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Edited by Jaspal Singh, Rajesh Bajpai, and Ravi Kumar Gangwar

Biotechnology in Environmental Remediation

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Editors

Prof. Jaspal Singh
Bareilly College
Department of Environmental Science
Kalibari Road
Bareilly
243001 Uttar Pradesh
India

Dr. Rajesh Bajpai
CSIR-National Botanical Research Institute
Plant Diversity Systematics & Herbarium
Rana Pratap Marg
226001 Lucknow
India

Mr. Ravi Kumar Gangwar
Hungarian University of Agriculture and Life Sciences
Department of Soil Science
Páter Károly u. 1
2100 Gödöllő
Hungary

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Preface

Remediation is the process of removing impurities from places that have been polluted by industrial, manufacturing, mining, and commercial activities. Remediation involves an all-encompassing process of land restoration from detection, investigation, assessment, determination of remedial measures, actual clean-up, as well as redevelopment of the area. It is known that the involvement of biological organisms in remediation is called bioremediation. Similarly, the involvement of biotechnology in environmental remediation can immediately help by modifying the solid, liquid, and gaseous waste either by recycling or making new products so that the end product is less harmful to the environment. Replacing chemicals with biological materials using biotechnology is another way to reduce our harmful impact on the environment. Biotechnology employs technology along with fungi, bacteria, and plants to decontaminate affected areas like soil, water, air, and sediments. It further aids in identifying some secondary metabolites. This is the central idea that can be found in this book. It is timely, acknowledging the lack of any such detailed work on biotechnology in the context of environmental remediation and also because of the current surge in the application of plants, microbes, and lichens in the context of bioremediation. To summarize, bioremediation is a vast topic, and a lot of research has already been done in the world, but the involvement, as well as the possibility of secondary metabolites, is least investigated. Biotechnology as a tool in the advancement of environmental remediation is a new directional research in diverse ecosystems. Considering that the knowledge of biotechnological application in environmental remediation is very limited, this book for the first time summarizes evidence-based multidisciplinary aspects of bioremediation, through a scientific approach comprising a team engaging 14 experts carrying out a multisite and multidisciplinary research on environmental remediation. The outcomes of the team research on bioremediation along with biotechnological aspects are published in an edited book form. Each chapter of the book reveals the outcomes of a complete study on specific aspects, thereby, the entire book provides a comprehensive account of the role of biological organisms in environmental remediation. Additionally, we aim to widen the scope of this book by bringing a more cohesive picture of bioremediation research in the country along with the USA, Ethiopia, Hungary, Nigeria, and Taiwan. With this inclusion, the book substantially (i) covers diverse aspects of pollutants remediation, (ii) improves global understanding of this hitherto less explored technology, and (iii) increases the availability and application of secondary metabolites from microbes' evidence to use as eco-friendly aspects in the remediation process. The focus of the book is on extraordinarily large spatial scales and various dynamics of environmental remediation through biotechnology as a tool.

It covers various aspects of environmental remediation, namely, the plant biotechnology in environmental aspects, remediation of petroleum hydrocarbons, heavy metals, soil contaminants, the role of plant growth-promoting rhizobacteria, lichen secondary metabolites diversity and applications, algal carbon sequestration, *in vitro* mycobiont culture and an eco-friendly approach, wastewater management, biofuel from waste, nanotechnology toward the clean environment, the role of fatty acid and proteins in remediation, chemical sustainability being the key areas. The book addresses a major gap in the field of environmental remediation through biotechnological approaches. The present book is of interest and useful to scholars, ecologists, general scientists, environmentalists, botanists, policymakers, academicians, researchers, and NGOs, who have an interest in contaminants remediation, and a valuable source of reference to the relevant researchers and policy planners. It establishes a landmark for any further investigation on the pollutants remediation and application of organisms and their secondary metabolites through biotechnological interventions.

