natural origin.

Protein-based biomaterials:

Protein-based biomaterials offer a particularly advantageous approach among natural biomaterials, given their essential role in biomechanical responses and the control of macromolecular structure and function. Their structural flexibility and biocompatibility make them ideal building blocks for biomaterials engineering. These biomaterials intrinsically possess binding sites for cell adhesion, eliminating the need for additional modifications to enhance cell attachment and proliferation. Proteins widely used as biomaterials are collagen, elastin, keratin, silk, and resilin.

The structural compositions of protein-based biomaterials are characterized by distinct attributes that determine their exceptional mechanical strength and elasticity. These proteins are built on tandem repeats of short amino acid sequences, a design that confers unique properties based on the arrangement of these amino acids. Silk, composed of fibroin and sericin, has remarkable mechanical properties due to tightly packed antiparallel β-sheets formed by regions rich in glycine and alanine. Elastin, a key mammalian structural protein, uses hydrophobic and hydrophilic domains in tandem repeats, facilitating its elasticity. Resilin, found in insects, has domains with different levels of resilience, enabling it to jump and fly efficiently. Collagen, the most abundant structural protein in mammals, assembles into complex supramolecular structures with a glycine-X-Y repeat structure. Keratin, found in a variety of animal structures, encompasses different helical structures based on α- or β-keratin types, while possessing attributes such as insolubility and cell adhesion sequences. **Figure 1.13** shows the chemical structure and some natural sources of the main protein-based biomaterials.

Protein-based materials, characterized by their adaptable chemical composition and morphology, hold exceptional promise for a variety of applications. Using a variety of processing techniques, proteins such as silk, elastin, and resilin can be tailored to specific needs, promoting biocompatibility and versatility. Trolling silk, a protein produced by spiders, has attracted attention for its resistance, prompting the development of recombination methods for traditional silk and trolling silk. Elastin-based hydrogels and fibers, resilin-based hydrogels, and collagen-based grafts are contributing to advances in tissue engineering, while protein-based materials have the potential for drug delivery and conductive applications. Chlorinated keratin offers antimicrobial properties in textiles, and the combination of several proteins, such as silk- and elastin-related peptides (SELPs), results in a variety of materials with applications ranging from tissue engineering to packaging.

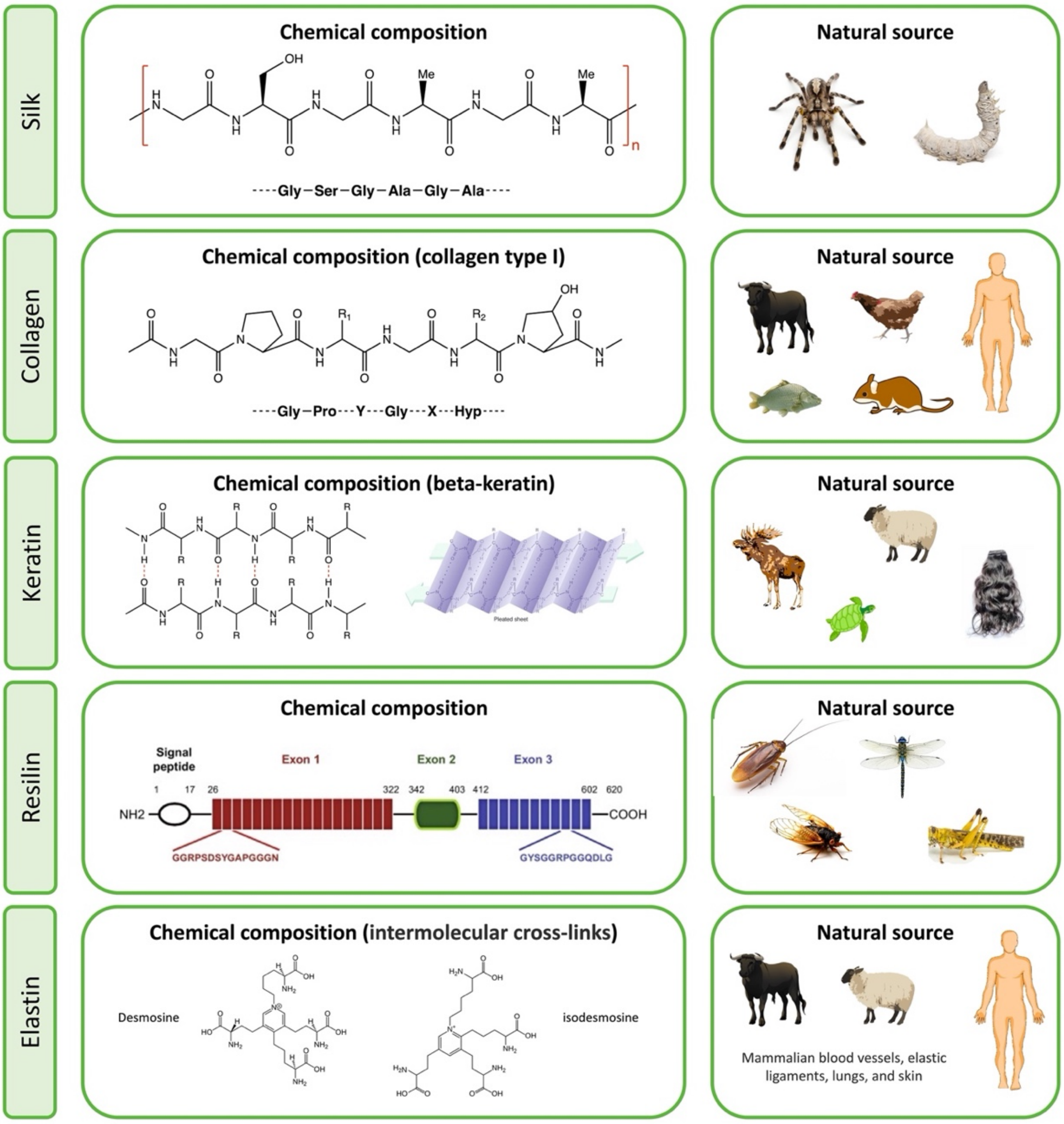


Figure 1.13. chemical structure and some natural sources of the main protein-based biomaterials.

Polysaccharide-based biomaterials:

Polysaccharide-based biomaterials are complex carbohydrates composed of repeating sugar units. These biomaterials are created by extracting or modifying polysaccharides present in natural sources such as plants, algae, fungi, and animals. Polysaccharide-based biomaterials have properties that make them suitable for use in wound dressings, drug delivery systems, tissue scaffolds, and coatings for tissue engineering. They are favored because of their biocompatibility, biodegradability, natural abundance, and ability to interact with biological tissues, cells, and growth factors. In addition, many polysaccharides exhibit a range of beneficial properties such as antimicrobial, anti-adhesive, and wound-healing capabilities, making them even more suitable for medical and regenerative purposes.

Polysaccharides are versatile biomaterials with properties such as chemical stability, pH sensitivity, and thermosensitivity, making them well suited to biomedical applications. They also have biochemical functionalities, gel-forming capabilities, and structural similarities with the extracellular matrix, making them even more attractive. The most important polysaccharides are glycosaminoglycans (GAGs), alginate, chitosan, and their derivatives. GAGs include complex anionic structures such as heparin, chondroitin sulfate, and hyaluronic acid, which play an essential role in various biomedical functions. Chitin can be converted into chitosan, which reacts to pH and has mucoadhesive attributes, offering the potential for drug delivery systems. Alginate, derived from marine sources, forms hydrogels by gelation induced by divalent cations, its composition influencing rigidity. **Figure 1.14** shows the molecular structure of some polysaccharides and their natural origin.

Decellularized extracellular matrix scaffolds:

Decellularized extracellular matrix (dECM) scaffolds are biological materials that are derived from tissues or organs by a process that removes cellular components while preserving the extracellular matrix (ECM) structure and composition. The extracellular matrix is a complex network of proteins, glycoproteins, and other molecules that provides structural support and signals for cell behavior in tissues (**Figure 1.15**).

Numerous approaches to decellularisation have been developed, classified into three main groups: physical, chemical, and biological methods. In the physical methods, techniques such as freeze-thaw cycles, high hydrostatic pressure, electroporation, ultrasonic waves, and supercritical CO_2 have been explored. Chemical approaches involve the use of ionic or non-ionic detergents, hypertonic or hypotonic saline solutions, acids, and bases. On the other hand, biological methods involve enzymes such as trypsin, dispase, phospholipase, as well as nucleases such as DNase.The careful selection of an appropriate decellularisation protocol is of paramount importance for each specific application. It is imperative to recognize that any treatment chosen may introduce certain limitations. For example, physical methodologies risk damaging the complex structure of the matrix, while chemical techniques could potentially alter the fundamental chemical composition of the decellularised extracellular matrix. It is important to emphasize that the primary objective of the decellularisation process is to preserve the original three-dimensional geometry of the tissue while leaving the overall integrity of the organ intact.

dECM scaffolds have received particular attention in the field of tissue engineering due to their potential applications in the regeneration of various organs and tissues. These scaffolds address the challenges of organ transplantation by offering alternatives for organ and tissue regeneration. Several medical applications of dECM scaffolds have been explored, including skin, bone, nerve, heart, lung, liver, and kidney regeneration. dECM shows promise in all areas of tissue engineering. In skin regeneration, they facilitate wound closure, reduce scarring, and improve tissue regrowth by preserving dermal architecture and bioactive molecules. In bone repair, dECM scaffolds preserve bone characteristics and immunomodulatory factors and promote endochondral ossification, which is crucial for the regeneration of large bone defects. Nerve tissue engineering benefits from preserved neural architecture, promoting neural stem cell proliferation, synapse formation, and axonal regrowth. Cardiac applications include preventing adverse remodeling, aiding functional recovery and regenerating cardiac tissue, and providing solutions to heart problems.

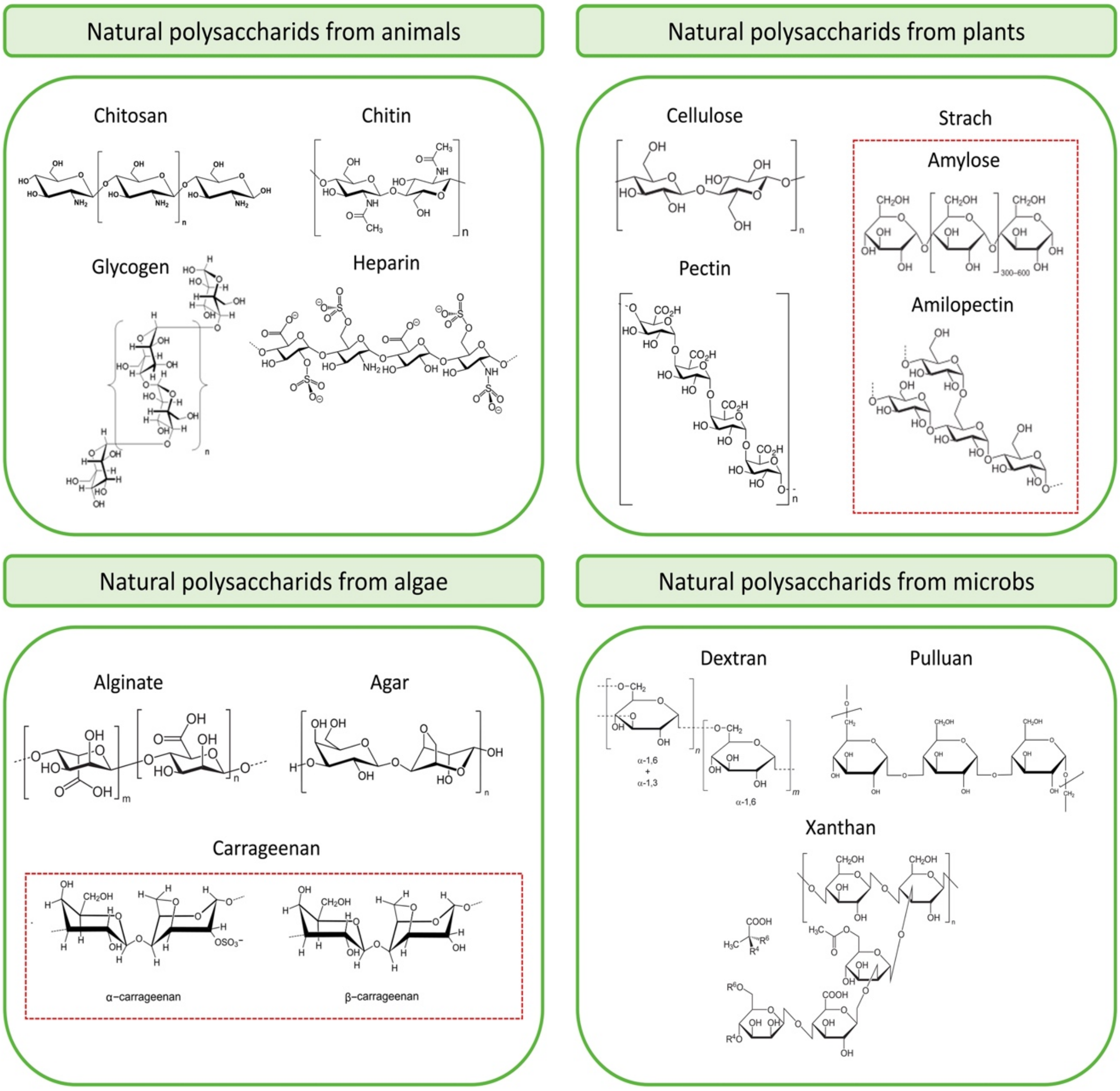


Figure 1.14. Molecular structure of some polysaccharides and their natural origin.

The use of dECM in tissue engineering is based on several key attributes. Firstly, as a tissue-derived biomaterial, decellularised extracellular matrix provides a basis for a variety of tissue engineering applications. Its intrinsic bioactive properties, capable of influencing cellular behavior and promoting tissue regeneration, further underline its potential. The versatility of dECM is demonstrated by its adaptability to various tissue engineering contexts, serving both as a complete tissue scaffold for structured growth and as a bio-ink for complex 3D printing. In addition, the incorporation of dECM consistently enhances regenerative capabilities, as evidenced by controlled laboratory conditions and dynamic living organisms. Remarkably, the role of dECM extends to directing stem cells to specific lineages, leading to tissue regeneration, often independently of external growth factors (**Figure 1.15**).

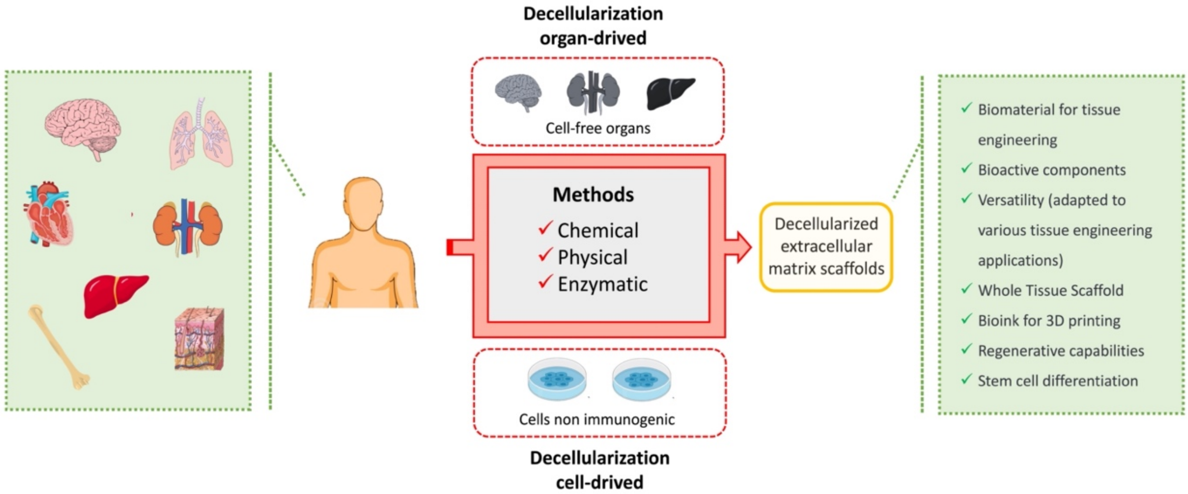


Figure 1.15. overview of decellularized extracellular matrix scaffolds.

Check your understanding

1. Provide a comprehensive definition of biotechnology based on your understanding.
2. Biotechnology in its simplest forms has been known to mankind since ancient times. Identify and describe one of the earliest forms of biotechnology.
3. Briefly explain the contributions of Gregor Mendel to the field of genetics.
4. Who is the scientist credited with laying the foundation for the modern understanding of heredity?
   a. Gregor Mendel
   b. Louis Pasteur
   c. Robert Koch
5. List the five main categories of biotechnology, and describe each in terms of its specific domain and potential applications.
6. Gray biotechnology concerns:
   a. Environment
   b. Solar energy conversion
   c. Biofuels
7. Explain how biotechnology is used in vaccine development.
8. Genetically modified organisms are products of:
   a. White biotechnologies
   b. Red biotechnologies
   c. Green biotechnologies
9. Answer true or false:
   a. Biotechnology is the exploitation of biological processes for industrial purposes.
   b. Red biotechnology primarily deals with medical processes, such as developing antibiotics.
   c. Green biotechnology is used in agricultural processes to produce more resilient crops.

d. Blue biotechnology is exclusively concerned with the development of marine-based cosmetics.
e. Virus-free plant production is part of green biotechnology.
f. Fuel and energy production are part of white biotechnology.
g. Red biotechnology plays a pivotal role in the development of disease-resistant plants.
h. Blue biotechnology is not involved in the enhancement of fish breeds for more efficient aquaculture.
i. Genetically engineered organisms are organisms that have had their DNA altered using genetic engineering techniques.
j. Biotechnology plays a crucial role in the discovery of new medicines and therapeutic agents.
k. Tissue engineering involves the creation of artificial organs and tissues for transplantation.
l. Biotechnology is used in wastewater treatment to break down harmful pollutants using microorganisms.
m. Escherichia coli is good bacteria because once in the human body, it produces insulin.

