ADVANCES IN

BIOTECHNOLOGY AND BIOSCIENCE

Editors

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Dr. G. Venkata Subbaiah
Dr. Rohan Vilas Gavankar
Dr. Sachin Hemant Raut

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Preface

The field of biotechnology and bioscience has witnessed unprecedented growth in recent decades, transforming the way we understand life processes and address global challenges. The edited volume "Advances in Biotechnology and Bioscience" is a comprehensive compilation of contemporary research, innovative methodologies, and multidisciplinary applications that reflect the dynamic progress in these domains. This book aims to provide a platform for researchers, academicians, and industry professionals to explore emerging trends and technological breakthroughs that are shaping the future of biological sciences.

The chapters included in this volume cover a wide spectrum of topics, ranging from nanobiotechnology to environmental sustainability. The discussion on recent progress in carbon nanotubes highlights their synthesis, functional properties, and diverse applications across scientific disciplines. Environmental and green biotechnology emphasizes eco-friendly solutions for sustainable development, while molecular approaches based on DNA technologies offer advanced tools for parasite identification and characterization, contributing significantly to medical and veterinary sciences.

Sustainability remains a central theme throughout this book. Chapters on waste-to-energy (WtE) technologies and biomass energy conversion explore innovative strategies for efficient resource utilization and waste management. The inclusion of research on phthalate esters in aquatic environments sheds light on emerging environmental pollutants, their ecological impact, and the urgent need for mitigation strategies. These contributions collectively underline the importance of integrating biotechnology with environmental conservation efforts.

Agricultural biotechnology is another key focus of this volume. The chapter on biotechnological interventions for enhancing stress tolerance in paddy (Oryza sativa L.) addresses critical challenges related to food security and *climate change. Additionally, the integration of image-based phenotyping and computer vision in agriculture demonstrates how modern computational tools are revolutionizing crop monitoring and precision farming practices.*

The book also highlights transformative advances in biotechnology and biological research, reflecting the convergence of traditional biological sciences with cutting-edge technologies such as nanotechnology, data science, and artificial intelligence. These interdisciplinary approaches are paving the way for novel solutions to complex biological and environmental problems.

This volume would not have been possible without the dedicated efforts of the contributing authors, whose expertise and scholarly insights have enriched the quality and scope of this work. We extend our sincere gratitude to all contributors for their valuable contributions and to the reviewers for their constructive feedback. We also acknowledge the support of the publishing team in bringing this work to fruition.

We hope that this book serves as a valuable resource for students, researchers, and practitioners, inspiring further research and innovation in biotechnology and bioscience. It is our sincere belief that the knowledge presented in this volume will contribute meaningfully to scientific advancement and sustainable development.

Editors

Advances in Biotechnology and Bioscience

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Advances in Biotechnology and Bioscience

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Recent Progress in Carbon Nanotubes: Synthesis, Functional Properties, and Multidisciplinary Applications

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Abstract

In the nanotube world, carbon nanotubes (CNTs) are considered nanotubes with unique properties, especially carbon nanotubes, which are considered to have excellent structural, electrical, and mechanical properties, making these nanotubes exceptionally promising in the development of nanotechnologies. The recent developments in carbon nanotube synthesis are seen in improving the purity of CNTs. Various synthesis methods, especially those involving chemical vapor deposition, arc discharge, and laser ablation, have also helped in producing nanotubes with different composition, structure, and properties. Much more important, however, are the properties of CNTs, especially its electrical, thermal, and chemical properties, which make carbon nanotubes extremely promising in the development of nanotechnologies. The review aims and objectives are to address issues regarding the synthesis of carbon nanotubes, properties, and its application in nanosciences, especially with regard to nanotechnology, relating to issues in recent developments in this field. Ultimately, this review aims to focus on issues related to the applications of nanotubes and what they might actually achieve in the coming days in terms of nanosciences and nanotechnologies.

Keywords: carbon nanotubes, nanotechnologie, carbon meta-nanotubes, sensing mechanisms.