June 2023

Jaspal Singh
Bareilly, India

Rajesh Bajpai
Lucknow, India

Ravi Kumar Gangwar
Gödöllő, Hungary

1

Biotechnology and Various Environmental Concerns: An Introduction

Ravi K. Gangwar[1], Rajesh Bajpai[2,3], and Jaspal Singh[4]

[1] *Institute of Environmental Science, Hungarian University of Agriculture and Life Sciences, Department of Soil Science, Páter Károly utca 1, Gödöllő, 2100, Hungary*
[2] *CSIR-National Botanical Research Institute (CSIR-NBRI), Plant Diversity Systematics and Herbarium Division, Rana Pratap Marg, Lucknow, Uttar Pradesh, 226001, India*
[3] *Biodiversity, Biomonitoring & Climate Change Division, Environment, Agriculture and Education Society, Anand Vihar, Bareilly, Uttar Pradesh, 243122, India*
[4] *Bareilly College, Department of Environmental Science, Kalibari Road, Bareilly, Uttar Pradesh, 243001, India*

1.1 Introduction

Károly Ereky, a Hungarian engineer, first used the term "biotechnology" in 1919 to describe the science and techniques that allow products to be made from raw materials with the help of living organisms. Biotechnology is broadly defined as using living organisms or the products of living organisms for human benefit (or to benefit human surroundings) to make a product or solve a problem [1]. This technology has been used for thousands of years and involves working with cells and bacteria to produce various products useful for mankind. The best examples include the fermentation used to make breads, cheese, yogurt, and alcoholic beverages such as beer and wine. However, modern biotechnology is a multidisciplinary subject that involves the sharing of knowledge between different areas of science such as cell and molecular biology, genetics, microbiology, anatomy and physiology, computer science, biochemistry, and recombinant DNA technology (rDNA technology).

The foundation of modern biotechnology was laid down with the advancements in science and technology during the eighteenth and nineteenth centuries. Between 1850 and 1860, Louis Pasteur developed the process of pasteurization. By 1860, he also concluded that organisms did not occur as a result of spontaneous generation; all cells arise from preexisting cells. At the beginning of 1857, Gregor Mendel developed genetics and the Principles of Heredity, where he cross-pollinated pea plants to examine traits such as petal color, seed color, and seed texture. In 1869, J. F. Miescher isolated "nuclein" from the nuclei of white blood cells, which contain nucleic acids. In 1882, German cytologist Walter Flemming described that during mitosis, thread-like bodies (chromosomes) were equally distributed to daughter cells during cell division. In 1896, Eduard Buchner showed that biochemical transformations can take place without the use of cells by converting sugar to ethyl

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alcohol using yeast extracts. In 1928, Alexander Fleming discovered *Penicillium* inhibited the growth of a bacterium called *Staphylococcus aureus*, responsible for skin disease in humans.

Many novel experiments were conducted during the 20th century, like identification of DNA as the genetic material by the classical Alfred Hershey and Martha Chase in 1952, followed by the double helical structure of DNA proposed by James Watson and Francis Crick in 1953. In 1978, Boyer was able to isolate a gene for insulin (a hormone to regulate blood sugar levels) from human genome using biotechnology. In 1997, Ian Wilmut was successful in cloning a sheep named "Dolly." In 2003, the Human Genome Project completed the sequencing of the human genome.

The multidisciplinary nature of modern biotechnology and the areas of its application are given in Figure 1.1 and Table 1.1.

Biotechnology has revolutionized diagnostics and therapeutics; however, lethal virus diseases like avian flu, Chikungunya, Ebola, influenza A, SARS, West Nile, Zika virus, and the most recent coronavirus have posed the greatest risks to humans. Scientists and researchers are continuously adapting to various biotechniques to tackle these threats. Moreover, biotechnology has provided novel opportunities for the sustainable production of various products and services. Additionally, environmental concerns encourage the use of biotechnology for biomonitoring as well as ecologically friendly chemical synthesis, waste minimization, and pollution management (decontamination of water, air, and soil).

In Chapter 2, the importance, contribution to agriculture and environment, and future prospects of plant biotechnology have been discussed. To ensure that