10. How biotechnology has contributed to the development of personalized medicine?
11. Insert a gene into a cell and then use this new cell to cure a patient it is:
    a. Genetic cloning
    b. Gene therapy
    c. Biotherapy
12. What is DNA recombinant molecule?
13. Which technique is used to amplify the sequences of DNA?
    a. Cloning
    b. Expression vectors
    c. Polymerase chain reaction (PCR)
    d. DNA splicing

14. What is introduced into an organism that lacks a specific DNA sequence during the production of recombinant DNA?
    a. A vector
    b. A restriction enzyme
    c. A sequence of interest from another organism
    d. A primer
15. Recombinant proteins are produced in the final step of:
    a. Polymerase chain reactions
    b. DNA amplification
    c. Recombinant DNA production
    d. DNA identification
16. Why might scientists modify genes in E. coli?
    a. To improve its ability to cause disease
    b. To produce a desired protein in large quantities
    c. To change its color to pink for aesthetic reasons
    d. To increase its natural lifespan by 100 years
17. What is the first step in producing insulin using recombinant DNA?
    a. Cellular transformation
    b. Expression and production
    c. Selection of the insulin gene
    d. Construction of an expression vector
18. Why is PCR used in the process of producing insulin through recombinant DNA?
    a. To create an expression vector
    b. To transform cells
    c. To ensure the gene is expressed in a controlled manner
    d. To amplify the insulin gene for obtaining sufficient DNA
19. What is the main purpose of an expression vector in this process?
    a. To grow bacteria rapidly
    b. To carry the gene of interest into a host cell
    c. To transform cells into insulin
    d. To isolate the insulin gene

20. At which levels can cloning be carried out?
    a. Only at the cellular level.
    b. Only at the molecular level.
    c. Both the cellular and molecular levels.
    d. The cellular, molecular, or whole organism levels.
21. What is cloning and what are the different levels at which it can be done?
22. Cloning refers to:
    a. Copying cells
    b. Reproducing genetic material
    c. Extracting DNA
    d. Isolating genes
23. Cloning of high-quality agricultural plants can help:
    a. Study plant diseases
    b. Produce consistent crop yields
    c. Conserve plant species
    d. Isolate plant genes
24. Who is considered the pioneer of cellular culture?
    a. Louis Pasteur
    b. Rudolf Virchow
    c. Wilhelm Roux
    d. Alexander Fleming.
25. What is required to maintain sterile conditions for cell culture?
    a. Laminar flow cabinet
    b. Dedicated laboratory
    c. Environmental monitoring
    d. All of the above
26. What is the primary purpose of fermentation in micro-organisms?
27. What is the role of sugars in the fermentation process?
28. What is the starting sugar used in lactic acid fermentation?
29. What is the starting sugar used in alcoholic fermentation?
30. What is DNA sequencing?

31. What enzyme is crucial for DNA sequencing?
32. What is the difference between dNTPs and ddNTPs used in DNA sequencing?
33. What is the role of dNTPs and ddNTPs in DNA sequencing?
    a. dNTPs are used to stop the addition of nucleotides, and ddNTPs are used for the regular addition of nucleotides to the growing DNA strand.
    b. dNTPs and ddNTPs both serve to accelerate the rate of DNA replication.
    c. dNTPs are used for the regular addition of nucleotides to the growing DNA strand, and ddNTPs are used to stop the addition of further nucleotides to the chain.
    d. dNTPs are used to initiate the reaction, and ddNTPs are used to catalyze the reaction.
34. Insulin is the key that allows
    a. Glucose to pass from our blood into our cells
    b. Sugar to pass from our blood into our cells
    c. Glucose to pass from our cells to our blood
    d. Sugar to pass from our cells to our blood
35. What is a bioactive material?
36. Transforming sugar into ethanol is:
    a. Saccharification.
    b. Fermentation.
    c. Transesterification
37. Biomaterial is:
    a. Any living material used in a medical device.
    b. Any non-living material used in a medical device.
38. Cite the three types of biomaterials.
39. Cite three desirable properties of biomaterials for implantation?
40. What are smart biomaterials?
41. Cite five areas of application of biomaterials:
42. Which of the following materials can be classified as biomaterials?
    a. Tricalcium phosphates.
    b. Cobalt.

c. Glasses.
d. Chrome.
e. Silicones.
f. Polyimines.
g. Carbon.
h. Mercury

43. Give five advantages of using ceramics as a biomaterial
44. Biocompatibility is the ability of a material to:
    a. Be rejected by the biological environment.
    b. Solve problems in biological environment.
    c. Degrade without biological intolerance.

Answers

1. Biotechnology refers to the creative and innovative use of biological processes, living organisms or their components to develop products, technologies, and solutions that benefit society
2. Fermentation: Fermentation is one of the oldest and most fundamental biotechnological processes. It involves the controlled use of microorganisms like yeast and bacteria to convert organic substances into more desirable products. Ancient civilizations, such as the Sumerians and Egyptians, used fermentation to produce alcoholic beverages like beer and wine.
3. Mendel established the laws of heredity, showing how characteristics are transmitted from one generation to the next. This laid the foundations for understanding gene transmission and paved the way for molecular biology
4. a
5. Red biotechnologies (health), green biotechnologies (agriculture and food), white biotechnologies (industry), gray biotechnologies (environment), blue biotechnologies (marine biodiversity).
6. a
7. La biotechnologie joue un rôle essentiel dans le développement des vaccins, permettant aux scientifiques de créer des vaccins qui protègent contre diverses

maladies infectieuses. Par exemple, la biotechnologie permet aux chercheurs d'utiliser la technologie de l'ADN recombinant pour cloner et produire des antigènes. Ce processus consiste à insérer le gène qui code pour l'antigène dans un organisme hôte, tel qu'une bactérie, une levure ou une cellule de mammifère, qui peut alors produire l'antigène en grande quantité. Cette opération est souvent réalisée à l'aide de vecteurs d'expression et de techniques de génie génétique.

8. b
9. a.true; b. true; c. true; d. false; e. true; f. true; g. false; h. false; i. true; j. true; k. true; l. true; m. false
10. Personalized medicine has been made possible by a growing understanding of genomics. Genetic testing can help predict susceptibility to disease, personalize treatment, and avoid undesirable side effects.
11. b
12. A DNA recombinant molecule is a genetically engineered DNA created by combining DNA from different sources. It involves cutting and inserting specific DNA sequences into a vector for various biotechnological applications.
13. c
14. c
15. c
16. b
17. c
18. d
19. b
20. d
21. Cloning is the process of generating genetically identical copies of organisms, cells, or DNA molecules. It can occur at various levels: reproductive cloning, which creates entire organisms with identical genetic material; molecular cloning, used to replicate and manipulate specific DNA sequences for research and biotechnology; gene cloning, which isolates and reproduces individual genes; and cellular cloning, replicating individual cells. Each level serves distinct

scientific and practical purposes, from producing genetically identical animals to studying genes and producing medically relevant proteins.

22. b
23. b
24. c
25. d
26. Fermentation in microorganisms primarily serves to produce energy and regenerate vital molecules like NAD+ in the absence of oxygen. This metabolic process is crucial for the survival and growth of microorganisms in anaerobic conditions.
27. Sugars act as the primary energy and carbon source for microorganisms during fermentation, enabling the production of energy-rich molecules like ATP and various end products depending on the microorganism and sugar type.
28. Lactose
29. Glucose
30. DNA sequencing is the process of determining the precise order of nucleotide bases in a DNA molecule.
31. DNA polymerase
32. ddNTPs are similar to dNTPs but lack the 3' hydroxyl group. This absence of the 3' hydroxyl group prevents further extension of the DNA strand when a ddNTP is incorporated into it.
33. c
34. a
35. A bioactive material is a substance or material that, when in contact with biological systems, induces a specific biological response or activity, such as tissue growth, cellular signaling, or therapeutic effects.
36. b
37. b
38. Metals and metal alloys, Ceramics and Polymers.
39. Three desirable properties of biomaterials for implantation are: biocompatibility, mechanical compatibility, degradability or stability

40. Smart biomaterials are materials that respond to external stimuli like temperature, pH, or electrical signals, allowing for controlled behavior.
41. Odontology, orthopedic surgery, cardiovascular, plastic surgery, and chemotherapy.
42. a, c, e
43. Biocompatibility, resemblance to human anatomy, corrosion resistance, mechanical strength, durability, and thermal stability
44. c

Chapter 2: Chemical biotechnology

2.1. Introduction

The dramatic advance of modern science and technology is the result of close interdisciplinary collaboration. An outstanding example of this convergence can be seen in the field of chemical biology, a cross-disciplinary field that merges the traditional domains of chemistry, biology, and physics. It relies on the application of chemical techniques, tools and analyses, often supported by synthetic chemistry, to explore and manipulate biological mechanisms. The exponential growth of chemical biology has led to the emergence of specialized sub-disciplines such as synthetic biology, chemical biotechnology* and chemical engineering.

Given the ever-increasing global population and the challenges of energy and the environment, synergy between these different disciplines is crucial to solving complex problems that exceed the capabilities of any single discipline. Chemical biotechnology, for example, takes advantage of small chemical molecules to influence and optimize biological processes. This innovative approach promises greener, more cost-effective production of a variety of chemicals, overcoming the resource and environmental crises facing conventional chemical biology. Moreover, it enhances natural biological processes that are not yet sufficiently robust for practical application.

The scope of chemical biotechnology is vast, ranging from macro-biomolecules to cells and plants in various contexts. It acts as a chemical modulator in enzymatic reactions, a chemical regulator in the regulation of non-canonical DNA structures, a biomimetic chemical cofactor in in vitro biosystems for the production of high-value chemicals and low-value biological products, and many other applications besides. As environmental and industrial challenges evolve, chemical biotechnology offers a promising prospect for meeting the needs of our constantly changing society (**Figure 2.1**).

* According to the American Chemical Society, biotechnology is the application of biological organisms, systems or processes by various industries to learn about the science of life and improve the value of materials and organisms, such as pharmaceuticals, crops and livestock. *Chemical biotechnology* involves using the tools and techniques of chemistry to understand and manipulate biological processes. It is one of the key technologies of the future, using enzymatic and microbial systems to produce a variety of bulk and fine chemicals, fuels, materials and pharmaceutical precursors from renewable raw materials.

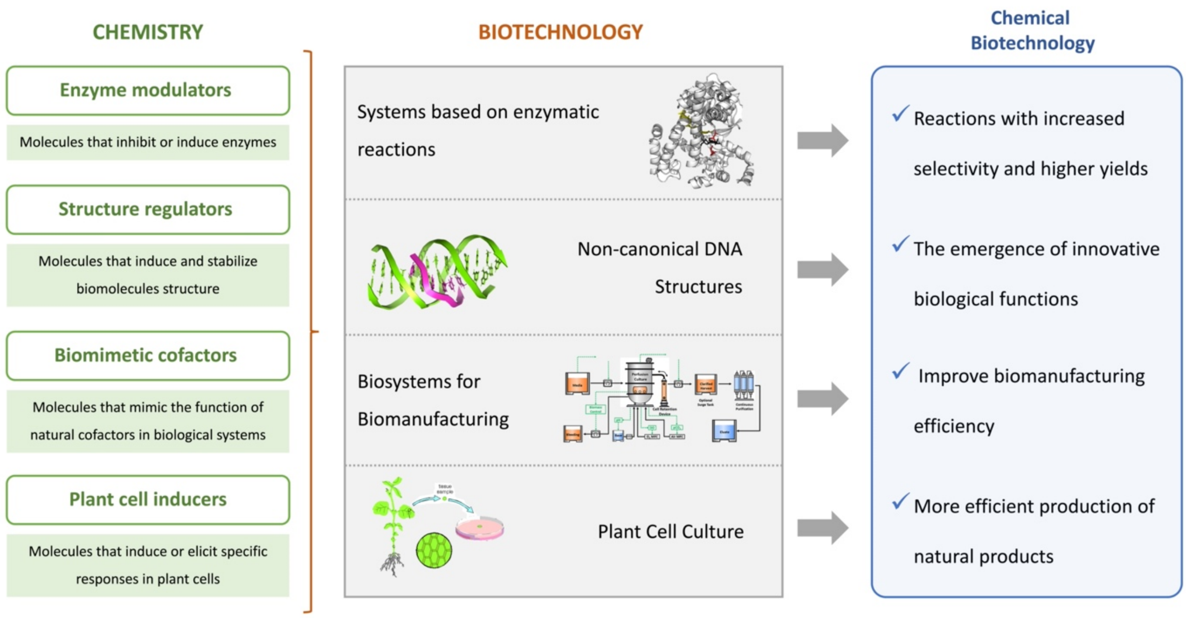


Figure 2.1. The emergence of chemical biotechnology in the context of chemistry and biotechnology.

2.2. Chemical biotechnology for enzymatic reactions

2.2.1. Asymmetric synthesis

Asymmetric synthesis, also known as enantioselective synthesis* or stereoselective synthesis†, is a method used in organic chemistry to produce chemical compounds with molecular asymmetry, i.e. compounds that have a specific spatial configuration and orientation of atoms around a central atom, often a chiral carbon atom.

Chiral compounds are molecules that exist as two enantiomers, which are non-superimposable mirror images of each other (**Figure 2.2**). These enantiomers have

* *Enantioselective synthesis* aims to selectively create one enantiomer of a chiral compound while minimizing the formation of the other enantiomer. This selectivity is crucial, as often only one of the enantiomers exhibits the desired biological activity or can be safely used in pharmaceuticals. Enantiomers are mirror isomers that are not superimposable, like a left hand and a right hand. Enantiomers have the same chemical formula and atomic connectivity, but differ in their three-dimensional arrangement.

† *Stereoselective synthesis* is a chemical process used to selectively produce a specific stereoisomer of a compound, stereoisomers being isomers that have the same atom connectivity but differ in their spatial arrangement (an be enantiomer or diastereoisomer). Stereoselective synthesis focuses on controlling the formation of stereoisomers, whether enantiomers (mirror isomers) or diastereoisomers (non-mirror isomers), during a chemical reaction.

different chemical and biological properties, which means they can have very different effects on chemical or biological reactions.

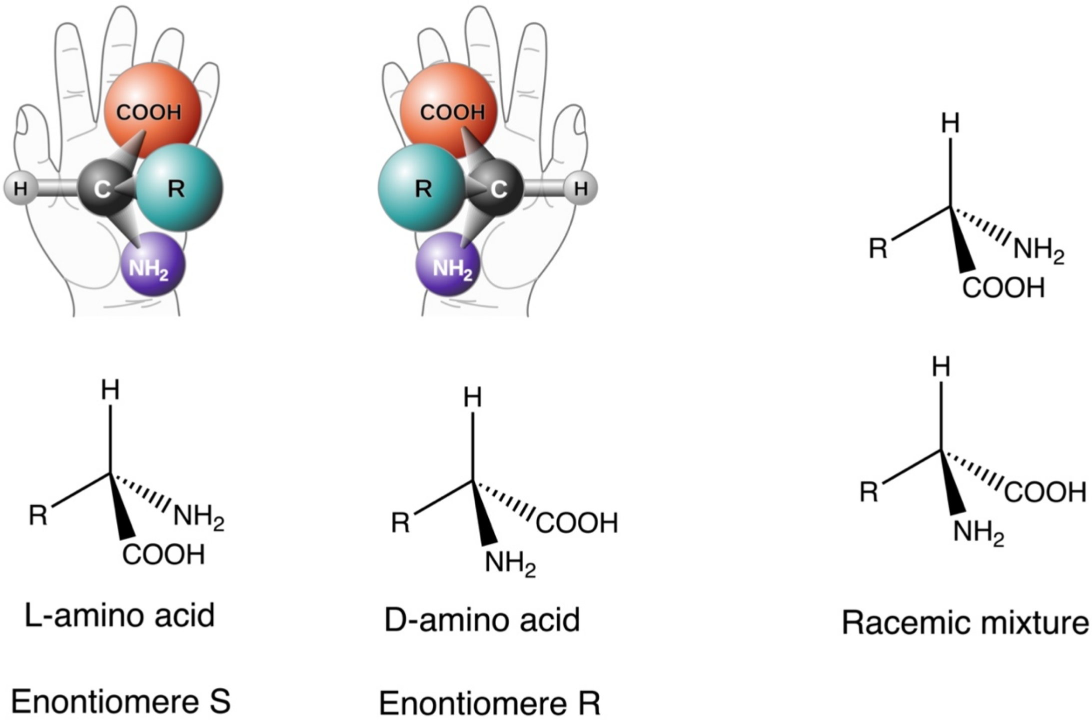


Figure 2.2. An example of enantiomers.

Asymmetric synthesis aims to selectively produce one of the enantiomers of a chiral compound while avoiding or minimizing the formation of the other enantiomer. This can be achieved using a variety of strategies, such as the use of chiral reagents, chiral catalysts, or by exploiting specific chemical reactions that discriminate between enantiomers.

This approach is of great importance in pharmaceutical chemistry, as it enables drugs to be produced in pure form, which is essential for guaranteeing their efficacy and safety. It is also used in many other areas of chemistry for the production of chemicals and compounds with significant molecular asymmetry.

Chirality is becoming increasingly widespread in pharmaceuticals and the fine chemicals industry. According to recent reports, 80% of FDA-approved small-molecule drugs are chiral, and of these, 75% consist of enantiomers or single diastereoisomers, with an average of two chiral centers. Under current regulations, a stereoisomeric purity of 99.5% is required for chiral drugs.

Chemical methods used in asymmetric synthesis can be associated with issues related to waste, cost, and the toxicity of reagents. An increasingly attractive approach is to employ enzymes for the production of pure chiral compounds. Biocatalysis using enzymes offers advantages in terms of selectivity, mild reaction conditions, environmental sustainability, and the potential for recycling.

Disadvantages of chemical methods:

i. Chemical methods can often generate unwanted by-products and produce waste, which can be costly and harmful to the environment.
ii. Some asymmetric chemical reactions require expensive chiral reagents, which can make production costly.
iii. Some chemical reagents used may be toxic or hazardous to human health and the environment.
iv. Chemical reactions can sometimes have selectivity problems, leading to the formation of unwanted enantiomers.

Advances in enzymatic biocatalysis

i. Enzymes are often highly selective and catalyze reactions specifically, minimizing the formation of undesirable enantiomers.
ii. Enzyme-catalyzed reactions generally take place under mild temperature and pH conditions, reducing the risks and costs associated with the use of high-tech equipment.
iii. Biocatalysis is considered more environmentally friendly because it uses natural catalysts and can reduce the production of hazardous waste.
iv. In some reactions, enzymes can be recycled, which can help reduce production costs.

Figure 2.3. shows some examples of biocatalyzed asymmetric synthesis.