Introduction

Nanotubes are members of the fullerene family of structures, like their cousins the buckyballs. While buckyballs are spherical in shape, while a nanotube is cylindrical with one end closed by a hemisphere of the buckyball structure. The names of the atoms are based on the size of the atoms, as the diameter of a buckyball molecule is equal to nanotube is of the order of a few nanometers, which is roughly 50,000 times smaller than the width of a human hair), while they can be up to several millimeters in length. Researchers at the University of The University of Cincinnati (UC) has developed a method of creating extremely long aligned nanotube arrays [1]. They have been able to produce 18-mm-long carbon nanotubes that could be used to produce nanofibers. There are two main types of nanotubes which are single-walled nanotubes and multi-walled Nanotubes (MWNTs). The discovery of fullerenes by Harold Kroto of Sussex University in the UK and Richard Smalley and coworkers at Rice University in the US stimulated researchers to explore carbon filaments further. Indeed, the realization that the ends of carbon nanotubes must be fullerene-like "caps" explained the fact that the diameter of a carbon nanotube could only be as small as a fullerene molecule Carbon nanotube, a nanomaterial, have been identified and understood as being the most prominent nanomaterial ever, mainly owing to the unique and specific properties of its structure and functions in comparison to other nanomaterials. It has also been identified, understood, and comprehended as being in the form of a cylindrical nanostructure of the material, which is rolled-up in layers of graphene [2]. The unique and specific properties of the material have rendered CNTs to become the focal point of modern nanosciences and nanotechnologies. The goal of CNTs is that it could be clearly and comprehensively evaluated as an opportunity in terms of developing new materials and technology in specific fields of science and technology. Based on a clear comprehension of a unique structure of the material, CNTs have also been clearly and comprehensively identified and understood as being in two forms of the nanomaterial: single-walled carbon nanotubes (SWCNTs) and multi-walled carbon nanotubes (MWCNTs). The unique and specific properties of a given material have been comprehended in association with its small scale, which could be considered as almost being contradictory in terms of the electrical properties of the material. Based on evolutionary developments, advancements in a unique structure-property correlation of the material [3].

Fullerenes

Fullerene – C60, C70, C84, etc. – possessed the essential characteristic of being a pure carbon cage, each atom bonded to three others as in graphite [4]. Unlike graphite, every fullerene has exactly 12 pentagonal faces with a varying number of hexagonal faces (e.g., buckyball – C60 – has 20) Show in fig.1.

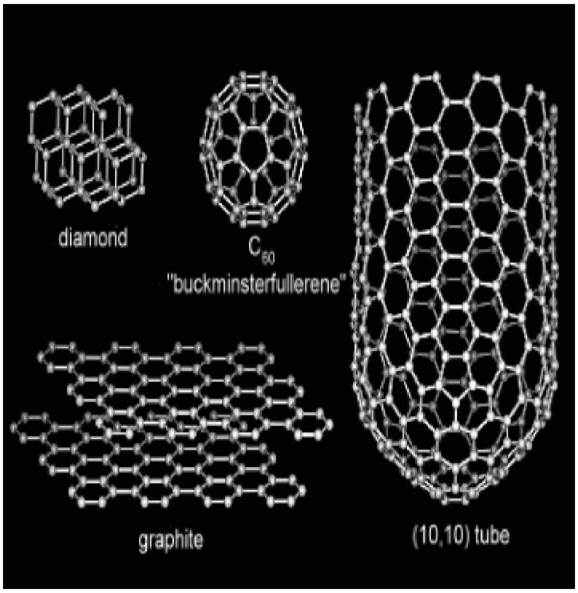

Fig. 1. Various forms of fullerenes

Single Wall Carbon Nanotube

A single wall carbon nanotube is a one-atom thick sheet of graphite (called graphene) rolled up into aseamless cylinder with diameter of the order of a nanometer . This results in a nanostructure where the length-to-diameter ratio exceeds 10,000. Such cylindrical carbon molecules have novel properties that make them potentially useful in a wide variety of applications in nanotechnology, electronics, optics andother fields of materials science[5].