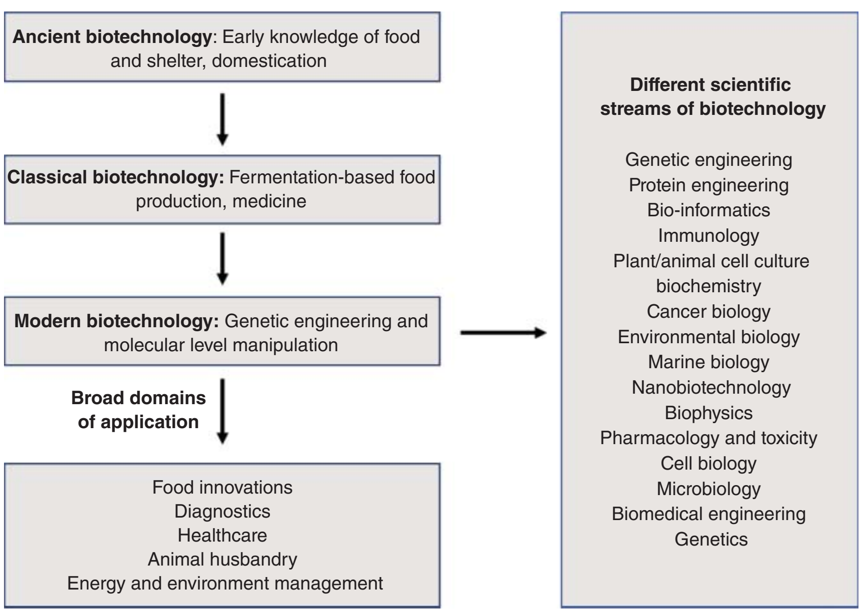

Figure 1.1 Multidisciplinary nature of modern biotechnology and the areas of its application. Source: NCERT [2].

Table 1.1 Some common areas of biotechnology.

Biotechnology area	Products/applications
Industrial (White) biotechnology	Enzymes, fermentation products, biochemicals, reagents, etc.
Agricultural (Green) biotechnology	Golden rice, drought-tolerant crops, transgenic crops, salt-tolerant crops, etc.
Marine (Blue) biotechnology	Aquatic organisms, aquaculture, increasing sea food, etc.
Medical (Red) biotechnology	Vaccines, antibodies, therapeutic proteins, antibiotics, stem cells, gene therapy, etc.
Environmental biotechnology	Pollution detection, composting, bioremediation, phytoremediation, bioextractions, transgenic modifications, etc.

Source: Adapted from Gupta et al. [3].

environmental resources are preserved for future generations, deliberate efforts must be undertaken to identify alternatives and ways to use them sustainably. This chapter seeks to provide an introduction to plant biotechnology, covering its fundamentals, where research has taken it thus far, the need for it, its place in agriculture, and how it might help solve agricultural and environmental problems. The chapter places a strong emphasis on debunking myths about genetically modified organisms (GMOs), including how they are made in the plant kingdom, the advantages they provide, and the problems they have. The reader will have a better grasp of how scientific ideas developed for the enhancement of crops for farmers and those who depend on agriculture are used in the actual world.

Furthermore, metals and hydrocarbons are considered hazardous materials because they have the potential to cause cancer and mutagenic consequences in humans. Forest fires, transportation, and various industrial operations are the main causes of this environmental pollution. These metals and hydrocarbons are major constituents of petroleum. The effects of petroleum and hydrocarbons on microbes, soils, plants, and human health are discussed in Chapter 3. As microorganisms extract xenobiotic organic and inorganic substances from the environment and totally mineralize them into carbon dioxide, water, and inorganic compounds during biodegradation, this chapter also discusses the functions of microorganisms in the breakdown of hydrocarbons and several processes used in the process, including enzymes, biosurfactants, and immobilization. Additionally, the function of aerobic and anaerobic pathways as well as several parameters for hydrocarbon breakdown are described.

Recent decades have seen an increase in environmental contamination as a result of several increasing anthropogenic activities. A popular and efficient technique for eliminating hazardous material from a contaminated environment is "bioremediation." Bioremediation is extensively involved in the degradation, eradication, immobilization, or detoxification of a wide range of chemical wastes and physically dangerous compounds from the environment through the all-inclusive activity of microorganisms. Heavy metals, nuclear waste, pesticides, greenhouse gases, and hydrocarbons are among the pollutants that threaten the environment and public health due to their toxicity. The organisms involved in bioremediation, as well as the variables influencing microbial bioremediation, are all covered in Chapter 4. Additionally, this chapter provides details on the types and methods of bioremediation. It also covers the advantages, disadvantages, and drawbacks of bioremediation.

Chapter 5 makes an attempt to evaluate the function of soil biodiversity in environmental sustainability as it relates to sustaining terrestrial life and biodiversity, climate change, hydrological dynamics, and environmental remediation. Numerous soil processes that provide a variety of ecosystem goods and services are driven by soil biodiversity. Soil organisms are closely linked to a number of ecosystem services, including nutrient cycling, climate regulation, water infiltration, and purification, through their activity and interactions. The effort to link soil biota to specific functions that support soil-based ecosystem services is difficult due to the complicated interaction between soil biodiversity and ecosystem function. Its ability to provide different ecosystem services is also significantly influenced by other environmental conditions and soil management practices. Therefore, in order to maximize biodiversity's potential for environmental sustainability, it must be comprehended, evaluated, and managed sustainably.