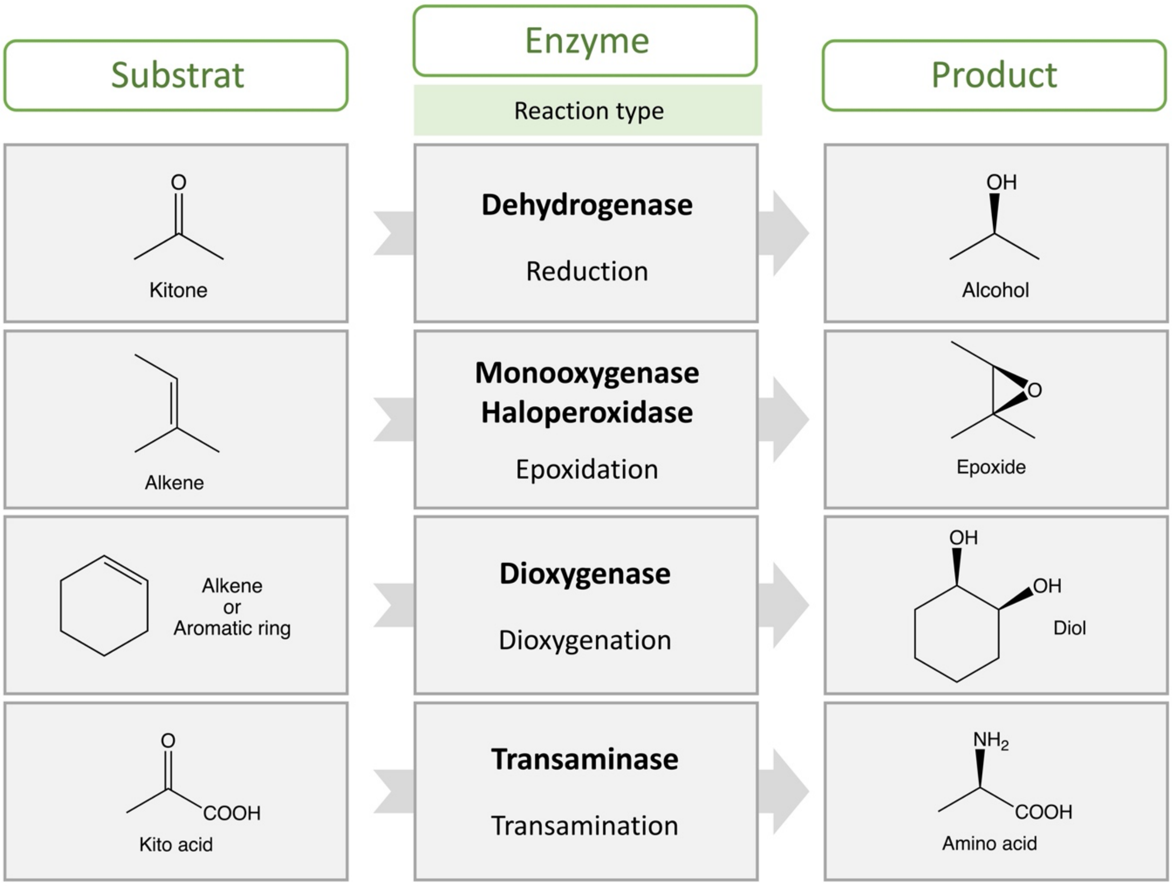


Figure 2.3. some examples of biocatalyzed asymmetric synthesis.

2.2.2. Chemical modulators of biocatalytic reactions

Chemical modulators of biocatalytic reactions are compounds or molecules that are used to influence or enhance the performance of enzymes in various biocatalytic processes. These modulators can have a variety of effects on enzyme activity, stability, selectivity, and efficiency.

Here are some common types of chemical modulators used in biocatalytic reactions:

i. *Cofactors and coenzymes:* These are small molecules that are often required for the proper functioning of enzymes. For example, NADH and NADPH are coenzymes that play essential roles in many enzymatic reactions, particularly in redox reactions.

ii. *Inhibitors:* Chemicals that can either inhibit or enhance enzyme activity. Competitive inhibitors, for instance, compete with the substrate for the enzyme's active site, while non-competitive inhibitors bind to another part of the enzyme and alter its shape or activity.

iii. *Activators:* Compounds that enhance the activity of enzymes. Allosteric activators, for example, bind to specific sites on the enzyme and increase its catalytic efficiency.

iv. *Stabilizers:* Chemicals that improve the stability and longevity of enzymes, especially in harsh reaction conditions. Examples include polyols, sugars, and certain salts.

v. *Solvents:* The choice of solvent can significantly impact enzyme activity. Some solvents are known to enhance enzyme stability and activity, while others may denature or inhibit enzymes.

vi. *pH modifiers:* Altering the pH of the reaction environment can affect enzyme activity. Some enzymes function optimally at specific pH levels, and adjusting the pH can be a way to modulate their performance.

vii. *Additives for chiral control:* In asymmetric synthesis and chiral biocatalysis, certain chemical additives may be used to enhance the enantioselectivity of the enzyme, allowing for the production of specific chiral products.

viii. *Buffer systems:* Buffers are used to maintain a stable pH during enzymatic reactions, ensuring that the enzyme remains active and selective throughout the process.

ix. *Substrate analogues:* Chemicals that mimic the enzyme's substrate can be used to competitively inhibit or modulate enzyme activity.

x. *Surfactants and detergents:* These can be added to enhance the solubility of substrates or stabilize enzymes in heterogeneous reactions.

One industrial example that involves the use of chemical modulators in biocatalytic reactions is the production of biofuels, specifically biodiesel, through enzymatic transesterification (**Figure 2.4**).

In the production of biodiesel, triglycerides (found in vegetable oils and animal fats) are converted into biodiesel (fatty acid methyl esters) and glycerol through a transesterification reaction. Enzymes such as lipases are commonly used as biocatalysts for this process. Chemical modulators are often employed to enhance the efficiency and selectivity of the enzyme-catalyzed transesterification.

One important chemical modulator used in this context is a co-solvent, typically methanol or ethanol. Methanol, for example, acts as a substrate and a chemical modulator. It helps to overcome mass transfer limitations by increasing the solubility of both the triglycerides and the enzyme, leading to more efficient reactions. It also helps to prevent the enzyme from aggregation or denaturation, thereby improving enzyme stability.

Additionally, chemical modulators like acyl acceptors (e.g., dimethyl carbonate) can be added to the reaction mixture to drive the transesterification reaction to completion and increase the yield of biodiesel.

The careful selection and optimization of these chemical modulators, including the type and concentration of co-solvents and acyl acceptors, play a crucial role in the commercial production of biodiesel using enzymatic transesterification. This approach not only improves the overall reaction efficiency but also ensures the cost-effectiveness of biodiesel production, making it a sustainable alternative to traditional fossil fuels.

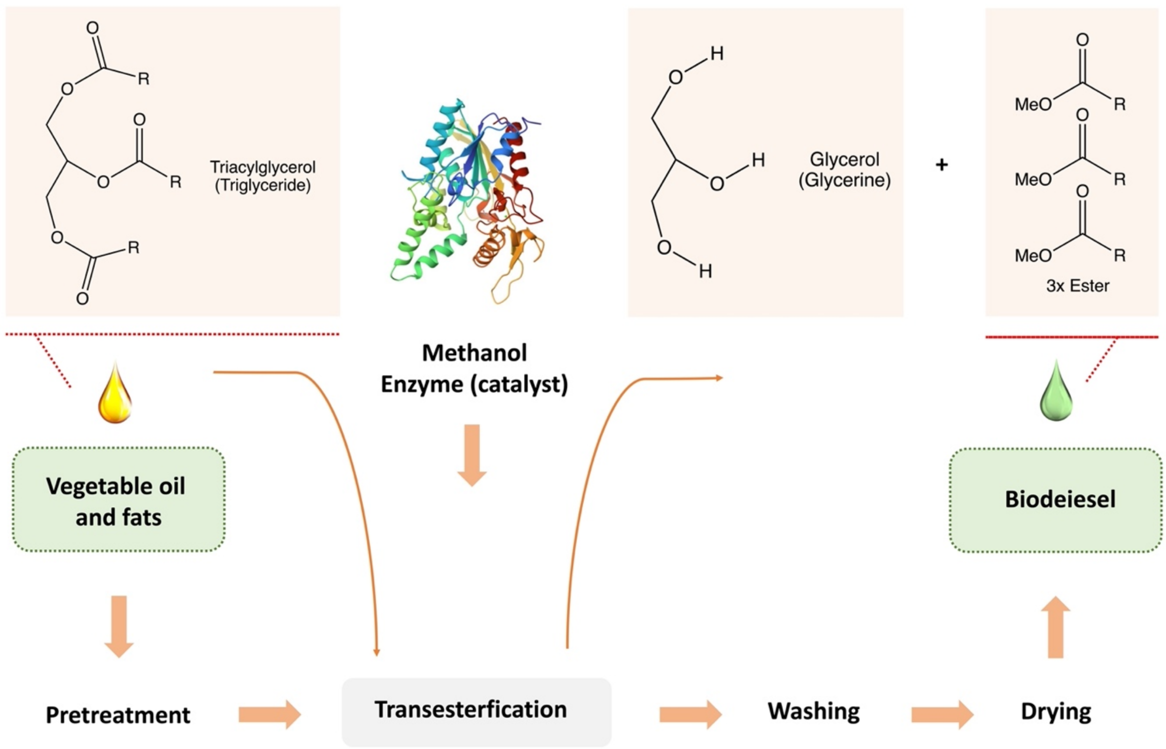


Figure 2.4. Process for the production of biodiesel from vegetable oils and fats using enzymes as catalysts.

Another industrial example involving chemical modulators in biocatalytic reactions is the production of high-fructose corn syrup (HFCS) using enzymes such as glucose isomerase.

High-fructose corn syrup is a widely used sweetener in the food and beverage industry. It is primarily produced by isomerizing glucose (found in corn starch) into fructose, which is sweeter and provides better flavor characteristics. Enzymatic isomerization using glucose isomerase is the preferred method for this conversion (**Figure 2.5**).

Chemical modulators play a vital role in optimizing the enzymatic isomerization process used in the production of HFCS. These modulators are employed in several ways to enhance the performance of glucose isomerase, the key enzyme in this process. Firstly, pH control is crucial since the enzymatic activity of glucose isomerase is highly pH-dependent. To ensure maximum efficiency, chemical buffers are regularly utilized to maintain the enzyme's optimal pH range, making pH adjustment a critical aspect of the process. Secondly, metal ions like calcium and magnesium, acting as cofactors, are introduced into the reaction mixture to augment glucose isomerase's activity, thereby improving its overall performance. Thirdly, maintaining precise temperature control is essential for preserving enzyme stability and activity, prompting enzymatic isomerization reactions to be conducted within specific temperature ranges. Moreover, chemical modulators are employed to counteract the inhibitory effects of certain compounds that can hinder glucose isomerase's function. Finally, precise control of glucose concentration in the reaction mixture is achieved through the use of chemical modulators, facilitating the optimization of fructose yield in HFCS production.

By carefully adjusting these chemical modulators in the enzymatic isomerization process, manufacturers can produce HFCS with specific properties, such as different fructose/glucose ratios, which are tailored to the needs of various food and beverage products. This optimization ensures the efficient and cost-effective production of high-fructose corn syrup for a wide range of applications in the food industry.

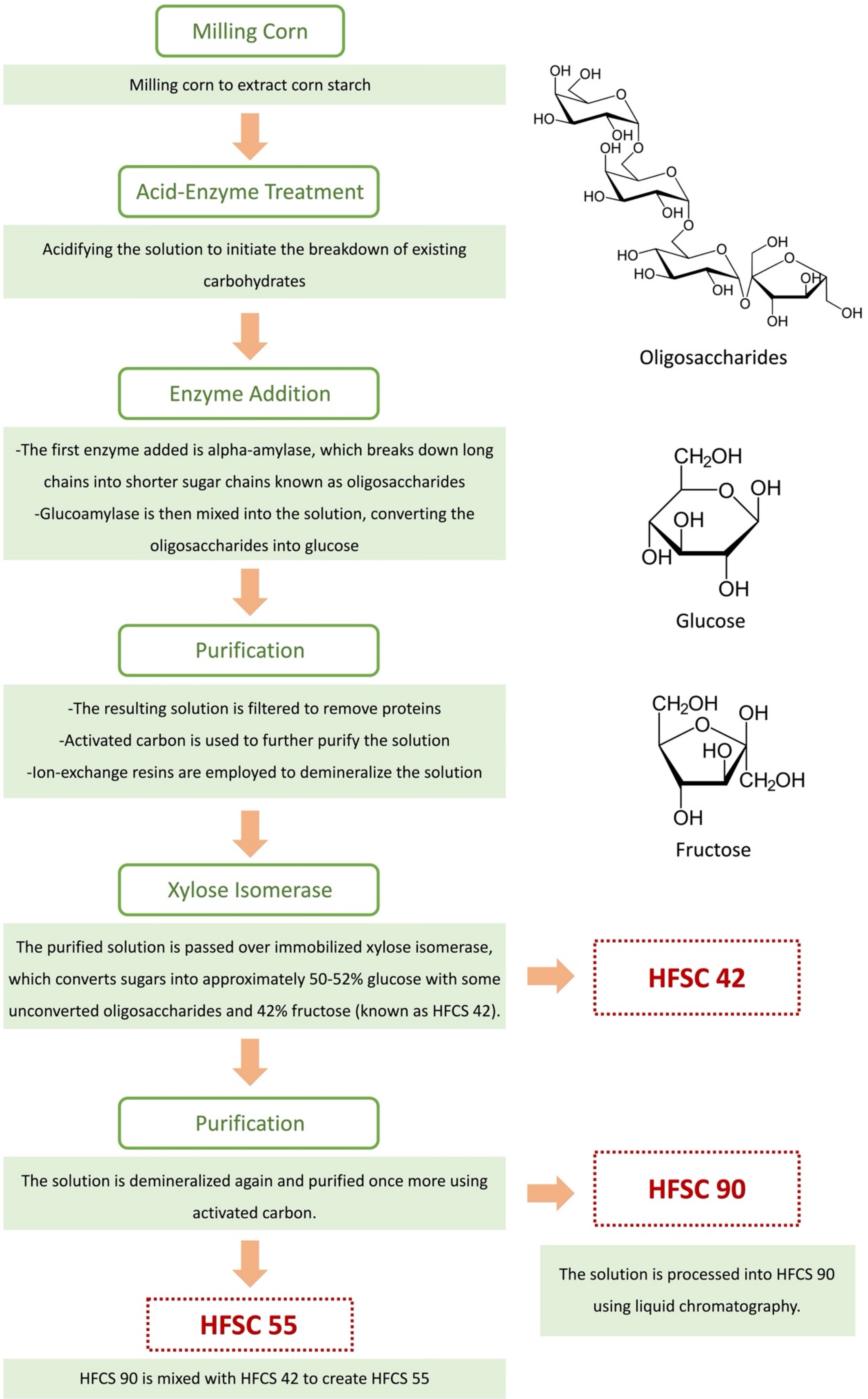


Figure 2.5. Process for producing high-fructose corn syrup (HFCS).

2.2.3. Biocatalysis in organic chemistry

Biocatalysis in organic chemistry represents a dynamic and sustainable approach to chemical synthesis, harnessing the remarkable catalytic power of biological molecules such as enzymes to facilitate complex chemical reactions. Enzymes, as nature's catalysts, offer several advantages over traditional chemical catalysts. They can operate under mild reaction conditions, exhibit exquisite selectivity, and often provide higher yields, thus reducing the environmental impact of chemical processes. Biocatalysis has revolutionized the synthesis of pharmaceuticals, fine chemicals, and biofuels, making it a key player in green chemistry initiatives. Researchers continue to explore and engineer enzymes for novel applications, driving innovation in the field and promoting greener and more efficient routes to valuable organic compounds.

In the 1990s, enzymes became widely used in various industrial processes, particularly for the production of fine chemicals like chiral compounds crucial for pharmaceuticals, plant protection agents, and fragrances. Several fermentation processes were established to obtain enantiomerically pure chiral compounds, such as L-amino acids, on a large scale. One notable example is BASF's method for producing chiral amines with high selectivity using appropriate lipases as catalysts. Dynamic kinetic resolution techniques, combining enzymes and racemizing catalysts, have also been developed for high enantioselectivity and full conversion. Enzymes found applications in the preparation of semisynthetic antibiotics, like Cephalexin, as well as bulk chemical production, such as the hydrolysis of acrylonitrile to ultrapure acrylamide using nitrile hydratase, as demonstrated by Nitto Chemical Industry and Lonza in Switzerland. These industrial processes exemplify the versatility and efficiency of enzyme-based catalysis in various chemical applications. **Figure 2.6** shows some organic products synthesized using biocatalysts.

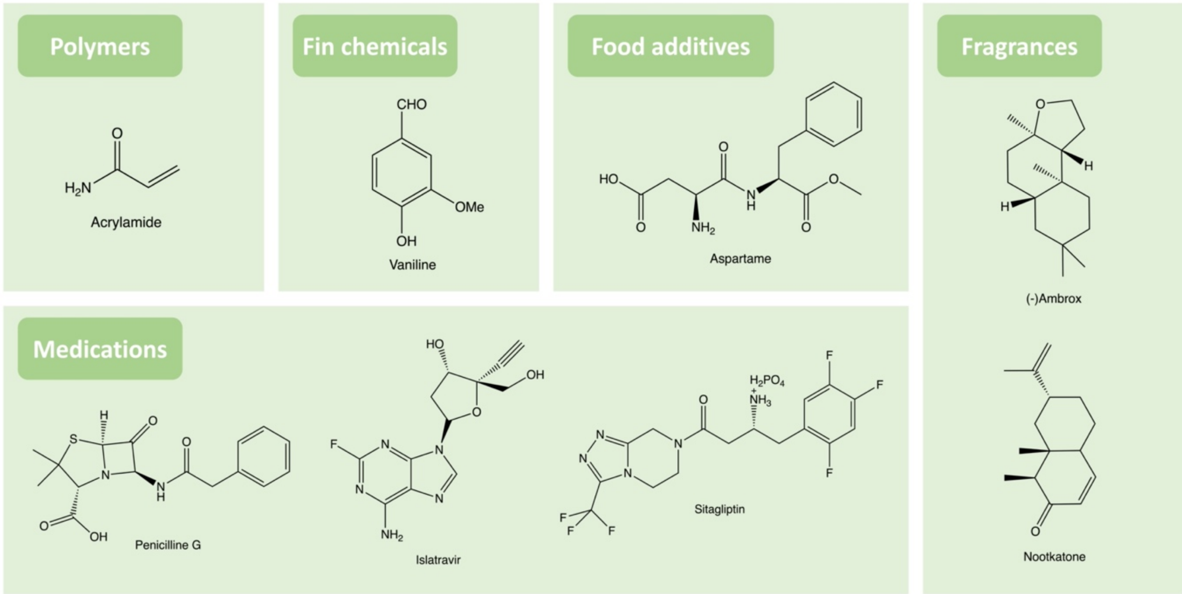


Figure 2.6. Some organic products synthesized using biocatalysts.