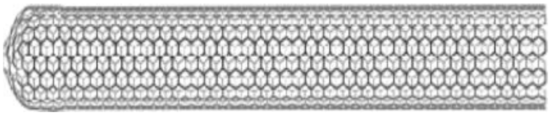

Fig. 2. Buckytube or carbon nanotube

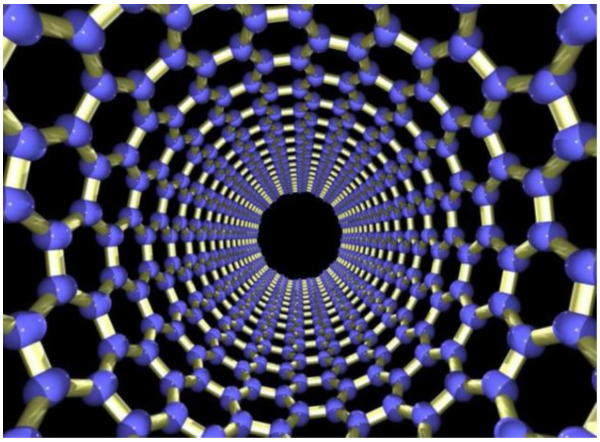

Fig. 3. Single walled carbon nanotube

Multiwall Carbon Nanotube

Among the nanotubes is the formation of different layers of rolled up graphite. Majorly, the nanotube is made up of different layers of rolled up graphite. tube shape. Two models exist, which are of greater relevance for the explanation of the definition of nanotubes: One of the models revolves around the formation of multi-walled nanotubes in fig.4: In the case of the Russian Doll Model, the formation of fullerenes takes the form of the rolled-up layers of graphite. Whereas the rolled-up layers of the graphite take the shape of concentric cylindrical structures as depicted in the formation of the (0,8) SWNT. A rolled-up graphite is then rolled up onto itself in a way that is almost as easy as rolling up the rolled-up state of a piece of newspaper or even just the rolled-up state of any ordinary piece of paper. Therefore, it is clear that the interlayer distance for the multi-walled nanotube is quite similar to the interlayer distance of the lattice of the Gamma Graphene sheet [6]. Unique role that has to be played by Double-Walled Carbon Nanotubes (DWNT) is particularly of greater relevance when considering it in the context of its emphasis, due.

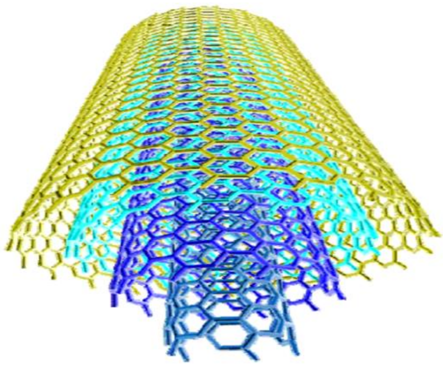

Fig.4. Multiwalled Carbon Nanotueb

Currently, some companies have established the commercial scale of production and utilization of these CNT nanomaterials, and these nanomaterials can be found at various suppliers throughout the world. Currently, several suppliers of CNT, who have mega-ton capacity production annually, are available, and new low-cost methods targeted at synthesizing CNTs are emerging, and this is bound to boost this field tremendously, as this demand is still on the increase. There has been a tremendous change in the synthesis of these CNTs, and various techniques have been developed targeted at improving these processes of synthesizing these products. Some of these methods that are currently being used in the synthesis of these materials are arc discharge, laser ablation, and chemical vapor deposition. New techniques have been developed targeted at improving the synthesis of these materials, including the synthesizing of these materials in low temperatures, as well as the improvement and optimization of its catalysts. This is in addition to introducing new techniques by changing the chemistry of these materials using intermediaries like functionalization and covalent/non-covalent chemistry [7].

CNT superior physicochemical properties have made them useful in a wide range of applications, including biomedical electronics, flexible and wearable sensors, energy storage and conversion devices, environmental detection and remediation, nanoelectronics, catalytic applications, and high-performance composites. New concepts in the development of emerging technologies have been brought about by their advantages in material reinforcement with increased strength, electrical conductivity, and thermal stability. However, there are still a number of obstacles to be addressed in the application of CNT [8].

Most current products with CNT are thought to have been recently introduced onto the consumer market. However, a vast increase may occur within the next few years depending on the regulatory actions across the world. Some currently actual and potentially attractive applications are electronics, functional fillers in various types of composites (ceramics, polymer materials and textiles), chemical sensors, gas-storage etc. Other applications, such as the use of CNT in Li-ion batteries, may have existed for decades now [9].