The main constituents of soil biodiversity are microorganisms. Plant growth-promoting rhizobacteria (PGPR), which are naturally occurring soil microorganisms, are helpful to plant growth and have the ability to encourage plant growth by colonizing the plant roots [4]. PGPR's roles such as the production of indoleacetic acid (IAA), ammonia (NH_3), hydrogen cyanide (HCN), catalase, and others are discussed in Chapter 6. PGPR promotes seed germination, seedling growth, and yield. Many microorganisms such as *Pseudomonas, Azospirillum, Azotobacter, Klebsiella, Enterobacter, Alcaligenes, Arthrobacter, Burkholderia, Bacillus*, and *Serratia* promote plant growth. Seed inoculation, alone or in combination with bacterial cultures/products, to increase nutrient availability through various processes such as phosphate solubilization, nitrogen fixation, and soil applications that are gaining popularity are presented in Chapter 6.

Chapter 7 focuses on the biological capture of CO_2 using microalgae to minimize carbon footprint and other methodologies to compare with biological CO_2 fixation. The drastic increase in CO_2 concentration in the atmosphere resulted in climate change. To tackle this problem, conventional carbon capture and storage (CCS) technology is not economically useful for its long-term application in mitigating climate change. These conventional approaches to reduce CO_2 concentration have several technological and economic limitations. Therefore, it is essential to identify suitable alternatives to the current technologies and to improve existing techniques in order to reduce CO_2. Hence, various biological carbon dioxide (CO_2)-capturing technologies, such as using microalgae, absorption, adsorption, membrane separation, and other biological methods, were discussed in Chapter 7. Out of which, microalgae have been very promising in the recycling of CO_2 into biomass, and they can assimilate 10–50 times more CO_2 than vascular plants without providing food to humans/animals [5]. It will also lessen the amount of additional carbon dioxide released into the atmosphere and reduce the burden on industries that produce high-value goods.

In Chapters 8 and 9, the importance of lichens is discussed. Lichens are remarkable alliances of nature, a composite organism arising from fungus and algae. They may live and flourish in a variety of climatic and biological settings all around the world. Lichens are extremely useful economically and commercially as ingredients for perfumes, colors, ayurvedic medicines, food, fodder, flavoring agents, spices, and sauces, among other products. Additionally, lichens have recently become significant to the pharmaceutical sector as a result of the discovery of physiologically active compounds in lichens. Due to their slow rate of growth in their natural habitat, lichens are vulnerable to extinction if their commercial use is not restricted. In order to conserve these organisms in nature, *in vitro* production of lichens or their constituent parts may offer a viable approach. Furthermore, *in vitro* culture of lichens and its components for studying the biosynthetic pathways and their manipulation to enhance the production of valuable secondary metabolites are presented in Chapter 8.

Another lichen, *Cladonia*, of the family Cladoniaceae is more dominant in the Indian Himalayan regions. Cladonioid community has fumarprotocetraric acid, which contains species that have special traits and prefers to grow on soil, dead decaying wood, and soil over rocks. The Cladoniaceae family is an intriguing source of bioactive substances that offer many prospects for developing new antioxidant, antibacterial, and anticancer medicines. Because of the potential uses suggested by the research, Cladoniaceae lichens may be useful in the food, pharmaceutical, and agricultural sectors. Chapter 9 discusses the structure and composition of the lichen family Cladoniaceae in six Himalayan states of India, together with a comparative assessment of functional traits varied in different habitats and metabolites. The qualitative assessment of secondary metabolites will also serve as a baseline for the development of green medicines in the near future through biotechnological applications.

Water is essential for all life forms. The quality of the water is getting worse every day due to the growing world population, excessive water usage, and overexploitation of the water resources. Industrial activities and agricultural activities, particularly the excessive and irrational use of fertilizers and other chemicals, damage freshwater bodies. This problem of wastewater treatment can be solved by biotechnology, which can also aid in the evaluation of water quality. The applications of biotechnology for wastewater remediation are an efficient and environmentally friendly strategy because they require fewer chemicals and fewer laborers to regulate. Chapter 10 focuses on the use of biotechnological methods for the remediation of wastewater. GMOs like *Pseudomonas* spp. (Super Bug) and biologically advanced systems for the remediation of polluted aquatic bodies for industrial wastewater treatment were also discussed. Various bioremediation processes that involve the use of micro- and macroorganisms along with activated sludge process, trickling filters, membrane bioreactors, and constructed wetlands are presented in Chapter 10.