2.3. Chemical regulation of non-canonical DNA structures

2.3.1. Non-canonical DNA structures

A non-canonical DNA structure, also known as an atypical DNA structure or non-B-DNA, refers to DNA arrangements or conformations that differ from the classic B-DNA double helix. The B-DNA structure is the most common and is characterized by two strands wound into a straight double helix. Non-canonical DNA structures can take several different forms, such as (**Figure 2.7**):

i. *Z-shaped DNA:* In this structure, DNA forms a left-hand (left) helix, as opposed to the right-hand (right) helix of B-DNA. This structure can form when DNA undergoes significant twisting or superwinding.

ii. *Cruciform DNA:* When inverted repeat sequences are found in close proximity to each other on a DNA strand, they can form a cross-shaped (or cruciform) structure where the strands spread apart and form loops. This may play a role in regulating gene expression.

iii. *DNA triplex:* In a DNA triplex, a third DNA chain attaches to a double helix, forming a three-stranded structure. This can have implications for the regulation of gene expression and the recognition of specific sequences.

iv. *G-quadruplex tetracodes:* These structures are formed when clusters of four guanine (G) bases form planar stacks, which can lead to the formation of a square or tetrad structure. G-quadruplex tetracodes are involved in the regulation of gene expression and may play a role in telomere regulation.

These non-canonical DNA structures may have specific biological functions, including regulation of gene expression, DNA replication, telomere protection*, and are often associated with specific genomic regions. One promising avenue of research is the targeting of non-canonical DNA chains. Using chemical molecules such as cisplatin and carbon materials such as carbon nanotubes (CNTs) and graphene oxides (GOs), scientists are exploring innovative methods for modulating these structures, opening the way to new medical treatments.

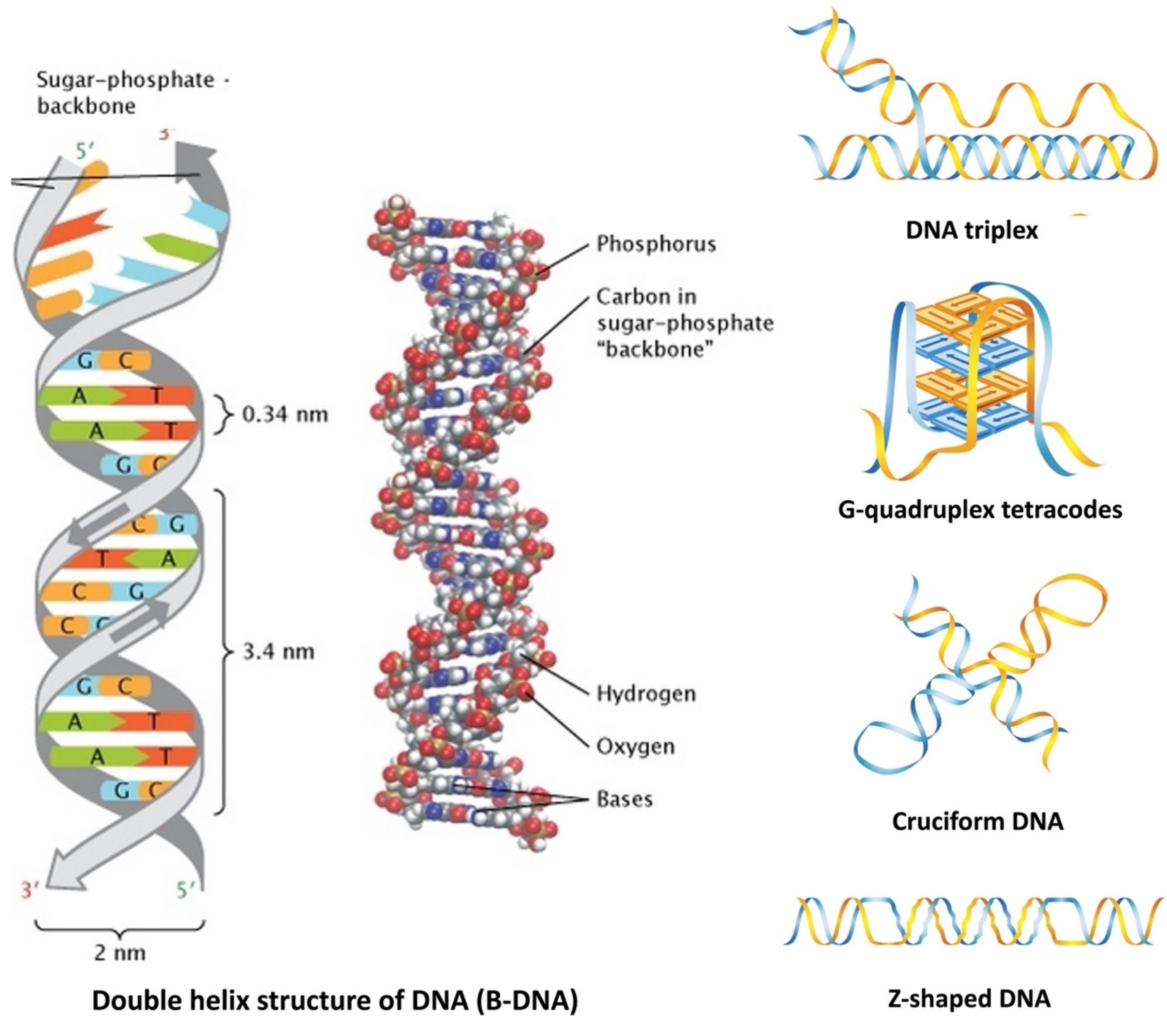


Figure 2.7. Commonly studied molecular structure of DNA.

* Telomere protection refers to the maintenance and preservation of telomeres, which are the protective caps at the ends of linear chromosomes. Telomeres play a crucial role in maintaining the stability and integrity of the genome. They consist of repetitive DNA sequences and associated proteins.

2.3.2. Chemical modulators of non-canonical DNA structures

Over the past decade, structure-based drug design has experienced remarkable growth, facilitated by advances in molecular and structural biology techniques and computer-aided drug design. While the majority of these efforts have focused on targeting proteins because of their identifiable structural features, nucleic acids, with their defined structures and biological functions, have received less attention as potential drug targets. This is evident in the decline in FDA-approved drugs targeting DNA, from 31% before 1982 to approximately 2% between 2010 and 2015. Despite this trend, DNA remains a promising therapeutic target for diseases linked to genetic instability, such as cancer. Recent studies have revealed that non-canonical DNA structures often coincide with mutation hotspots linked to diseases associated with genetic instability.

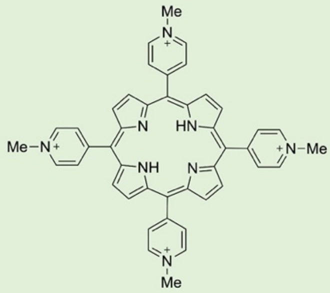
Small molecules have been used to regulate these non-canonical DNA structures. Due to their unique three-dimensional structures, small molecules can stabilize or alter these structures, potentially regulating their biological functions. Methods for inducing the formation of non-canonical structures have been developed using small molecules and oligonucleotides. These techniques are being used for the development of innovative therapeutic strategies against diseases like cancers and neurological disorders. They are also being used in the development of new functional non-canonical nucleic acid structures for applications in the diagnosis and therapy of human diseases. **Figure 2.8** shows some examples of chemical molecules used to regulate DNA structure and their biological activity.

Modulation of G-Quadruplex DNA Structures

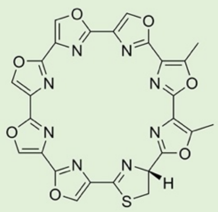
G4 DNA structures are implicated in the regulation of gene expression. Small molecules that stabilize G4 structures such as telomestatin and TMPyP4 have been explored as potential anticancer agents by targeting telomeres and oncogene promoters.

Telomestatin is a macrocyclic chemical compound that acts by inhibiting the telomerase activity of in vitro cancer cells. Upon formation of G4 structure there will be a decrease in the activity of the telomerase, which is involved in the replication of the telomeres and as a result the cell dies.

TMPyP4 is a potent inhibitor of human telomerase. It binds strongly to DNA quadruplexes by stacking on the G-tetrads at the core of the quadruplex, resulting in telomerase inhibition.

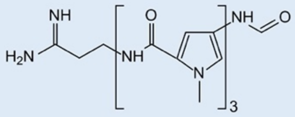


TMPyP4



Telomestatin

Modulation of Z-DNA Structures



Distamycin

Z-DNA is a left-handed helical form of DNA. Small molecules, such as the antibiotic distamycin and certain metal complexes, have been investigated for their ability to modulate Z-DNA formation. This has potential applications in controlling gene expression and understanding immune responses.

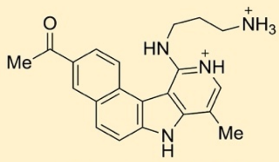
Modulation of triplex DNA Structures

Triplex DNA structures, also known as H-DNA, are involved in genetic regulation. Small molecules that can stabilize or destabilize triplex structures have been studied for their impact on gene expression and as potential therapeutic agents. Examples include benzopyridoindole derivatives and coralyne.

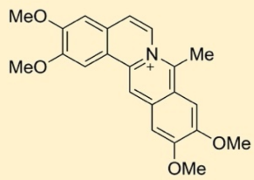
Benzopyridoindole derivatives are a type of chemical compounds that have been identified as inhibitors of the protein kinase CK2. These compounds have shown a good pharmacologic profile, causing a marked inhibition of CK2 activity associated with cell cycle arrest and apoptosis in human cancer cells. In addition, in vivo assays demonstrate antitumor activity in a mouse xenograft model of human glioblastoma. The research on these compounds lays the foundation for the development of clinically useful CK2 inhibitors derived from a well-studied scaffold with suitable pharmacokinetics parameters.

Coralyne is a natural alkaloid compound found in certain plant species, particularly in the genus Corydalis. It is a compound that can stabilize DNA triplexes which is of interest in the field of molecular biology and has potential applications in areas such as drug development and gene therapy.

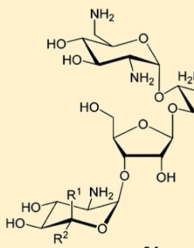
Neomycin is an antibiotic belonging to the aminoglycoside class. It is known for its broad spectrum of activity against bacteria, particularly gram-negative bacteria. It works by inhibiting protein synthesis in bacteria, leading to their death. Neomycin is effective in stabilising DNA triplexes. More specifically, neomycin selectively stabilises triplex DNA without affecting the DNA duplex. Neomycin is therefore a molecule capable of recognising and stabilising the DNA triplex rather than the double-stranded duplex structure. This property makes neomycin attractive for potential applications in molecular biology and medicine.



Benzopyridoindole



Coralyne



Neomycin	R^1	R^2
B	CH_2NH_2	H
C	H	CH_2NH_2

Neomycin

Figure 2.8. Some examples of chemical molecules used to regulate DNA structure and their biological activity.

2.4. Chemical biotechnology for biomanufacturing

Enzymes have been used for over 50 years to manufacture a variety of products, including fructose, chemicals, and antibiotics. Cell extracts have been studied to produce vaccines, vitamins and proteins over the last two decades. Whole cells, especially microbes, have long been used for the production of foods, beverages, medicines and more.

In vitro biosystems involve the assembly of multiple enzymes and coenzymes from different sources to produce the desired products. They offer advantages over whole-cell biomanufacturing, including (i) high yield, (ii) rapid productivity, (iii) easy product separation, (iv) better tolerance to toxins and solvents, (v) the possibility of carrying out unnatural reactions, and (vi) the ability to modify the reaction equilibrium in favor of product formation.

Cofactors are non-protein compounds required for enzymatic activity. Organic cofactors such as NADP (nicotinamide adenine dinucleotide phosphate), FAD (flavin adenine dinucleotide), NAD (nicotinamide adenine dinucleotide), CoA (coenzyme A), and ATP (adenosine triphosphate quinone compounds) are commonly used, but may not be stable for long-term processing (**Figure 2.9**). Biomimetic[*] cofactors, similar in structure and function, can be synthesized chemically at low cost and offer greater stability in solution.

[*] Biomimicry is an approach that draws inspiration from nature to solve human problems or create innovative designs. Biomimicry involves imitating biological systems, processes or structures to find sustainable and effective solutions.

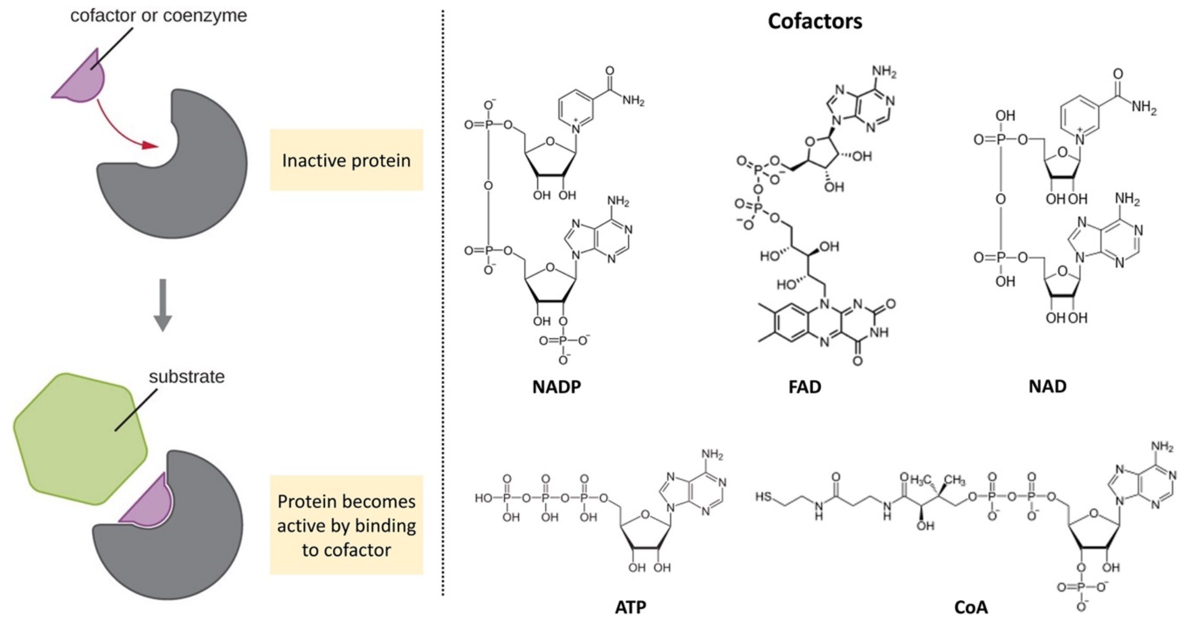


Figure 2.9. The operating principle of cofactors as well as the chemical structure of common cofactors.

2.4.1. Biomimetic nicotinamide adenine dinucleotide phosphate (NADP)

Nicotinamide adenine dinucleotide phosphate (NADP) is a coenzyme whose structure consists of two nucleotides linked by phosphate groups. One of these nucleotides is linked to adenine, while the other is linked to nicotinamide. NADP has an additional phosphate group compared to NAD (**Figure 2.10**).

In biology, NAD and its phosphorylated form, NADP, play a crucial role in various energy-related metabolic processes, such as respiration and photosynthesis. They are mainly used by oxidoreductase enzymes to transfer electrons and protons. NADP is generally associated with anabolic processes, while NAD is more often used in catabolic reactions.

During oxidation reactions, two hydrogen atoms are removed from the reactant, in the form of a hydride ion and a proton. This process converts NADP into NADH. NAD is often produced by microbial transformation using the recovery pathway, involving nicotinamide and ATP. NADP can be generated by enzymatic phosphorylation of NAD. Their reduced forms, NADH and NADPH, can be obtained by chemical or enzymatic processes. However, NADP and NADPH present a number of technical challenges. They are relatively expensive to produce, with NAD being more cost-effective. In addition, these coenzymes are unstable and susceptible to decomposition under various conditions, which limits their practical applications.

To address these challenges, researchers have engineered enzymes to change their cofactor preference from NADP to NAD. Additionally, biomimetic cofactors, smaller and more stable compounds, have been explored as alternatives to NADP. Examples include nicotinamide mononucleotide (NMN) and 1-benzyl-3-carbamoyl-pyridinium chloride (BCP). The use of biomimetic cofactors offers cost-effectiveness and improved stability **Figure 2.10**.

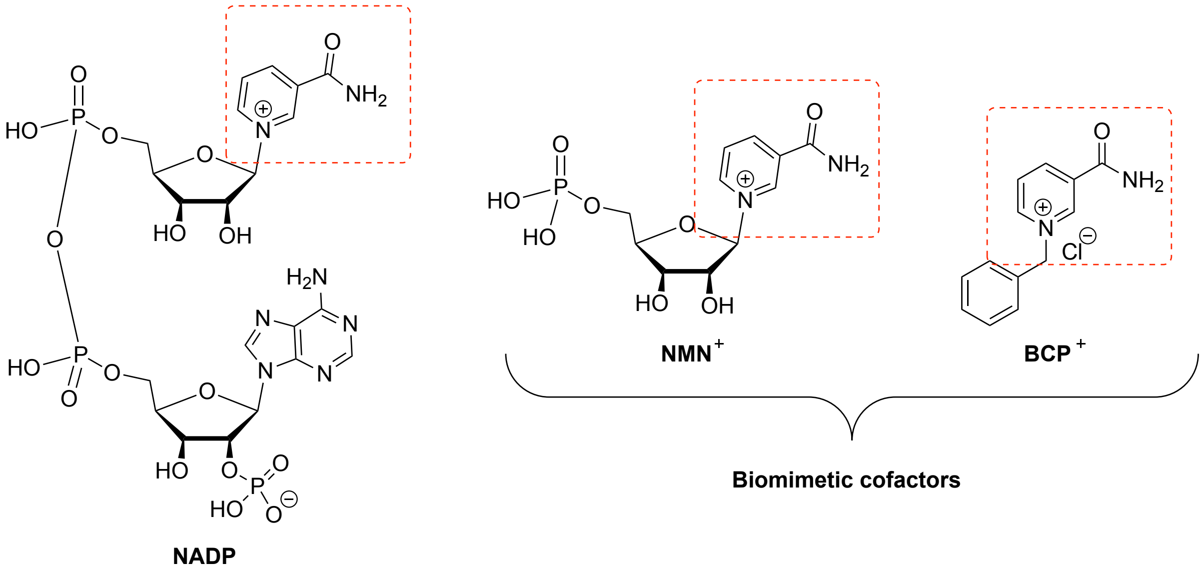


Figure 2.10. Active site of NADP and its biomimetic cofactors NMN and BCP.

2.5. Plant cell culture for chemical production

Plant cell culture technology has its origins in the theory of cellular totipotency, proposed by the German botanist Haberlandt in 1902. According to this theory, a single cultivated plant cell, containing all the necessary genetic information, has the potential capacity to regenerate the entire plant. This paved the way for the possibility of in vitro cultivation of plant cells.