Synthesis Methods

The structural quality and chemical purity of CNT depend on the synthesis technique used and purification procedures followed during the synthesis. The majority of the CNT used presently appears to be synthesized through the catalyst-assisted technique of chemical vapor deposition (CVD). The CVD technique is commonly used [10]. A gaseous compound rich in carbon is passed through the surface of a large number of catalysts having nanometer sized pores or particles in a chamber under a very specific atmospheric composition and pressure. The nanoparticles are formed during the floating catalyst technique. The type of CNT formed, structure, and size are mainly determined by the catalyst size and type, gas(es), and temperature. CVD allows a wide range of possibilities for the formation of well-controlled tube dimensions, but the tube formed tends to be structurally defective. Carbon arc discharge synthesis is another important technique in the synthesis of CNT. Evaporation of C from a graphite pole and condensation of CNT on the other pole of a cathode and anode, respectively, is the process followed here [11]. The technique is simple and relatively inexpensive, but the degree of purity of the compound produced is very low due to the formation of several kinds of carbon.Other synthesis methods exist but are preferentially used for research work or synthesis of highly specific CNT types. One example of such methods is the pulsed laser ablation method, where a graphite target is evaporated together with metallic catalysts by a high intensity laser beam in an inert atmosphere. The vaporized carbon atoms start forming CNT in the vapor phase and the CNT material is deposited on a cooled substrate.

Functionalization

There has been significant work towards the development of means for altering the surface chemistry of CNT in ways that enhance solubility of the CNT substance in certain fluids or increase its potential for undergoing a chemical reaction with nearby components of a composite material in a solid matrix. The topic of the functionalization of CNT is arguably one of the most critical areas towards biological uses of CNT. One can refer to for more advanced reviews concerning the functionalization of CNT. More recently, functionalized CNT, in line with the designation throughout this report, was designated as belonging to the third generation of CNT, or Carbon Meta-nanotubes [12].

As elucidated in the discussion above, it is dependent on certain types, as well as purification and annealing, if any. When graphitized, the surface will have smoothness and hence will be polar. Therefore, the surface will be insoluble in both organic and aqueous solvents since half of the surface is composed of electrons of π-orbitals residing at the outer surface of the SWCNTs, which provides it with the ability to reach the state of attaining one more molecule and forming a covalent bond. In addition, it is because of this reason that the agglomeration is formed, especially due to the presence of the electrons of the π-orbitals residing at the outer surface of the SWCNTs, as it is the main key to form a bond, non-covalent Van der Waals bond, where several SWCNTs will get attached to each other with a firm grip and form a rope, where several SWCNTs will get attached and form a rope due to the interaction of the π-orbitals. Therefore, as elucidated in the discussion above, as demonstrated in the above figure, as discussed in section 2.2, the defects will form components of π-orbitals, as they contributed to the formation of the aforementioned agglomeration. Different types of functionalization may be applied depending on the designated use. One may discriminate between:

- Non-covalent functionalization (specific CNT and molecule interaction)
- Defect-group functionalization (bonding at vacancy or sw-defects)
- Direct covalent side-wall functionalization (bonding forming topological defects)
- Endohedral functionalization (tube-caged molecules, elements or metals)

Non-covalent functionalization is highly attractive when the physical properties of the CNT should be affected as little as possible. In non-covalent functionalization, one takes advantage of the high adsorption capacity of the CNTs, which is facilitated by their high surface energy. The procedure includes wrapping of CNT by polymers, polypeptides, and DNA. Interaction of the π-orbitals of CNT with aromatic moieties such as pyrene has also been widely used for non-covalent functionalization. The larger the π-orbital system on the aromatic molecule, the better they are attached [13]. In toxicological studies, non-covalent functionalization using proteins is also often used for making steric stabilization of CNT in dispersions.

Defect group and direct covalent functionalization: In the same way as the tube ends and the tube walls, the surface modification can be achieved. Although the defect group and the covalent CNTs will manifest lower levels of tensile and electrical conductivity compared with the raw material, the surface modification has been investigated.

As discussed above, i.e., in the above section, it has been clearly identified and discussed that the CNT in the solution of oxidizing acid will get reacted, thereby producing COOH end groups on the end of the tube and on the defects at hexagonal rings in the chain of carbon. "Oxygen-containing" surface groups refer to different raw materials for different surface modificiations, and it has been identified that fluorine and hydrogenation have been made for surface modification on a large scale. Furthermore, it has also been identified and proven that different organic chemical reactions, which are largely used, hold large application potential for their usage with CNTs. Those reactions include Grignard reactions conducted for organic metal chemistry, i.e., regarding organometallic reactions, along with other reactions such as radical addition reactions and electrophile addition reactions.