With a growing population, energy demand is increasing unprecedentedly. To meet this demand, crude oil is currently acting as a primary source of energy. However, the consumption of a vast quantity of these nonrenewable fossil fuels resulted in the release of various environmental contaminants. Thus, incorporation of clean and green technologies with the aim of achieving sustainable development is now of great concern. Biotechnological and microbiological techniques have shown great potential for biofuel production using crops and other organic waste, the generation of wind and solar energy, and the development of alternative energy through renewable, nonpolluting resources. The contribution of biotechnology to energy production has significant potential to increase not only in biofuel production but also in producing energy from petro-crops, biochar, petroleum production, petroleum upgrading, and biogas production. Chapter 11 focuses on the developments in biotechnological applications to create more advanced biofuels and offer a better and more sustainable fuel economy globally.

Another biotechnological approach to remediate environmental pollutants for a cleaner environment is "nanotechnology." A number of organic and inorganic pollutants that are harmful to both the environment and human health have been introduced into the environment as a result of growing industry, urbanization, and population growth worldwide. The potential of nanotechnology in environmental cleanup has been demonstrated in Chapter 12. Pollutants are removed from the environment using a variety of nanomaterials, including carbon-based, inorganic, and polymeric-based compounds. Nanomaterials can be used to successfully remove synthetic colors, heavy metals, pesticides, organophosphorus chemicals, chlorinated organic compounds, and volatile organic compounds. These environmental contaminants could potentially alter the composition of the environment and pose health risks to humans [6]. Nanomaterials can be used in water, soil, or air because of their adaptability and wide range of forms. In this chapter, the use of nanoparticles for environmental cleanup is briefly covered. It presents the most recent developments in the use of metal, carbon, polymer, and silica nanoparticles in the treatment of wastewater, soil, and air.

In the modern era, microorganisms are frequently used in bioremediation to remove organic and inorganic pollutants from our environment. Microbes are widely applied in various fields like pollution mitigation, biofuel and enzyme production, food industries, agriculture, and medical science. Microbes' interactions with their surroundings, in particular, are influenced by the surface of their cells. Biomolecules found on the surface of microbial cells control their fluidity or hydrophobicity, which determines whether they are attracted to hydrophilic or hydrophobic substances. Higher hydrophobicity in microbial cells has been linked to a variety of microbial behaviors, including cell aggregation, biodegradation of organic pollutants, biofilm development, and resistance to hydrophobic substances. To effectively utilize these potent natural agents for their various applications, it is important to understand the biomolecules that influence cell surface hydrophobicity (CSH). Chapter 13 discusses the different cell surface biomolecules (fatty acids and proteins) that are being reported as responsible for the alteration of CSH, along with the environmental factors that influence microbial cell hydrophobicity.

For a healthy environment, chemical sustainability is essential, as these are a necessary part of everyday life. Humans are widely exposed to toxic chemicals present in domestic, environmental, and occupational setups. This exposure may be of the acute, brief-duration type, or it may be persistent. People working in various industries that deal with asbestos, battery recycling facilities, tanneries, lead smelting plants, paint factories, etc. are more likely to be exposed to hazardous chemicals at the workplace. Chemical sustainability, commonly referred to as sustainable chemistry, is the effective introduction of novel chemicals, processes, and products while sustaining environmental and public health protection. The Organisation for Economic Co-operation and Development (OECD) is greatly concerned about these global issues, and it helps countries by implementing regulations that support economic growth and the smart and efficient use of resources. The use of biomass as an energy source should also be encouraged as one of the ways to reduce dependence on fossil fuels. Enzyme technology advancements through protein engineering and bioremediation technology have also been very successful in reducing urban waste management. Chapter 14 will cover the scope of chemical sustainability, current research in chemical sustainability, regulatory requirements, significant regulatory contributions in various countries, particularly India, potential benefits, and hidden cons.

References

1 Thieman, W.J. (2009). *Introduction to Biotechnology*. Pearson Education India.

2 NCERT (2022–2023). National Council of Education Research and Training. https://ncert.nic.in/textbook/pdf/kebt101.pdf.

3 Gupta, V., Sengupta, M., Prakash, J., and Tripathy, B.C. (2017). An introduction to biotechnology. In: *Basic and Applied Aspects of Biotechnology* (ed. V. Gupta, M. Sengupta, J. Prakash, and B.C. Tripathy), 1–21. Singapore: Springer https://doi.org/10.1007/978-981-10-0875-7_1.