Gottlieb Haberlandt
1854-1945

2.5.1. Plant secondary metabolites

Secondary metabolites, also known as secondary products or natural compounds, are small molecules produced by organisms to survive in hostile environments or to resist the physical aggression of pathogens, even if they are not essential to the organism's survival. These compounds can be synthesised by numerous micro-organisms, plants, fungi and animals.

Of the 30,000 or more natural products identified worldwide, around 80% come from plant sources. The number of known chemical structures of secondary plant metabolites is four times greater than that of known microbial secondary metabolites. These secondary plant metabolites are widely used in various fields such as medicine (e.g. paclitaxel, vinblastine, camptothecin, ginsenosides, artemisinin), food additives, flavourings, spices (e.g. rose oil, vanillin), pigments (e.g. Sin red and anthocyanins), cosmetics (e.g. aloe polysaccharides) and bio-pesticides (e.g. pyrethrins).

Currently, around a quarter of all medicines prescribed in industrialised countries are derived directly or indirectly from plants, either by direct extraction or semi-synthesis. In addition, 11% of the 252 drugs considered essential by the World Health Organisation (WHO) are derived exclusively from plants. Depending on their biosynthetic pathways, secondary metabolites are generally classified into three main families of molecules: phenols, terpenes and steroids (**Figure 2.12)**.

2.5.2. Plant cell culture technology

Plant cells in culture can efficiently convert natural or artificial compounds into secondary metabolites through a variety of reactions. This method of biosynthesis is safe, controllable and cost-effective compared with traditional field cultivation. Over the last 50 years, plant cell culture technology has progressed rapidly, with the study of more than 400 plants and the isolation of more than 600 secondary metabolites from their cultured cells. Notably, the yield of secondary metabolite products from plant cell cultures often exceeds that of the corresponding original plants.

In 1983, shikonin (**Figure 2.13**) was successfully produced on a large scale from plant cell cultures, marking a significant breakthrough. Efforts focused on the use of plant cell, tissue and organ cultures as an alternative method to whole plant cultivation for the production of pharmacologically important secondary metabolites. Bioreactor culture of adventitious roots has shown promising results, offering high proliferation rates and stable production of valuable compounds.

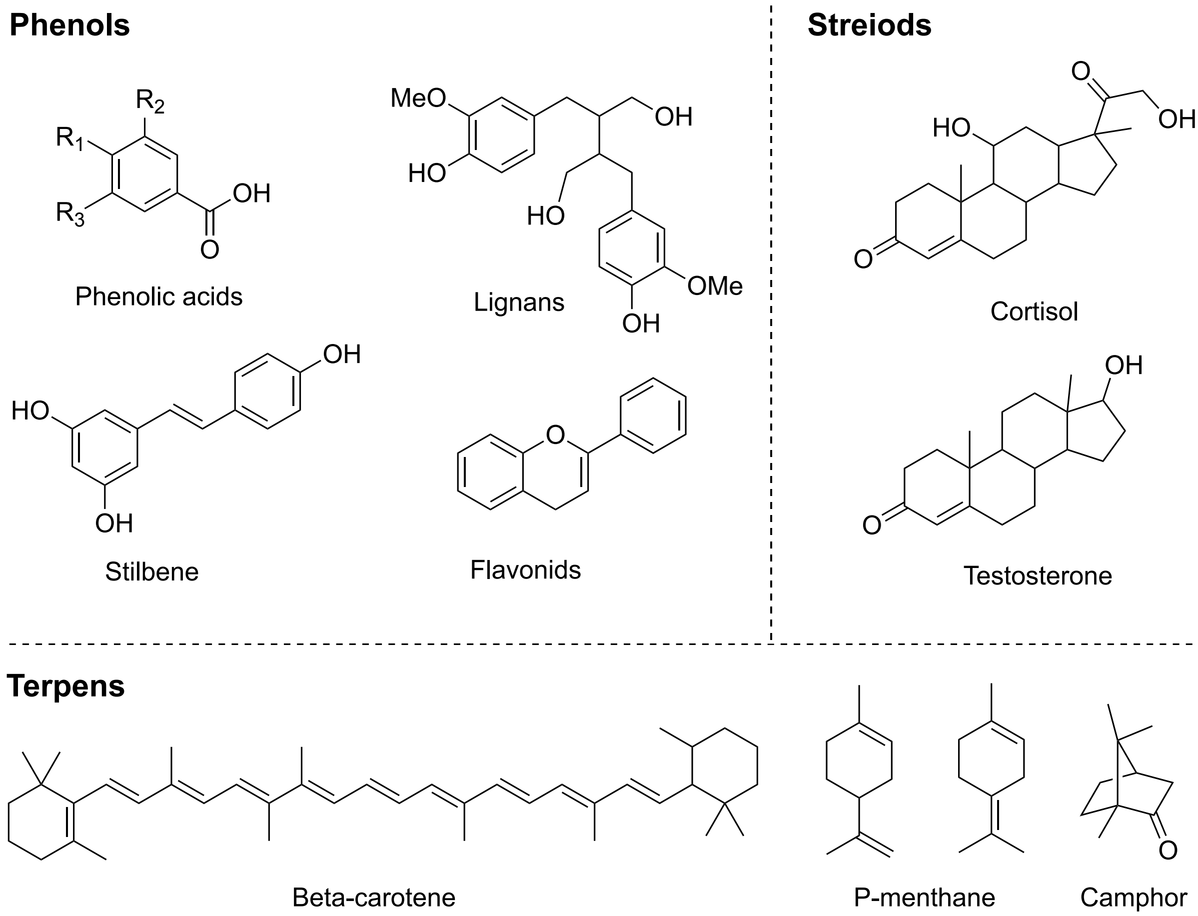
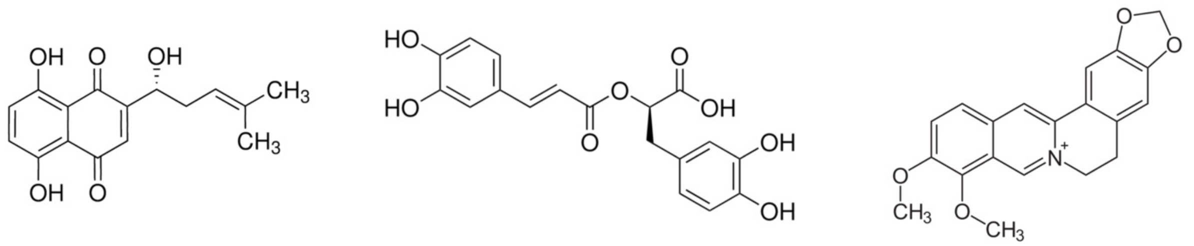


Figure 2.11. Molecular structure of represented molecules of the three main families of plant secondary metabolites.

Plant cell culture technology, such as the production of rosmarinic acid and berberine (**Figure 2.12**), has been commercialised. However, challenges remain, including the limited industrial application of the production of natural active compounds due to low yields. Researchers are working to resolve this problem through the breeding of high-quality cell lines, training and genetic improvement.

Chemical synthesis of compounds from plant resources is often economically impractical due to their complexity. Biotechnological production in plant cells or organ cultures offers an alternative, but the low content of these compounds limits their commercial application. Direct extraction of secondary metabolites from naturally growing plants carries the risk of resource depletion. Large-scale cultivation for the production of secondary metabolites in plants is difficult to control in terms of quality and yield. It is therefore necessary to explore better methods for the sustainable production of plant secondary metabolites.



Shikonin **Rosmarinic acid** **Berberine**

Figure 2.12. Molecular structure of shikonin, rosmarinic acid, and berberine.

2.5.3. Strategies for improving the production of secondary metabolites in medicinal plants

In the quest to optimize the production of secondary metabolites from medicinal plants, various biotechnological strategies have been implemented with promising results. These innovative approaches include the selection of high-yield cell lines, the adjustment of culture conditions, precursor feeding and the application of elucidation treatments. Each method is strategically designed to raise the efficiency and yield of valuable compounds.

Selection of high-yield cell lines: The identification and establishment of high-yielding, fast-growing cell lines is a crucial strategy. This involves identifying cell lines capable of exceeding the production levels observed throughout the plant. In addition, inducing mutations to improve metabolite levels presents a viable route, with the careful selection of analogues playing a crucial role in diversifying the range of products produced.

Optimal culture conditions: Modifying the nutritional ingredients of plant cells, such as minerals and natural hormones, is a powerful way of improving the way they grow. By adjusting the quantity, proportion and form of these ingredients, you can significantly influence the total amount that plants produce. Maintaining ideal conditions is crucial, as imbalances, such as too much of a certain hormone which stimulates growth but blocks the production of other important substances, can interfere with the way it all works.

Precursor feeding: The precursor feeding strategy is based on increasing the concentrations of intermediates or initial compounds in the biosynthetic pathway. This increases the potential to improve the yield of the final product. Successful examples include the use of ferulic acid in Vanilla planifolia cell culture, resulting in increased vanillin accumulation, and the restoration of anthocyanin synthesis in Daucus carota by the addition of dihydroquercetin (naringenin).

Elucidation treatment: Elicitors, defined as substances that initiate plant cell stress responses and influence cell metabolism processes at minimal concentrations, serve as a valuable tool. Elucidation is used to induce or amplify the biosynthesis of secondary metabolites by introducing minute quantities of these elicitors. Although cell cultures from various plants often show low production of secondary metabolites, the application of elicitors such as methyl jasmonate, salicylic acid, chitosan and heavy metals has proved effective in improving the productivity of undifferentiated cells.

2.5.4. Elicitors in plant cell culture

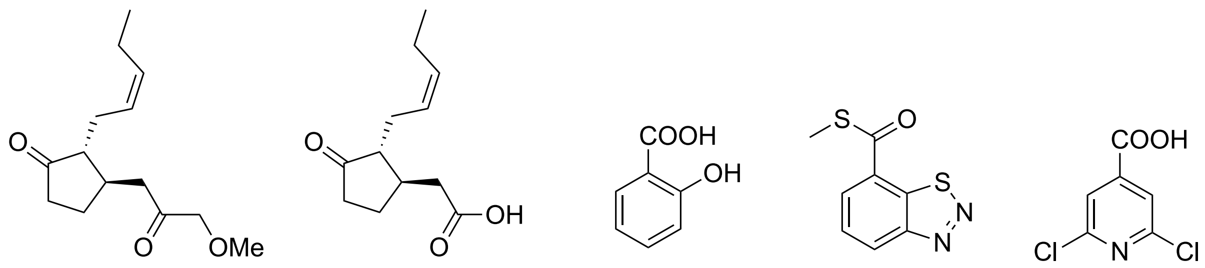
Elicitation is a process by which plants induce or enhance the synthesis of secondary metabolites that are essential to their survival and competitiveness in response to environmental changes. This is achieved by adding elicitors, substances that selectively induce the expression of specific genes in plants, leading to the accumulation of particular secondary metabolites. In the field of plant cell culture technology, successful application relies on improving the production of these valuable compounds. Elicitor treatment has proved to be one of the most effective approaches, significantly increasing the yield of useful secondary metabolites. The mechanism of action involves elicitors acting as external stimuli, binding to cell membrane receptors, inducing changes in the membrane, regulating gene expression and acting as secondary messengers to facilitate changes in enzyme activity.

2.5.5. Examples of chemical elicitors

Since 1962, (−)-jasmonic acid methyl ester (−)-MJA has been recognized as a fragrant component in the essential oils of Jasminum and other plants. The free acid form (−)-JA was isolated in 1971 and identified as a growth inhibitor in various higher plants (**Figure 2.13**). Currently, JA and its stereoisomers are major representatives of jasmonates, a group of native plant regulators with widespread presence in the plant kingdom. Jasmonates play crucial roles in plant physiology, affecting growth, development, environmental responses, and defense against herbivores and pathogens. Recent studies have contributed to understanding the biosynthesis, metabolism, and modes of action of natural JA analogues. Among various elicitors, exogenous MJA has been confirmed as effective in inducing secondary metabolites in plant cell cultures. Studies have demonstrated its role in significantly increasing the production of compounds such as paclitaxel in Taxus cuspidata and various alkaloids in different plant species.

In addition to jasmonates, other compounds known for inducing systemic acquired resistance (SAR) in plants have been identified as elicitors in plant cell cultures. Salicylic acid (SA, **Figure 2.13**), synthesized through the phenylpropanoid pathway, serves as a key signaling component activating plant defense responses. While considered a potential plant hormone, high concentrations of SA can be toxic to plants, limiting its application in disease control. Exogenous SA has been shown to stimulate secondary metabolism in certain plant species, enhancing the production of phytoalexins[*] and influencing the synthesis of various compounds. Another SAR activator, S-Methyl benzo-1,2,3-thiadiazole-7-carboxylate (BTH, **Figure 2.13**), shares defense response induction with SA and has been found to induce secondary metabolism in plant cell cultures. Similarly, compounds like 2,6-dichloro-isonicotinic acid (INA, **Figure 2.13**) and its methyl ester can induce SAR in plants, although INA's cytotoxicity poses limitations even within its effective concentration range.

[*] Phytoalexins are antimicrobial compounds produced by plants in response to infection or other forms of stress. These substances serve as a part of the plant's defense mechanisms against pathogens such as bacteria, fungi, and viruses. When a plant is under attack or stress, it can activate specific biochemical pathways that lead to the synthesis and accumulation of phytoalexins.



SA BTH INA

(–)-MJA (–)-JA

Figure 2.13. Molecular structure of some chemical elicitors.

2.5.6. Production of paclitaxel as an example of the application of chemical elicitors

Paclitaxel is a chemotherapy drug commonly used to treat various types of cancer, including breast cancer, ovarian cancer, lung cancer and Kaposi's sarcoma. It belongs to a class of drugs known as taxanes and is derived from the bark of the Pacific yew tree (Taxus brevifolia, **Figure 2.14**). Paclitaxel acts by interfering with the normal functioning of microtubules, which are cellular structures involved in cell division and replication.

In the quest for efficient and sustainable production of paclitaxel, various methods, such as total chemical synthesis, semi-synthesis, and extraction from natural sources, have been explored. However, challenges, including the complexity of the molecule and environmental concerns related to mass deforestation for yew tree extraction, have prompted the exploration of alternative methods. Among these, plant cell culture, particularly through the use of elicitors, has emerged as a promising avenue for paclitaxel production.

Cell culture processes have been adapted to cultivate plant cells and produce paclitaxel and its taxane precursor, baccatin III (**Figure 2.14)**. While initially promising, the yields obtained from cell cultures were insufficient for industrial applications. Consequently, there has been a pressing need to enhance paclitaxel production on an industrial scale.

Elicitation has played a crucial role in improving paclitaxel yields in cell cultures. Researchers, such as Yukimune et al., have demonstrated the significance of elicitation in enhancing paclitaxel production. For instance, the addition of 100 µM Methyl Jasmonate (MJA) resulted in a 5.1-fold increase in paclitaxel production, reaching 115.2

mgL^{-1}. This breakthrough suggests that elicitation can be a key factor in overcoming the limitations of plant cell cultures.

To further optimize elicitation strategies, researchers have explored synthetic derivatives of MJA. Novel compounds, such as 2-hydroxyethyl jasmonate (HEJA) and trifluoroethyl jasmonate (TFEJA), were synthesized and evaluated as elicitors. The results indicated that these derivatives significantly increased the accumulation of taxuyunnanine C (Tc). For example, the addition of 100 μM HEJA or TFEJA induced high Tc production, reaching 47.2 mg g^{-1} or 39.7 mg g^{-1}, compared to 13.7 mg g^{-1} for the control. These findings suggest that the development of novel jasmonate analogues holds great potential for enhancing paclitaxel production in plant cell cultures.

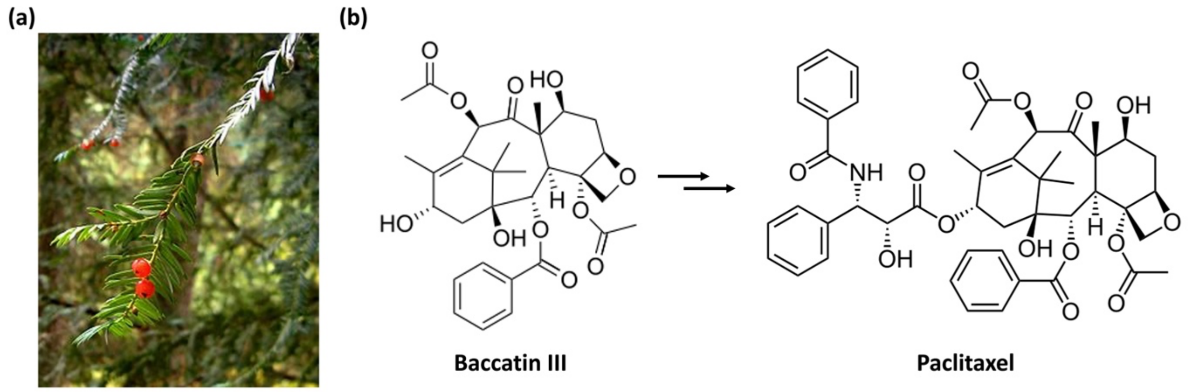


Figure 2.14. (a) illustration of *Taxus brevifolia* and (b) chemical structure of paclitaxel and its natural precursor baccatin III.

Moreover, the application of unnatural elicitors, such as BTH derivatives and INA derivatives, has proven successful in regulating the biosynthesis of secondary metabolites in Taxus cells. This chemical bioengineering strategy not only lays the foundation for the industrial production of paclitaxel but also holds promise for other bioprocess systems. The exploration of elicitors in plant cell cultures represents a crucial step toward developing sustainable and scalable methods for paclitaxel production, addressing both environmental concerns and the growing demand for this important chemotherapy agent.

Check your understanding

1. Which of the following is not a component of chemical biotechnology?
    A. Chemistry
    B. Biology
    C. Physics
    D. Geology
2. What is the potential of chemical biotechnology in agriculture?
    A. Enhancing the production of plant secondary metabolites
    B. Decreasing the production of plant secondary metabolites
    C. No effect on the production of plant secondary metabolites
    D. Increasing the environmental concerns of plant secondary metabolite production
3. What is the potential of chemical biotechnology in industry?
    A. No effect on the production of chemicals
    B. More expensive and less sustainable production of chemicals
    C. Greener and more cost-effective production of chemicals
    D. Increased use of non-renewable resources in the production of chemicals
4. What is the potential of chemical biotechnology in overcoming resource depletion?
    A. Developing better methods for sustainable production
    B. Increasing resource depletion
    C. No effect on resource depletion
    D. Decreasing the demand for chemicals
5. What is the potential of chemical biotechnology in addressing environmental concerns?
    A. Developing greener production methods
    B. Increasing environmental concerns
    C. No effect on environmental concerns
    D. Decreasing the demand for chemicals
6. Which of the following is not a benefit of chemical biotechnology?

A. Greener production
B. More cost-effective production
C. Overcoming resource and environmental crises
D. Increased use of non-renewable resources

7. What is the significance of elicitation in plant cell cultures?
    A. No effect on plant cell cultures
    B. Reducing the production of secondary metabolites
    C. Overcoming limitations of plant cell cultures
8. What progress has been made with the use of elicitation strategies?
    A. Increased production of secondary metabolites
    B. Decreased production of secondary metabolites
    C. No effect on production of secondary metabolites
9. What is the potential of exploring elicitors in plant cell cultures?
    A. Decreasing the demand for natural products
    B. No effect on natural products production
    C. Developing sustainable and scalable methods for natural products production
    D. Increasing the environmental concerns of natural products production
10. What is chemical biotechnology?
11. What is the potential of chemical biotechnology in agriculture?
12. What is the significance of elicitation in plant cell cultures?
13. What is the potential of chemical biotechnology in industry?
14. What are non-canonical DNA structures?
15. What is the potential of chemical biotechnology in addressing environmental concerns?
16. What is the potential of exploring elicitors in plant cell cultures?
17. What is the significance of exploring plant cell cultures in drug discovery?
18. What is a secondary metabolite?
19. What is an elicitor?
20. What is an elicitation strategy?
21. What is a biocatalyst?

22. What is a transgenic organism?
23. What is a host-vector system?
24. What is a fermentation process?
25. What is a gene expression system?
26. What is a recombinant protein?
27. What is a transfection?
28. Answer true or false to the following statements
    A. Transgenic organisms are organisms that have been genetically modified by introducing foreign DNA into their genome, often for the purpose of expressing a desired trait or producing a specific compound.
    B. Enantioselective synthesis produces chemical compounds with a symmetrical molecular structure.
    C. Fermentation is a metabolic process in which microorganisms convert a substrate, such as sugar, into a desired product, such as ethanol or lactic acid, under controlled conditions.
    D. Metabolic engineering is the practice of modifying the metabolic pathways of microorganisms or other organisms to enhance the production of desired compounds, such as biofuels or pharmaceuticals.
    E. In biodiesel production, triglycerides are converted to biodiesel and glycerol by a transesterification reaction. The latter (glycerol) can be considered as a chemical modulator of the reaction.
    F. The non-canonical DNA structures are typical DNA structures (B-DNA).
    G. A biomimetic cofactor is a natural coenzyme.
29. Chemical biotechnology acts as a chemical modulator in:
    A. DNA replication
    B. Enzymatic reactions
    C. Photosynthesis
    D. Cell division
30. The scope of chemical biotechnology includes:
    A. Only macro-biomolecules
    B. Only cells

   C. Only plants
   D. Macro-biomolecules to cells and plants in various contexts

31. Chemical biotechnology serves as a biomimetic chemical cofactor in:
   A. Cellular respiration
   B. In vitro biosystems for high-value chemicals
   C. Protein synthesis
   D. Photosynthesis
32. What is the primary objective of asymmetric synthesis in the context of chemical biotechnology for enzymatic reactions?
   A. To produce symmetric molecules
   B. To produce molecules with identical chirality
   C. To selectively produce one enantiomer over the other
   D. To increase the overall reaction rate
33. How do enzymes contribute to asymmetric synthesis in chemical biotechnology?
   A. By speeding up all reactions equally
   B. By selectively catalyzing reactions to favor the formation of one enantiomer
   C. By inhibiting reactions to prevent racemization
34. Which term describes a molecule that is not superimposable on its mirror image?
   A. Isomer
   B. Enantiomer
   C. Diastereomer
   D. Racemate
35. Why is the selective production of a single enantiomer important in pharmaceuticals and agrochemicals?
   A. It is not important; both enantiomers are equally effective.
   B. The other enantiomer is toxic.
   C. Different enantiomers may exhibit different biological activities.
   D. It reduces the overall yield of the reaction.
36. What is the role of cofactors and coenzymes in biocatalytic reactions?
   A. They inhibit enzyme activity

B. They enhance enzyme stability
C. They compete with substrates for the active site
D. They are not involved in biocatalysis.

37. Explain the role of pH modifiers in modulating enzyme performance in biocatalytic reactions.
38. In the context of enzymatic transesterification for biodiesel production, how does the addition of co-solvents, such as methanol, act as a chemical modulator?
39. Describe the significance of substrate analogues in biocatalytic reactions. How can they be used to modulate enzyme activity?
40. Provide examples of chemical modulators used in the production of biodiesel through enzymatic transesterification. Explain their roles in enhancing the efficiency and selectivity of the reaction.
41. Discuss the importance of careful selection and optimization of chemical modulators in the commercial production of biodiesel through enzymatic transesterification. How does this contribute to sustainability and cost-effectiveness?
42. How do stabilizers contribute to biocatalytic reactions?
    A. By inhibiting enzyme activity
    B. By decreasing enzyme stability
    C. By improving enzyme stability in harsh conditions
    D. By competing with substrates for the active site
43. What is the primary role of biocatalysis in organic chemistry?
    A. To inhibit chemical reactions
    B. To enhance enzyme stability
    C. To accelerate and regulate chemical transformations
    D. To replace enzymes with chemical catalysts
44. Which class of enzymes is commonly involved in biocatalysis for organic synthesis?
    A. Ligases
    B. Oxidoreductases
    C. Isomerases

D. Kinases

45. What is a key advantage of using biocatalysts in organic synthesis?
    A. Limited substrate specificity
    B. Harsh reaction conditions
    C. Low reaction rates
    D. High stereoselectivity and enantioselectivity
46. What is the primary focus of chemical regulation in the context of non-canonical DNA structures?
47. Which type of chemical molecules are commonly involved in the regulation of non-canonical DNA structures?
    A. Lipids
    B. Proteins
    C. Enzymes
    D. Small molecules and ligands
48. What is the role of biomimetic NADP in white biotechnology?
    A. It inhibits enzyme activity
    B. It acts as a substrate in enzymatic reactions
    C. It enhances enzyme stability
    D. It functions as a cofactor in redox reactions
49. How does biomimetic NADP differ from natural NADP in enzymatic reactions?
    A. It has a different chemical structure
    B. It is less stable
    C. It is not involved in redox reactions
    D. It mimics the structure and function of natural NADP
50. In which type of reactions is biomimetic NADP commonly involved?
    A. Hydrolysis
    B. Oxidation-reduction
    C. Polymerization
    D. Isomerization
51. How does the yield of secondary metabolite products from plant cell cultures typically compare to the corresponding original plants?

A. It is lower
B. It is equal
C. It is unstable
D. It often exceeds that of the original plants

Answers

1. D
2. A
3. A
4. B
5. A
6. D
7. C
8. A
9. C
10. Chemical biotechnology is an interdisciplinary field that combines the principles of chemistry, biology, and physics to explore and manipulate biological mechanisms.
11. Chemical biotechnology has the potential to enhance the production of plant secondary metabolites, which can be used in the development of new drugs, flavors, and fragrances.
12. Elicitation is a process of stimulating plant cells to produce secondary metabolites by exposing them to chemical or physical stress. This process can overcome the limitations of plant cell cultures and enhance the production of secondary metabolites.
13. Chemical biotechnology promises greener, more cost-effective production of a variety of chemicals, overcoming the resource and environmental crises facing conventional chemical biology.
14. Non-canonical DNA structures refer to DNA arrangements or conformations that differ from the classic B-DNA double helix

15. Chemical biotechnology can develop greener production methods that reduce the environmental impact of chemical production.
16. Exploring elicitors in plant cell cultures can develop sustainable and scalable methods for the production of plant secondary metabolites, which can be used in the development of new drugs, flavors, and fragrances
17. Plant cell cultures can produce secondary metabolites that can be used in the development of new drugs and therapies.
18. A secondary metabolite is a compound produced by an organism that is not essential for its growth or reproduction but may have other functions, such as defense against predators or attraction of pollinators.
19. An elicitor is a substance that can stimulate plant cells to produce secondary metabolites by inducing stress responses and metabolic changes
20. An elicitation strategy is a method of stimulating plant cells to produce secondary metabolites by exposing them to chemical or physical stress, such as elicitors or precursors.
21. A biocatalyst is a biological molecule, such as an enzyme, that can catalyze chemical reactions in a living organism or in vitro.
22. A transgenic organism is an organism that has been genetically modified by introducing foreign DNA into its genome, often for the purpose of expressing a desired trait or producing a specific compound.
23. A host-vector system is a system used for the expression of recombinant proteins or other molecules in a host organism, such as a bacterium or yeast, using a vector, such as a plasmid or virus.
24. A fermentation process is a metabolic process in which microorganisms, such as bacteria or yeast, convert a substrate, such as sugar, into a desired product, such as ethanol or lactic acid, under controlled conditions.
25. A gene expression system is a system used to express a gene or a recombinant protein in a host organism, such as a bacterium or yeast, using a promoter, a ribosome binding site, and other regulatory elements.

26. A recombinant protein is a protein that is produced by genetically modifying a host organism, such as a bacterium or yeast, to express a foreign gene encoding the protein of interest.
27. Transfection is the process of introducing foreign DNA or RNA into eukaryotic cells, often for the purpose of expressing a recombinant protein or studying gene function.
28.
    A. True.
    B. False.
    C. True.
    D. True.
    E. False
    F. False
    G. False
29. B
30. D
31. B
32. C
33. B
34. B
35. C
36. B
37. pH modifiers play a crucial role in modulating enzyme performance in biocatalytic reactions by influencing the enzyme's catalytic activity, stability, and substrate specificity. Enzymes typically exhibit an optimal pH range at which they function most efficiently. Deviations from this optimal pH can result in reduced activity or denaturation.
38. The addition of co-solvents, such as methanol, in enzymatic transesterification for biodiesel production serves as a chemical modulator by enhancing the solubility of reactants, improving mass transfer, and altering reaction kinetics. Co-solvents can create a more favorable environment for enzyme-substrate

interactions, ultimately increasing the efficiency of the transesterification process.

39. Substrate analogues can modulate enzyme activity by influencing the conformation of the enzyme or by acting as cofactors. Modifying the structure of the analogue can refine the activity of the enzyme, making it more selective or more efficient at catalysing specific reactions.
40. During the production of biodiesel by enzymatic transesterification, several chemical modulators are used to improve efficiency and selectivity. Two key examples are co-solvents, in particular methanol, and acyl acceptors, such as dimethyl carbonate.

    Co-solvents (e.g. methanol):

    Role: Methanol acts both as a substrate and as a chemical modulator in enzymatic transesterification.

    Solubility enhancement: Methanol increases the solubility of triglycerides and enzymes, overcoming mass transfer limitations. The result is better interaction between the enzyme and substrate, improving reaction efficiency.

    Prevention of aggregation and denaturation: Methanol helps prevent enzyme aggregation and denaturation, contributing to greater stability during the transesterification process.

    Efficient reactions: By facilitating the solubility of the triglycerides and enzyme, methanol ensures more efficient reactions, resulting in higher biodiesel yields.

    Acyl acceptors (e.g. dimethyl carbonate) :

    Role: Acyl acceptors are chemical modulators added to the reaction mixture to complete transesterification and increase biodiesel yield.

    Completion of transesterification: Dimethyl carbonate, as an acyl acceptor, reacts with glycerol to form methyl carbonate and monoacylglycerol, completing the transesterification reaction.

    Increased yield: The addition of acyl acceptors ensures that all triglycerides are fully converted to biodiesel and glycerol, maximising biodiesel yield.
41. The selection and optimisation of chemical modulators in enzymatic transesterification for biodiesel production is crucial for sustainability and

profitability. Well-chosen modulators improve enzymatic activity, substrate conversion and overall efficiency, leading to higher biodiesel yields. This contributes to sustainability by maximising resource use and minimising waste. In addition, optimised modulators reduce production costs, making enzymatic transesterification economically viable and promoting the competitiveness of biodiesel on the market.

42. C
43. C
44. B
45. D
46. The primary focus of chemical regulation in non-canonical DNA structures is to use small molecules to stabilize or alter these structures, aiming to regulate their biological functions. This approach is employed for the development of therapeutic strategies against diseases linked to genetic instability, such as cancer, and for applications in diagnosis and therapy.
47. D
48. D
49. A
50. B
51. D

Chapter 3: Environmental biotechnology

3.1. Introduction

Environmental biotechnology is a type of science that uses the ideas of biotechnology to solve different environmental problems. It began by focusing on cleaning up waste water, but today it helps in many areas such as soil remediation, air purification and water pollution control. In simple terms, it's a combination of science and engineering that uses microscopic living organisms such as microbes and plants to improve the environment. The main aim is to use resources wisely and reduce damage to the environment.

Environmental biotechnology is very important for water purification, as well as preventing the formation of viscous films, rapidly identifying and controlling microscopic living organisms and using biological processes to clean up industrial wastewater. Scientists, engineers and industry experts are working together in this field to create new and intelligent technologies in sectors such as agriculture, industry and healthcare.

One example of an application is the use of genetically modified bacteria that consume oil in the oceans and clean up oil spills, showing how environmental biotechnology can find intelligent solutions to major environmental problems. Thus it is a link between what we know scientifically and what we do in practice to preserve the safety and health of our environment.

3.2. Applications of environmental biotechnology

Currently, advanced technology is allowing us to create treatment methods that generate less waste and conserve natural resources. They can also be used to eliminate and break down harmful contaminants through the use of micro-organisms. Here are the main kinds of applications for environmental biotechnology:

3.2.1. Bioenergy

Bioenergy, a category encompassing fuels like biogas, biomass, and hydrogen, is utilized for both industrial and domestic purposes. This form of energy is considered a clean and competent alternative, addressing the growing need for sustainable energy resources. An exemplary instance of bioenergy is the production of energy from biomass, a prime example of green energy that offers eco-friendly solutions to pollution problems.

3.2.2. Bioremediation

Bioremediation stands as a pollution control technology that harnesses the power of natural biological species to catalyze the degradation of harmful chemicals into less toxic forms. Microorganisms, including bacteria, fungi, and algae, play a crucial role in breaking down and transforming pollutants such as azo dyes in wastewater. This approach is not only cost-effective but also environmentally friendly, providing an efficient method for cleaning up toxic pollutants.

3.2.3. Biotransformation

Biotransformation, a biological process, involves altering complex compounds from toxic to non-toxic forms. Particularly relevant in manufacturing industries where toxic by-products are released, biotransformation serves as a valuable tool for mitigating the environmental impact of industrial processes. By transforming toxic substances into harmless forms, this method contributes to reducing environmental harm.

3.2.4. Biomarker

Biomarkers, as defined in environmental biotechnology, are observable properties within an organism that indicate variations in cellular or biochemical components. These measurable indicators play a crucial role in estimating past exposure, identifying changes and assessing the sensitivity of an organism to environmental stressors. As quantitative measures, biomarkers provide early warnings of biological effects, contributing to our understanding of how chemicals interact with molecular targets. They form part of a continuum, from exposure to clinical disease, and can include markers of internal dose, biologically effective dose and early biological effects. Validation is crucial, as it involves sensitivity and specificity assessments. Despite the difficulties associated with biomarker validation, biomarkers are invaluable tools, as demonstrated by their successful use in aflatoxin exposure risk assessment[*].

[*] The risks of exposure to aflatoxins relate to the potential health hazards associated with eating food contaminated with aflatoxins, toxins produced by some fungi. Aflatoxins, particularly aflatoxin B1, are known carcinogens and can lead to liver cancer.Chronic exposure can suppress the immune system and cause growth problems.Acute poisoning can cause symptoms such as abdominal pain and vomiting.Prevention involves agricultural practices, proper storage and regulatory measures.Biomarkers, such as aflatoxin-albumin adducts and DNA adducts, can be used to assess exposure levels and the resulting health effects.

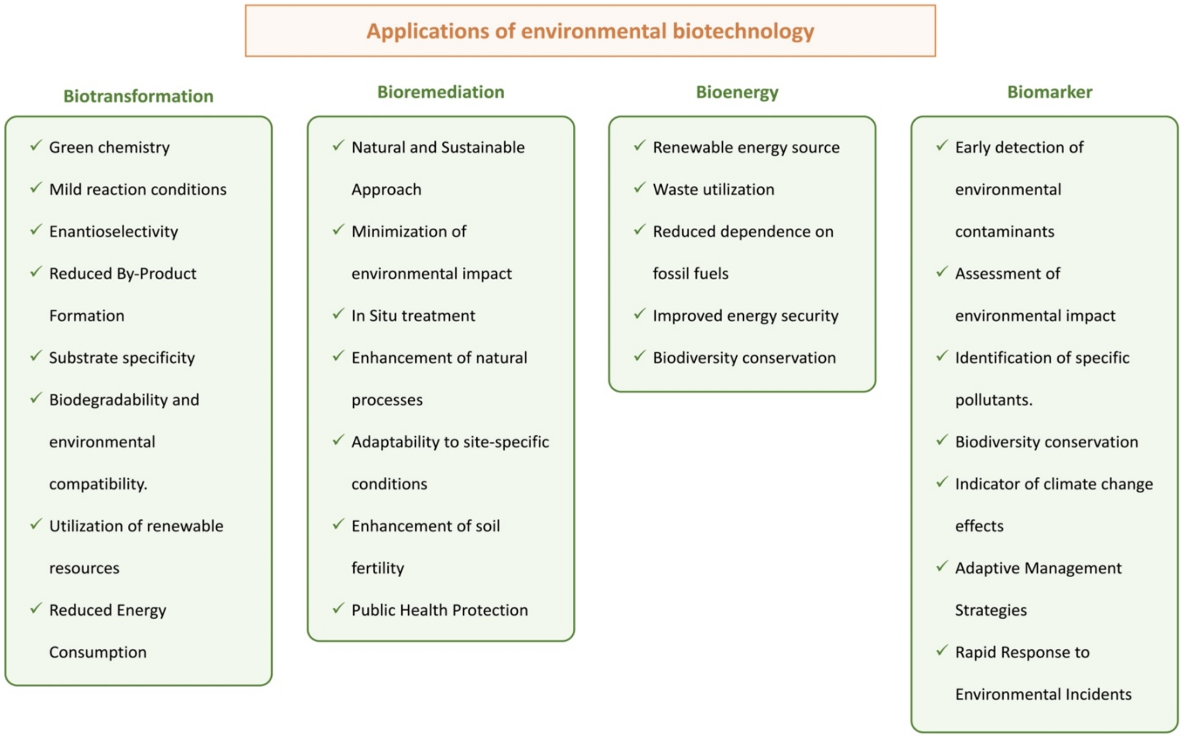


Figure 3.1. Main applications of environmental biotechnology and some of their benefits to human society and the environment.