4 Prasad, R., Kumar, M., and Varma, A. (2015). Role of PGPR in soil fertility and plant health. In: *Plant-Growth-Promoting Rhizobacteria (PGPR) and Medicinal Plants*, Soil Biology, vol. 42 (ed. D. Egamberdieva, S. Shrivastava, and A. Varma), 247–260. Cham: Springer https://doi.org/10.1007/978-3-319-13401-7_12.

5 Lam, M.K., Lee, K.T., and Mohamed, A.R. (2012). Current status and challenges on microalgae-based carbon capture. *International Journal of Greenhouse Gas Control* 10: 456–469.

6 Kampa, M. and Castanas, E. (2008). Human health effects of air pollution. *Environmental Pollution* 151 (2): 362–367.

2

Plant Biotechnology: Its Importance, Contribution to Agriculture and Environment, and Its Future Prospects

Jeny Jose[1,2] *and Csaba Éva*[2]

[1] *Hungarian University of Agricultural and Life Sciences, Doctoral School of Plant Sciences, Páter Károly u. 1, Gödöllő 2103, Hungary*
[2] *Eötvös Loránd Research Network, Centre for Agricultural Research, Agricultural Institute, Department of Biological Resources, Brunszvik utca 2, Martonvásár 2462, Hungary*

2.1 Where do Environment and Biotechnology Meet?

2.1.1 Introduction

Environment Merriam Webster [1] defines environment as

> *The complex of physical, chemical, and biotic factors (such as climate, soil, and living things) that act upon an organism or an ecological community and ultimately determine its form and survival.*

The environment is precious to us and should be rightfully protected, not only because of the vast resources it provides for our sustenance but also because human activities are depleting the resources at an alarming rate, putting them on the verge of scarcity in future generations. Since humankind has had the power to innovate, science and technology have and can work in a congruous manner to solve big environmental problems.

Biotechnology is a wide area of biology, involving the use of living systems and organisms to develop or make products. The term was first used by Károly Ereky in 1919 intending to highlight living organisms that could potentially help in production of goods from raw materials. But over the years, the term has expanded in its meaning and has incorporated scientific fields like genomics and pharmaceutical revisions, and, depending on the tools and applications, it often overlaps with related scientific fields.

Environmental biotechnology. The concept of utilizing the existing biological processes in the environment for commercial use whereby eventually giving back to the environment forms the backbone of environmental biotechnology. It aims to

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study, understand, and use the natural environment. The International Society for Environmental Biotechnology [2] defines it as

> *The development, use and regulation of biological systems for remediation of contaminated environments (land, air water), and for environment friendly processes (green manufacturing technology and sustainable development)*

The journal Nature [3] defines it as

> *The branch of biotechnology that addresses environmental problems, such as the removal of pollution, renewable energy generation or biomass. It includes a broad range of applications such as bioremediation, prevention, detection and monitoring, genetic engineering for sustainable development and better quality of living.*

2.1.2 Chief Applications

Major sectors where environmental biotechnology can be applied includes:

- **Biomarker**: It is referred to as a substance or a compound that enables quantifying the effect/damage by measuring the generated response to the pollutant/chemical. A biomarker is a change in biological responses, ranging from molecular, cellular, and physiological responses to behavioral changes, which can be related to exposure to a toxicant in the environment. With the advancement of biotechnology, the sensitivity and efficiency of biomarkers have increased, indicating the exposure and sensitivity effects of aquatic and terrestrial pollution. Biological assays (bioassays), which involve a living plant or animal (*in vivo*/*in vitro*) or tissue or cell cultures (*in vitro*), are used to determine the effect of a hormone/drug.
- **Biosensor**: Biosensors are primarily analytical reader devices incorporating a biological element sensing sensitive variation such as that in organelles, cell receptors, enzymes, and nucleic acids.
  DNA biosensors, among other biosensors, are a promising approach in the fields of agriculture and environmental cleanup efforts.
- **Biofuel**: With the increasing need for clean energy sources, biofuel has been a focus for exploration in the industrial, domestic, and space sectors. Biofuels are fuels derived from biomass, for instance, bioethanol (corn, sugarcane), ethyl *tert*-butyl ether (ETBE, converted from ethanol), and biodiesel (e.g. transistorized canola oil).
- **Bioremediation**: It refers to the use of the metabolism of microorganisms to remove pollutants both *in situ* and *ex situ*. It practically converts hazardous substances into nontoxic compounds using microorganisms.
- **Biotransformation**: It refers to transforming/changing a particular biological compound in the environment into nontoxic compounds. It particularly finds use

in the manufacturing sector, where toxic substances are converted into nontoxic by-products. It involves changes in compounds even within an organism.