3.3. Bioremediation concept

Bioremediation is a contemporary, environmentally friendly strategy for purifying ecosystems of harmful pollutants. The US Environmental Protection Agency defines bioremediation as "the use of living organisms to clean or remove pollutants from soil, water or wastewater". This involves the use of non-harmful insects to combat agricultural pests and diseases affecting trees, plants and garden soil. Bioremediation involves green plants and micro-organisms working together to detoxify or eliminate inorganic pollutants from the environment. Acting as natural purifiers, green plants absorb pollutants through their roots and store them in their leaves, facilitating a sustainable purification process. Unlike conventional methods such as soil excavation or incineration, bioremediation provides a sustainable solution on site rather than simply moving the problem.

This innovative technique has proven its effectiveness in the remediation of heavy metals, metalloids and various inorganic pollutants present in soil and water. This approach is not only effective, but also cost-effective, opening up a new, environmentally-friendly era in pollution mitigation. Using solar technology, bioremediation has gained widespread public acceptance, unlike traditional engineering methods such as soil washing, rinsing and solidification. Its success is based on the complex interaction of factors involving plants, microbes and the physico-chemical properties of the soil or water. The effectiveness of bioremediation underlines the ability of living organisms to absorb, accumulate, sequester, translocate and detoxify pollutants, making it a promising solution for a cleaner, more sustainable future.

3.4. Types and strategies of bioremediation

The concept of bioremediation encompasses plant- and microbe-based approaches, specifically phytoremediation and microorganism remediation. These strategies exhibit distinct processes and mechanisms through which plants and microbes immobilize, remove, or degrade pollutants. The main bioremediation strategies are summarized in **Figure 3.2**.

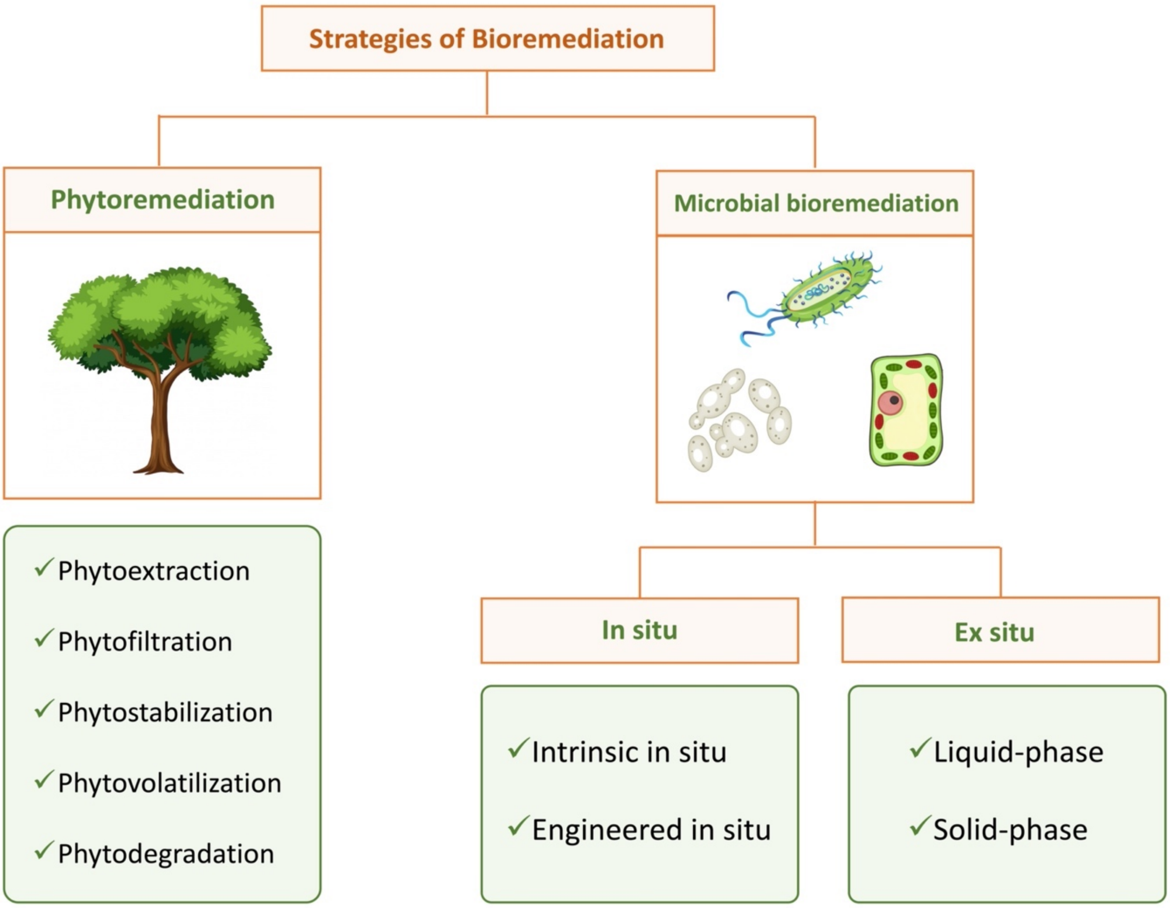


Figure 3.2. Main strategies of bioremediation.

3.4.1. Phytoremediation

Some of the primary phytoremediation methods utilized for the restoration of polluted environments are depicted in **Figure 3.3.**

3.4.1.1. Phytoextraction

Phytoextraction is a method where plants remove pollutants from soil or water through their roots, moving them to the aboveground parts, like shoots, which are then harvested. This process is crucial for effective phytoextraction because harvesting root biomass is usually impractical. There are five main steps involved: pollutant mobilization in the rhizosphere, uptake by plant roots, translocation to aboveground parts, and storage in plant tissues. Ideal plants for phytoextraction, called hyperaccumulators, have traits like rapid growth, high aboveground biomass, tolerance to pollutants, well-distributed roots, adaptability to the environment, pollutant translocation ability, and resistance to diseases. The effectiveness of phytoextraction is influenced by factors like root depth, seasonal weather, and climate conditions.

3.4.1.2. Phytofiltration

Phytofiltration, also known as rhizofiltration, involves the adsorption or precipitation of pollutants onto plant roots or their absorption into the root zone. Chemical synthesis within the roots enhances adsorption, with terrestrial plants preferred for their extensive root architecture. The success of rhizofiltration relies on a thorough understanding of contaminant speciation and interactions. Ideal plants for rhizofiltration should possess rapidly growing roots capable of removing contaminants over extended periods.

3.4.1.3. Phytostabilization

Phytostabilization, or phytoimmobilization, employs plants to decrease the mobility or bioavailability of pollutants, preventing their leaching into groundwater or entry into the food chain. This process aims to stabilize pollutants in the root zone, reducing their hazard to human health and the environment. Although not a permanent solution, phytostabilization has advantages over other techniques, such as lower translocation to shoots, making it effective for rapid immobilization and preservation of ground and surface waters.

3.4.1.4. Phytovolatilization

Phytovolatilization involves the uptake of contaminants by plants, conversion into volatile compounds, and release into the atmosphere through transpiration. This strategy is applicable to organic pollutants and certain gaseous heavy metals. Genetically modified plants are often used to enhance metal volatilization. However, phytovolatilization has limitations, as it does not completely remove pollutants but transfers them from soil or water to the atmosphere, posing potential return to the ecosystem through precipitation. Despite controversy, its advantages include minimal erosion and no disposal of contaminated plant biomass.

3.4.1.5. Phytodegradation

Phytodegradation, or phytotransformation, involves capturing contaminants from water, sediment, or soil and chemically modifying them through plant metabolism. Some plants can degrade absorbed contaminants into less toxic compounds. This metabolic strategy contributes to the detoxification and degradation of contaminants within plant tissues.

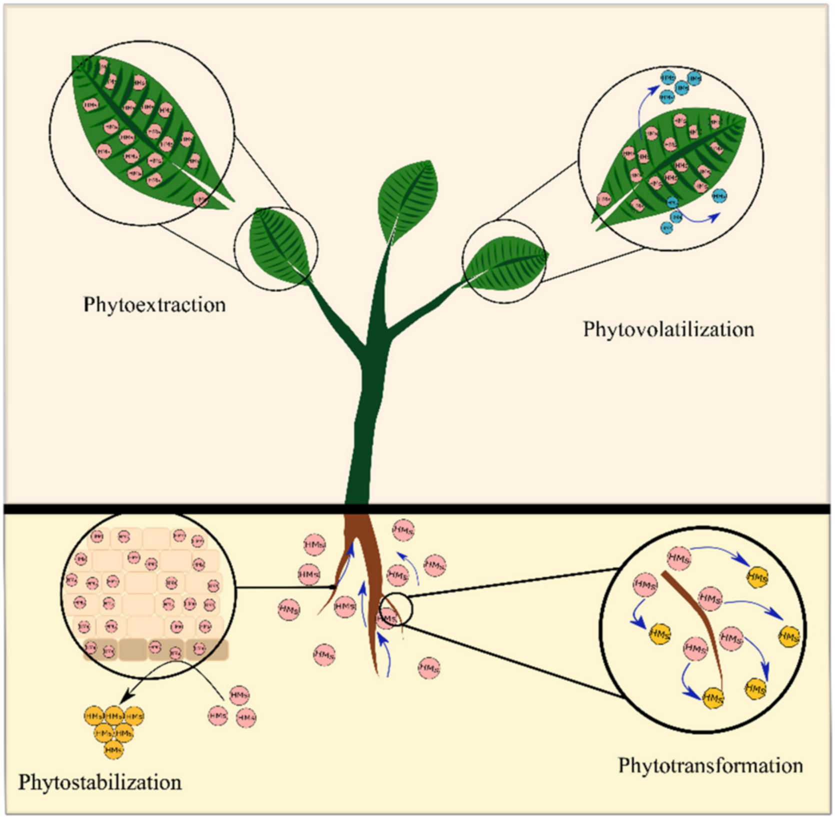


Figure 3.3. illustration of the primary phytoremediation techniques employed for remediating polluted environments. From: Int. J. Mol. Sci. 2022, 23(9), 5031.

3.4.2. Microbial bioremediation

Microbial bioremediation is an environmentally friendly method that uses a variety of microbes, including bacteria and fungi, to sequentially neutralise and detoxify toxic contaminants. Essentially, it accelerates natural metabolic processes, with micro-organisms or their enzymes breaking down pollutants into less harmful by-products such as carbon dioxide, water, microbial biomass and inorganic salts. This transformation process makes microbial remediation the technology of choice for environmental decontamination, enabling contaminated sites to be effectively restored under sustainable conditions. The modalities of bioremediation can be divided into two main categories, in situ and ex situ

3.4.2.1. In situ microbial bioremediation

In situ bioremediation is an environmentally important approach that refers to a process carried out at the site of origin of the contamination, primarily targeting pollutants in saturated soils and groundwater. This method involves the introduction of oxygen (O_2) and essential nutrients, mainly sources of carbon and nitrogen, into the contaminated environment, which promotes microbial growth and increases the rate of biodegradation. The microbial consortia involved may be indigenous or deliberately introduced because of their specialized expertise in degrading pollutants. The phenomenon of chemotaxis* plays an essential role in in situ bioremediation, where microbes move towards or away from specific chemicals, contributing to the efficient in situ degradation of pollutants.

In categorising in situ bioremediation, two main types are identified: **Intrinsic in situ bioremediation** and **engineered in situ bioremediation**. Intrinsic in situ bioremediation, also known as natural attenuation or passive bioremediation, illustrates a degradation process whereby organic compounds present in contaminated soil undergo natural attenuation by indigenous microbial communities, without artificial

* The phenomenon of chemotaxis refers to the directed movement of cells, such as microorganisms like bacteria or other single-celled organisms, in response to chemical gradients in their environment. Essentially, these cells can sense and respond to changes in the concentration of certain chemicals, either by moving toward the source of an attractive chemical or away from a repellent one.
In the context of bioremediation chemotaxis plays a significant role. Microbes with chemotactic abilities can actively move toward areas where contaminants are present, thus enhancing the in situ degradation of pollutants. This movement allows the microorganisms to efficiently locate and target the pollutants, contributing to the overall effectiveness of bioremediation processes in cleaning up contaminated sites.

augmentation. The success of intrinsic bioremediation depends on four fundamental prerequisites:

- a robust population of biodegrading microbes,
- the availability of the necessary nutrients,
- the maintenance of ideal environmental conditions,
- the allocation of sufficient time for natural microbial processes to deplete contaminants.

Intrinsic bioremediation is conceptually aligned with monitored natural attenuation, in which various natural processes act synergistically to reduce the toxicity, mass, volume, mobility or concentration of pollutants, without direct human intervention.

In contrast, engineered in situ bioremediation, also known as accelerated in situ bioremediation, involves the deliberate introduction of indigenous microbes into contaminated sites, enhancing the biodegradation process by optimizing physico-chemical conditions. Artificial bioremediation encompasses techniques such as *bioventing*, *biosparging*, *bioslurping*, *biostimulation* and *bioaugmentation*, strategically employed to address various contaminant scenarios. These methods illustrate the confluence of scientific innovation and environmental management, showing that it is possible to mitigate pollution and restore ecosystems to a state of sustainable equilibrium.

3.4.2.2. Ex situ microbial bioremediation

Ex situ bioremediation, a complex method for cleaning up contaminated environments, involves extracting polluted materials such as soil, sediment or sludge and enhancing the microbial degradation of the pollutants. This method can be applied in two ways: **liquid-phase bioremediation** and **solid-phase bioremediation**.

In liquid-phase bioremediation, contaminated soil, sludge or sediment is mixed with water and chemicals in a special container called a bioreactor. This device creates three phases - liquid, solid and gas - and maintains conditions that help the microbes to break down the pollutants more quickly. Oxygen and nutrients are then added to the mixture to create ideal conditions for microbial activity. Once the process is complete, we remove the water, assess the treated soil and reinject it into the environment. This method is faster because the closed environment is easier to manage and control.

Solid-phase bioremediation, on the other hand, treats contaminated soil in an above-ground area that is closely monitored to ensure optimum treatment. Although easy to maintain, it requires more space and takes more time than liquid-phase bioremediation. In solid-phase bioremediation, polluted soil is broken down mechanically in a closed container, ensuring continuous contact between nutrients, microbes, pollutants, oxygen and water. Techniques such as *biopiling*, *land farming* and *composting* are used to detoxify and break down hazardous contaminants.

Biopiling involves mixing excavated soil, sludge or sediment with soil improvers and using forced aeration to accelerate the activity of aerobic microbes. This method is particularly useful for cleaning up various materials contaminated by hydrocarbons.

Land farming involves spreading contaminated soil, sediment or sludge over a prepared bed and turning it over to allow aeration. This allows aerobic microbial activity to break down the pollutants, particularly in the top layer of soil.

Composting is a process whereby organic waste is transformed into stable organic matter, or compost, by microbes. In this method, contaminated soil is mixed with organic matter, creating an environment conducive to the decomposition of pollutants by microbes. Composting is an effective way of transforming organic waste into safe compost.

3.5. Different forms of environmental pollution

The various forms of pollution caused by intoxication depend on the polluted environment and the types of pollutants involved. These various forms of environmental damage include:

Atmospheric pollution: This is characterised by the presence of noxious particles or gases in the air, and can cause a variety of problems depending on their concentration. Examples: carbon, sulphur and nitrogen oxides.

Biological pollution: Resulting from the introduction of foreign species or genetically modified organisms. Examples: invasive species, algal blooms.

Chemical pollution: caused by the presence of chemical substances in the environment that are normally absent or present in small quantities. For example, organic pollution caused by organic pollutants, such as sewage, manure and organochlorine chemicals.

Chronic pollution: Permanent pollution resulting from repeated or continuous emissions of pollutants. Example: radioactive waste.

Diffuse pollution: Caused by the dispersion of multiple pollutants over time and space, it is less visible than accidental pollution. Examples: nitrates, pesticides.

Water pollution: Occurring in oceans, seas, lakes, rivers, etc., it involves the presence of toxic elements. Examples: industrial effluents, agricultural chemicals.

Electromagnetic pollution: Resulting from excessive exposure to electromagnetic fields, potentially harmful to health and the operation of equipment. Also known as electromagnetic smog.

Genetic pollution: Introduction of foreign or modified genes into a wild species, in particular through transgenic genetically modified organisms

Industrial pollution: Linked to industrial activities, it affects the ecosystem. Examples: gas emissions, chemicals.

Light pollution: Resulting from an overproduction of light at night, it can disrupt the environment and biological rhythms. Mainly caused by urban street lighting.

Radioactive pollution: Resulting from contamination by radioactivity, which can be caused by nuclear explosions, accidents in nuclear power stations or radioactive waste.

Soil pollution: caused by the infiltration of polluted water, often of industrial or agricultural origin.

Land-based pollution: Pollution from land-based sources in seas and oceans, transported by rivers and sewers.

Thermal pollution: Increase in water temperature due to cooling water discharges, particularly from thermal and nuclear power stations.

3.6. Environmental contaminants treated by bioremediation

Bioremediation techniques can be used to treat a wide range of contaminant chemicals that have been introduced into water, soil and air, causing significant environmental problems. As illustrated in **Figure 3.4** these hazardous chemicals includes:

Petroleum Hydrocarbons: This includes compounds found in crude oil, such as benzene, toluene, ethylbenzene, and xylene (BTEX), as well as polycyclic aromatic hydrocarbons (PAHs). Microorganisms can metabolize these hydrocarbons, breaking them down into less harmful substances.

Heavy Metals: Certain bacteria and plants have the ability to accumulate and detoxify heavy metals, such as lead, mercury, cadmium, and chromium.

Pesticides and Herbicides: Agricultural chemicals, such as pesticides and herbicides, can be degraded by microorganisms. For example, certain bacteria can break down organophosphates and other synthetic chemicals commonly used in agriculture.

Chlorinated Solvents: Compounds like trichloroethylene (TCE) and perchloroethylene (PCE), which are common industrial solvents, can be treated through bioremediation. Some bacteria can use these compounds as a source of carbon and energy.

Nitrates and Nitrites: Contamination of groundwater with nitrates and nitrites, often from agricultural runoff, can be remediated by bacteria that convert these compounds into nitrogen gas through a process called denitrification.

Organic Solvents: Various organic solvents, including industrial chemicals like chloroform and benzene, can be broken down by microorganisms in the environment.

Polychlorinated Biphenyls (PCBs): These are synthetic organic chemicals that were commonly used in electrical equipment. Certain bacteria have the ability to break down and detoxify PCBs.

Radioactive Contaminants: Some microbes can assist in the remediation of environments contaminated with radioactive materials. They can either immobilize or solubilize certain radionuclides.