- **Molecular Ecology**: Molecular ecology is a field of evolutionary biology that deals with applying molecular population genetics, molecular phylogenetics, and genomics to answer ecological questions. More attention is given to species diversity, species–environment relationship, and behavioral ecology. Biological techniques like DNA fingerprinting can be used to determine extinction of species or introduction of new species and map the evolutionary development of plant and animal families.

Environmental biotechnology helps the organisms and the engineers aid in adapting to environmental changes, keeping the environment clean, and reversing the damages done by human activity.

Biotechnology also holds the potential to be used in conservation of the natural resources and meet the rising demands for food and land; for instance, enabling better utilization of the nutrients present in feed reduces the nutrient runoff into water bodies. Production of hardier crops to withstand harsh climatic conditions that require fewer inputs for production is also possible via biotechnology.

2.2 Understanding Agricultural Biotechnology

2.2.1 Introduction

Agriculture is defined as

> *All forms of activities connected with growing, harvesting and primary processing of all types of crops, with the breeding, raising and caring for animals, and with tending gardens and nurseries*

by the International Labour Organization [4].

To address and acknowledge the existing agricultural roadblocks in achieving food security and addressing climate change and to ensure advancement in that sector, the International Assessment of Agricultural Knowledge, Science and Technology for Development (IAASTD) called for the promotion of small-scale agroecological farming systems and technology. Biotechnology found and consolidated its way into agriculture, leading to a pragmatic, solution-centric approach to agricultural problems.

As found in the BRIEF 2004 issue by Cornell University [5],

> *Agricultural biotechnology, also known as agritech, is an area of agricultural science involving the use of scientific tools and techniques, including genetic engineering, molecular markers, molecular diagnostics, vaccines, and tissue culture, to modify living organisms: plants, animals, and micro-organisms.*

Agricultural biotechnology as defined by U.S. Department of Agriculture [6] is

> *A range of tools, including traditional breeding techniques, that alter living organisms, or parts of organisms, to make or modify products; improve plants or animals; or develop microorganisms for specific agricultural uses.*

Broadly agricultural biotechnology is composed of **animal biotechnology** and **plant biotechnology**.

Farmers have, since around 10,000 years ago, had the aim of improving wild crops and animals and have practiced careful selection and breeding to achieve the desired traits. The fruits we see in the market today did not necessarily look like this years ago. This led to the domestication of plant and animal species, the products of which can be spotted in today's agriculture. Modern breeders share the same aim as early farmers: to produce superior crops or animals. Biotechnology today is equipped with the tools of genetic engineering. The prevalent food system necessitates a global response to supply demands. A system that works along the lines of sustainability is prepared for exigencies and leaves better environmental footprints.

2.2.2 Main Components of Agricultural Biotechnology

1. **Genetic engineering**: It involves moving genes that correspond to useful characteristics from one organism to another. The developed GM crops have traditionally focused on increasing productivity by eliminating losses due to weeds, diseases, or insects.
2. **Molecular markers**: Rather than basing selection of plants/animals on visible/measurable parameters for breeding, molecular markers are used to select traits. Information in the DNA reveals markers that are associated with the trait of interest even when they are visibly absent, thereby making breeding more precise and efficient.
3. **Molecular diagnostics**: These are precisely curated methods to detect genes in order to identify crop/livestock diseases.
4. **Vaccines**: To cure serious illnesses, biotechnology-derived vaccines have found their way to both livestock and humans, like the vaccine against hemorrhagic septicemia, which was developed to prevent the deaths of cattle and water buffalo.
5. **Tissue culture**: It refers to the regeneration of plants from sterile planting material *in situ*.

   It, therefore, makes multiple disease-free planting materials available and also is a cardinal part of generating any new variety *in situ* [5].

2.2.3 Applications of Agricultural Biotechnology

Agricultural biotechnology offers tools to farmers to make production processes cheaper and more manageable. To keep up with the growing demand for food and fodder crops without adding financial pressure or exploiting the environment, biotechnological intervention in agriculture has proven to be a beneficial tool [6]. Modern molecular plant breeding techniques such as marker-assisted selection (MAS) assist plant breeders in identifying better traits in plants faster than conventional breeding. An understanding of the DNA offers scientists possibilities to find solutions to agricultural problems with the identification of genes that are the cause or the genes that can be helpful to solve the problem at hand. Identification and precise working with such genes corresponding to certain traits enhances the ability of the breeders to make improvements for both crops and livestock. Though agricultural biotechnology is looked upon with skepticism, several biotechnology-derived crops and artificially inseminated animals have been adopted by farmers with proven results thereafter. Many gene technologies for crops and animals are in the research and development stages, as the development of biotechnology has its own challenges and undergoes thorough examination before release. The stringency followed for the release of a variety ensures its quality and productivity for future use.