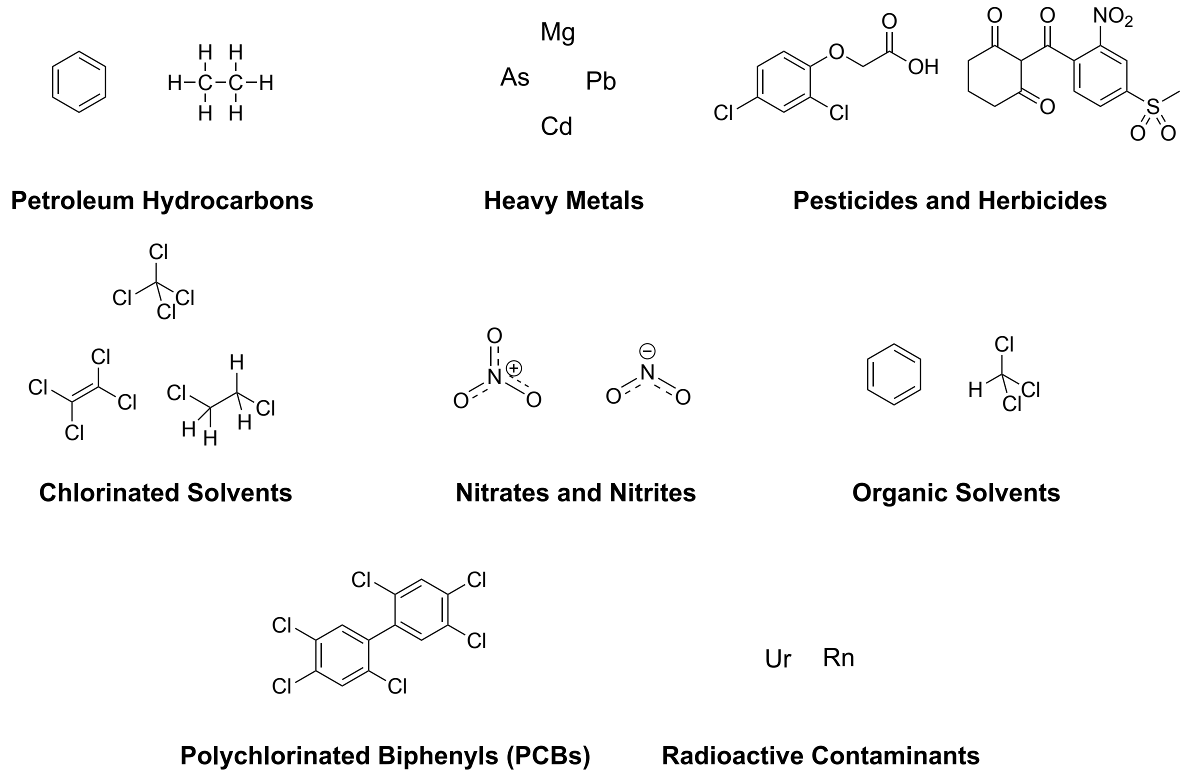


Figure 3.4. Chemical structure of some environmental contaminants treated by bioremediation

3.7. Biological wastewater treatment

Wastewater is defined as water that has been used in various human activities, such as industry, agriculture, households and public services, and that has undergone chemical, physical and biological alterations during its use. This wastewater can contain a variety of contaminants from different sources. Common contaminants include organic matter, such as food waste and excrements, industrial chemicals, heavy metals, nutrients such as nitrogen and phosphorus, microbial pathogens, and other potentially harmful substances. Human activities, such as manufacturing industry, intensive agriculture and the widespread use of chemicals, contribute significantly to wastewater pollution.

Wastewater treatment is a process that generally involves several stages, ranging from primary treatment, which aims to eliminate solid debris and suspended particles, to biological treatment (secondary treatment), where micro-organisms break down organic matter. Tertiary treatment may also be involved to remove residual contaminants, and advanced methods such as reverse osmosis or activated carbon adsorption may be applied to achieve higher quality standards. The ultimate aim of wastewater treatment is to produce treated water that complies with environmental standards, facilitating its safe discharge into the environment or reuse for specific purposes. This process plays a crucial role in preserving water resources, protecting public health and conserving the ecosystem.

Biological wastewater treatment is an ecologically viable and effective method of purifying water using natural processes. This approach results in the conversion of dissolved and suspended organic pollutants into biomass, as well as emitted gases such as CO_2, CH_4, N_2 and SO_2, which can be separated from the treated water. This separation ensures the production of substantially cleaner and less polluted water. In addition, the system is designed to break down the pollutants dissolved in the effluent through the action of micro-organisms. These micro-organisms use these substances as nutrients, promoting their growth and reproduction. This functional duality contributes not only to water purification, but also to the promotion of a healthy aquatic ecosystem.

Biological treatment of wastewater typically encompasses two distinct processes: aerobic and anaerobic. The term "aerobic" denotes a process in which oxygen is present, facilitating the metabolic activities of microorganisms. Conversely, "anaerobic" characterizes a biological process devoid of oxygen, where microorganisms adapt to alternative modes of energy production.

3.7.1. Aerobic biological wastewater treatment

The general process of an aerobic treatment system involves several stages designed to purify wastewater using aerobic micro-organisms. Here are the main stages:

- *Pre-treatment:* Removal of large solids and undesirable substances, often using screens or sieves to prepare the water for biological treatment.
- *Aeration:* Wastewater is exposed to air or oxygen, encouraging the growth of aerobic bacteria. These micro-organisms consume the organic waste present in the water.
- *Settling:* Undigested solids settle to the bottom of the treatment tank, forming a sludge. This stage separates the solids from the clarified liquid.
- *Disinfection:* Chlorine or other disinfectants can be used to eliminate any remaining bacteria and ensure water safety. UV disinfection is an alternative

where the water is exposed to ultraviolet light to kill pathogenic micro-organisms.

- *Sludge removal:* The sludge resulting from decantation must be periodically removed from the system to prevent excessive build-up.

These steps may vary depending on the specific type of aerobic treatment system, such as activated sludge, trickling filters, biological filters, etc.

A prominent example of an aerobic biological treatment method is the activated sludge process, a widely adopted approach to the secondary treatment of wastewater, whether from the domestic or industrial sector. This process is particularly well suited to the treatment of waste streams with a substantial organic or biodegradable content. It is frequently applied in municipal wastewater treatment, as well as in the industrial context, encompassing effluents generated by pulp and paper mills, food industries such as meat processing, and industrial waste streams containing carbon molecules. The process consists of several methodical stages:

- *Mixing:* A bacterial suspension rich in active micro-organisms is intimately associated with the raw effluent. This combination encourages the elimination of organic compounds present in the water.
- *Agitation*: The resulting mixture undergoes careful agitation to prevent the formation of sediments and ensure a homogenous distribution of bacteria in the liquid mass.
- *Aeration*: The controlled introduction of air is designed to provide an essential source of oxygen for the aerobic micro-organisms involved in the decomposition of organic substances.
- *Bioreactor*: The mixture is directed into a bioreactor, a stirred and aerated tank that is conducive to the development and metabolic activity of the microorganisms. These metabolise the biological contaminants present in the water as nutrient substrates.
- *Secondary decanter*: The treated solution is sent to a secondary decanter where the resulting sludge is separated from the clarified liquid. This sludge is recycled to the biological reactor to ensure continuity of the process, while the excess is evacuated.

The process is essentially self-cleaning, replicating the natural phenomena observed in aquatic ecosystems, but in the controlled environment of a reactor. The micro-organisms, grouped together in a restricted space, catalyse the decomposition of organic compounds. The effectiveness of the treatment depends on the adequate presence of organic matter and nutrients, while excessive quantities of these compounds, heavy metals or salts can hinder or compromise the efficiency of the process. In addition, the activated sludge system offers mechanical advantages, such as improved liquid retention, reduced wear and tear on equipment, more efficient sludge agitation, simplified cleaning maintenance, and a safe working environment.

3.7.2. Anaerobic biological wastewater treatment

Anaerobic wastewater treatment is based on the principle of decomposing organic contaminants in the absence of oxygen. Unlike aerobic treatment, which involves the use of oxygen, anaerobic treatment takes place in an oxygen-free environment. The anaerobic process can generally be broken down into three main phases:

- *Hydrolysis:* In this first phase, hydrolytic bacteria break down complex organic matter into simpler substances, such as fatty acids and soluble compounds.
- *Acetogenesis:* The products of the hydrolysis phase are transformed into acetate, hydrogen and carbon dioxide by acetogenic bacteria.
- *Methanogenesis:* Methanogenic bacteria then convert these compounds into methane (CH_4) and carbon dioxide (CO_2), producing biogas.

The biogas generated can be recovered and used as a renewable energy source. The anaerobic process offers a number of advantages, including reduced sludge production, greater energy efficiency than aerobic methods, and the ability to treat wastewater with a high organic content.

Anaerobic reactors, such as biodigesters and anaerobic sludge bed reactors, are commonly used to implement this principle of anaerobic wastewater treatment. These systems offer a sustainable alternative for reducing organic pollution in wastewater while recovering the energy produced during the methanogenesis process.

Check your understanding

1. What is the definition of environmental biotechnology?

   A. The study of environmental science and technology

   B. The use of biotechnology to solve environmental problems

   C. The development of new technologies for industrial processes

2. What is the main aim of environmental biotechnology?

   A. To use resources wisely and reduce damage to the environment

   B. To develop new technologies for space exploration

   C. To create genetically modified organisms for agricultural purposes

   D. To improve the efficiency of industrial processes

3. What is bioremediation?

   A. The use of physical methods to remove pollutants from the environment

   B. The use of chemicals to treat contaminated environments

   C. The use of living organisms to clean or remove pollutants from soil, water, or wastewater

   D. The use of nanotechnology to clean up pollution

4. What is the difference between phytoremediation and microorganism remediation?

   A. Phytoremediation involves the use of plants, while microorganism remediation involves the use of microbes

   B. Phytoremediation involves the use of microbes, while microorganism remediation involves the use of plants

   C. Phytoremediation and microorganism remediation are the same thing

5. What is the role of biomarkers in environmental contexts?

   A. To remove pollutants from soil, water, or wastewater

   B. To indicate the exposure, sensitivity or effects of organisms, providing information on the impact of environmental factors on living systems.

   C. To break down complex compounds into less toxic forms

   D. To generate sustainable energy resources

6. What is the ultimate aim of wastewater treatment?

   A. To reuse wastewater for industrial purposes

B. To produce energy from wastewater

C. To remove all contaminants from wastewater

D. To produce treated water that complies with environmental standards

7. What is the main application of bioenergy?

   A. To control water pollution

   B. To purify air

   C. To produce sustainable energy resources

   D. To remediate contaminated soil

8. What are some examples of environmental contaminants treated by bioremediation?

   A. Heavy metals, pesticides and herbicides, and radioactive contaminants

   B. Organic solvents, polychlorinated biphenyls (PCBs), and petroleum hydrocarbons

   C. Chlorinated solvents, nitrates and nitrites, and pesticides and herbicides

   D. All of the above

9. What is the difference between chronic pollution and diffuse pollution?

   A. Chronic pollution is caused by the dispersion of multiple pollutants over time and space, while diffuse pollution is permanent pollution resulting from repeated or continuous emissions of pollutants

   B. Chronic pollution is less visible than accidental pollution, while diffuse pollution is more visible

   C. Chronic pollution is caused by industrial activities, while diffuse pollution is caused by agricultural activities

   D. Chronic pollution and diffuse pollution are the same thing

10. What is the main benefit of using bioremediation for pollution control?

    A. It is a quick and easy method of pollution control

    B. It is a cost-effective and environmentally friendly solution

    C. It does not require the use of living organisms

    D. It is effective for all types of pollution

11. Answer with True or False

    A. Bioremediation involves the use of natural biological species to catalyze the degradation of harmful chemicals into less toxic forms.

B. Biotransformation is a biological process that involves altering complex compounds from non-toxic to toxic forms.
C. Environmental biotechnology primarily focuses on cleaning up wastewater and does not contribute to other areas such as soil remediation and air purification.
D. Phytoremediation and microorganism remediation are two distinct strategies of bioremediation with different processes and mechanisms.
E. Environmental biotechnology addresses various types of pollution, including genetic pollution and light pollution.
F. Environmental biotechnology is contributing to the production of sustainable energy resources through bioenergy, which includes fuels like biogas, biomass, and hydrogen.
G. Aerobic biological treatment of wastewater uses micro-organisms in the absence of oxygen.
H. Bioremediation techniques can be used to treat a wide range of contaminant chemicals introduced into water, soil, and air, causing significant environmental problems.
I. In situ and ex situ are the two main categories of microbial bioremediation.
J. Plants cannot be used in the context of bioremediation.

12. What is the definition of bioremediation in the context of environmental biotechnology?
13. How does biotransformation contribute to mitigating the environmental impact of industrial processes?
14. How is environmental biotechnology contributing to the production of sustainable energy resources?
15. What are some examples of environmental contaminants treated by bioremediation?
16. What is the difference between in situ and ex situ microbial bioremediation?
17. What is the difference between aerobic and anarobic biological wastewater treatment?
18. What are the main applications of environmental biotechnology and their benefits to human society and the environment?

19. How does environmental biotechnology contribute to the treatment of wastewater?
20. What are the two distinct processes involved in the biological treatment of wastewater, and how do they differ in terms of the presence or absence of oxygen?

Answers

1. B
2. A
3. C
4. A
5. B
6. D
7. C
8. D
9. A
10. B
11. Answers
    A. True
    B. False
    C. False
    D. True
    E. True
    F. True
    G. False
    H. True
    I. True
    J. False
12. Bioremediation is defined as the use of living organisms to clean or remove pollutants from soil, water, or wastewater
13. Biotransformation involves altering complex compounds from toxic to non-toxic forms, thereby reducing the environmental harm caused by industrial processes.

14. Environmental biotechnology is contributing to the production of sustainable energy resources through bioenergy, which includes fuels like biogas, biomass, and hydrogen, addressing the growing need for sustainable energy resources 1.
15. Environmental contaminants treated by bioremediation include petroleum hydrocarbons, heavy metals, pesticides and herbicides, chlorinated solvents, nitrates and nitrites, organic solvents, polychlorinated biphenyls (PCBs), and radioactive contaminants.
16. In situ microbial bioremediation involves treating pollutants at the site of contamination, while ex situ microbial bioremediation involves removing contaminated materials to be treated elsewhere.
17. Aerobic biological wastewater treatment relies on oxygen-dependent micro-organisms to break down organic pollutants, while anaerobic biological wastewater treatment uses oxygen-free conditions with anaerobic bacteria to break down organic matter.
18. The main applications of environmental biotechnology include bioenergy, bioremediation, biotransformation, and biomarker analysis, which offer benefits such as sustainable energy production, pollution control, and environmental monitoring.
19. Environmental biotechnology contributes to the treatment of wastewater through biological wastewater treatment, which involves the use of micro-organisms to break down organic matter and remove contaminants from wastewater
20. The two distinct processes involved in the biological treatment of wastewater are aerobic and anaerobic. Aerobic treatment occurs in the presence of oxygen, facilitating the metabolic activities of microorganisms, while anaerobic treatment characterizes a biological process devoid of oxygen, where microorganisms adapt to alternative modes of energy production.

Conclusion

In conclusion, biotechnology is a field that uses biological systems, organisms or their derivatives to develop and improve products and processes in a variety of applications, from medicine to agriculture and environmental management. It exploits the capabilities of living organisms, such as bacteria and enzymes, to make valuable advances in a wide range of industries.

Through the first chapter of this course, an in-depth introduction to the field of biotechnology was presented, covering a wide range of topics, from the basics of DNA sequencing to the applications of biotechnology in various industries. This chapter highlighted the importance of understanding fundamental biological processes and the crucial role of biotechnology in advancing scientific research and technological innovation. From providing the historical context of biotechnology to the latest automated sequencing techniques, this section offered a comprehensive overview of the evolution of the field and its impact on society.

For the second chapter on chemical biotechnology, we explored innovative strategies and advances aimed at optimising biological processes for sustainable production. By examining elucidation techniques, synthetic derivatives of key compounds and the use of non-natural elucidators, researchers are paving the way for improved production of valuable secondary metabolites in plant cell cultures. The emphasis on selecting high-yielding cell lines, adjusting culture conditions and using elucidator treatments underlines the importance of biotechnological approaches for maximising the efficiency and yield of bioactive compounds. These advances offer great promise not only for the industrial production of essential compounds such as paclitaxel, but also for sustainable solutions to address environmental concerns and the growing demand for these critical resources. Thus, chemical biotechnology continues to play a crucial role in the future direction of bioprocessing systems and offers a pathway towards more environmentally friendly and efficient production methods in biotechnology.

Finally, in the third chapter we have seen how environmental biotechnology plays a crucial role in solving various environmental challenges by using the principles of biotechnology to develop innovative solutions. From bioremediation to the biological treatment of wastewater, this field offers sustainable and effective methods for cleaning up pollutants and preserving the health of our environment. By harnessing the power of living organisms such as microbes and plants, environmental biotechnology contributes to water purification, soil remediation, air purification and pollution control. Applications in bioenergy, bioprocessing and biomarkers highlight the diverse benefits of environmental biotechnology in creating a cleaner, more sustainable future.

Resources

For more information on this subject, please consult the following books:

- *Biotechnology for Beginners*, Second Edition, 2016, Reinhard Renneberg, Viola Berkling and Vanya Loroch, Elsevier
- *Biotechnology Applying the Genetic Revolution,* Second Edition, 2015, David P. Clark and Nanette J. Pazdernik, Elsevier
- *Biotechnology: Special Processes*, 2001, Prof. Dr. H.-J. Rehm, Dr. G. Reed, WILEY
- *Omics Technologies and Bio-Engineering Towards Improving Quality of Life,* 2017, Debmalya Barh and Vasco Azevedo, Elsevier
- *Biomaterials, An Introduction,* 1992, Joon B. Park, Roderic S. Lakes, Springer
- *An Introduction to Biotechnology the Science, Technology and Medical Applications,* 2015, W.T. Godbey, Elsevier
- *Tissue Scaffolds*, 2022, Naveen Kumar, Vineet Kumar, Sameer Shrivastava, Anil Kumar Gangwar, Sonal Saxena, Springer
- *Novel Biomaterials for Regenerative Medicine,* 2018, Heung Jae Chun, Kwideok Park, Chun-Ho Kim, Gilson Khang, Springer
- *Handbook of Bioremediation Physiological, Molecular and Biotechnological Interventions,* 2020, Mirza Hasanuzzaman and Majeti Narasimha, Elsevier
- *Chemical Biotechnology and Bioengineering,* 2015, Xuhong Qian, Zhenjiang Zhao, Yufang Xu, Jian-He Xu, Y.-H. Zhang, Jingyan Zhang, Yang-Chun Yong, Fengxian Hu, Royal Society of Chemistry
- *Environmental Biotechnology: Principles and Applications,* 2001, Bruce E. RittmannPerry L. McCarty, McGraw-Hill Education