2.3 Animal and Plant Biotechnology

2.3.1 Animal Biotechnology

Animal biotechnology falls under the umbrella of agricultural biotechnology. Artificial insemination was one of the initial modern forms of assisted reproductive technology (ART) that pushed forward advancement in selective breeding. It also led to the development of industrial animal production, prominently in dairy and poultry, where more than 70% of all U.S.-bred Holstein cows are artificially inseminated. Estrus synchronization, which accurately monitors and controls the females when she is in heat, also contributes in increasing the efficiency of AI.

Embryo transfer (ET), where the donor cow of superior breeding is identified and chemically induced to superovulate and then fertilized, following which the developed embryo is later implanted in a recipient cow, is also a growing technique in animal production. Apart from these, as in plant research, transgenics have found their way into animal biotechnology. Transgenic animals are produced by means of genetic engineering to obtain animals expressing desired traits. Transgenic animals are currently only used for research, primarily done on mice to bring forth knowledge in medical research. There are several more approaches and practices, leaving behind a tremendous scope for growth. Genes to increase wool production in sheep, for disease resistance, and methods for identifying heifers and bulls via embryo sexing have all been and are being researched among many others. The two cardinal sectors that benefits from growing biotechnology are the livestock industry and drug research. Most of the environmental concerns are speculative due to the lack of commercialized products, but public and regulatory acceptance can be expected in the near future [7].

2.3.2 Plant Biotechnology

2.3.2.1 Introduction

Plant biotechnology is considered the application of genetic engineering and tissue culture for the improvement of crop plant traits [8]. Breeders have always aimed to improve the characteristics (yield, stress resistance, nutritional quality, etc.) of crop plants, however, desirable modifications in the genome can often be made slowly with traditional breeding. Traditional breeding also frequently produces plants that show new constitutive phenotypes linked to reduced yield, due to gene knockouts made by transposition of resident mobile elements and also because of random mutations [9]. However, genetic engineering is fast, and it offers the fine-tuning of gene expression by knockout (most often by Clustered regularly interspaced palindromic repeats [CRISPR]/Cas system) or overexpression (via classical GMO) of only the appropriate gene, often in a tissue-specific manner. With these techniques, it was possible to harness the available knowledge in plant physiology and plant molecular biology to improve crop traits.

2.3.2.2 Traditional Breeding and Genetic Modification

The yield of crop plants is dependent on environmental factors as well as the genetic potential of the given species/variety. Farmers might change the environment (e.g. through pest control and irrigation) in the plant's favor while breeders try to improve the genetic make-up of the plant. Breeding, the improvement of existing crop plant cultivars or developing new ones, requires changing the genetic background. It may be performed in traditional ways (i.e. traditional breeding) by looking for plants with desirable characteristics, maintaining the genotype, and crossing the useful genes into other varieties. Crossing might be assisted by using genetic mapping (biparental quantitative trait locus [QTL]-mapping or genome-wide association studies) of the chromosomal position of the gene of interest, followed by crossing and MAS for an advantageous trait among progeny plants [10, 11].

However, it is often ineffective and time-consuming to simply look for plants that have new desirable traits or have lost undesirable ones. Historically, the mutation rate was often increased using gamma or X-rays (radiation breeding). The semi-dwarf barley variety, Golden Promise was produced this way, and it proved to be a very successful variety of the 1970s and 1980s [12]. Interestingly, it is now favored by molecular biologists due to its high amenability for genetic transformation [13, 14]. The method of radiation breeding then gave way to chemical mutagenesis using ethyl methane sulfonate (EMS). EMS induces guanine-cytosine (GC) to adenine-thymine (AT), as well as AT to GC changes in the genome, as well DNA breaks, insertions, and deletions [15]. EMS is used in TILLING (Targeting Induced Local Lesions in Genomes) programs, originally developed by McCallum et al. [16]. TILLING is random chemical mutagenesis, followed by the identification of attractive mutants and the screening for causal genetic modification [17, 18]. A more recent example was the generation of powdery mildew-resistant wheat using the TILLING approach [19]. Another way for equipping the crop plant with useful genes (abiotic/biotic stress resistance and nutrient uptake) would be through