Applications

The most important applications are in various types of composites, electronics, and energy. The CNT properties used is currently mainly their extraordinary tensile strength, their electrical conductivity and current carrying capacity, gas and energy storage capacity, as well as well as tunable chemical reactivity from being inert to reactive with applications in sensors and catalysis [14].

The most important industrial sectors are the automotive, energy, paint and coatings, and electronics. The oldest use may be in Li-ion batteries where they constitute 1 - 3 weight percent of the graphite electrodes. Another, apparently well-established use is the application of CNT is as conducting filler in base resins and thermoplastics where it may reduce the tendency of the plastic parts to become charged by static electricity and enabling electrostatic painting without further treatment of the materials. The use of CNT in base resins due to their strength-enhancing properties is also well-known. Another more application is as reinforcing agent in advanced polymer composites used for sports equipment, e.g. rackets, golf-clubs, and ice-hockey sticks, but also high-performance bicycles, small boats and windmill blades containing CNT are on the market. There is however still technical challenges to be solved before the extreme strength and stiffness of CNT can be fully utilized in composites and this is one of the reasons for the still relatively limited use of CNT for specific enhancement of mechanical properties of materials [15]. Composite materials other than polymers are also being investigated, e.g. aluminium CNT composites and ceramic CNT composites. Only sparse information on these materials and their applications is available, and most likely their actual use is currently very limited.

Conclusion

Carbon nanotubes (CNTs) have proven to be one of the most promising and versatile nanomaterials because of their remarkable mechanical, electrical, thermal properties, and accessibility. Advances in synthesis methods, including chemical vapor deposition, arc discharge, and laser ablation, have addressed structural issues and made CNT production more cost-effective. Despite these advantages, problems remain in implementing defect-free and bulk synthesis. CNTs have been successfully surface-modified and hybridized to exhibit exceptional properties, making these nanomaterials highly soluble, selective, and compatible for application in sophisticated systems. CNTs have become essential in multidisciplinary areas of interest, including sensing, energy storage devices, composites, catalyzing chemical reactions, environmental applications, and medical devices. CNTs can transfer electrons and detect specific chemical interactions, making them the best solution for developing futuristic sensing mechanisms.

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Environmental and Green Biotechnology

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Abstract

Environmental and green biotechnology are interdisciplinary fields that harness biological systems to address environmental challenges and promote sustainable development. This chapter provides a comprehensive overview of key principles, technologies, applications, and recent advances in environmental and green biotechnology. It covers bioremediation strategies, bioenergy production, waste valorisation, sustainable agriculture, and regulatory and ethical considerations. Emphasis is placed on emerging molecular tools, engineered microbial consortia, and plant-based solutions that contribute to pollution control, resource recycling, and climate change mitigation. The chapter concludes with future prospects, challenges, and directions for research, including the role of digital technologies and systems biology in enhancing environmental biotechnological applications.

Keyword: Environmental biotechnology, green biotechnology, bioremediation, bioenergy, bioplastics, microbial ecology, sustainable agriculture, circular bioeconomy

Introduction

Environmental degradation including soil and water pollution, biodiversity loss, and climate change poses serious risks to ecosystems and human health. Conventional approaches to pollution control and resource management have limitations related to cost, efficiency, and sustainability. Environmental biotechnology utilizes living organisms or their derivatives to monitor, analyse, and mitigate environmental contaminants. Green biotechnology, closely aligned with sustainable and eco-friendly practices, focuses on leveraging biological systems to produce renewable products while minimizing environmental impact.

Together, these fields support a transition toward a circular bioeconomy, where biological resources are used efficiently, waste is minimized, and ecological integrity is preserved. Innovations in molecular biology, systems ecology, and engineering have expanded the toolkit available to researchers and practitioners. This chapter explores the scientific foundations, technological innovations, and real-world applications that define environmental and green biotechnology today.

Historical Context and Evolution

Environmental and green biotechnologies emerged from early efforts in microbial ecology and plant science. Early applications such as composting and nitrogen fixation were traditional biotechnological practices. With advances in genetic engineering and omics technologies, researchers began to design organisms and systems targeted for specific environmental functions.

The concept of bioremediation using microorganisms to degrade pollutants became widely recognized during the late 20th century following oil spill responses and soil contamination studies. Green biotechnology evolved with agricultural biotechnology, focusing on crops engineered for drought tolerance, pest resistance, and enhanced nutrient utilization.

Bioremediation Technologies

Microbial Bioremediation

Bioremediation uses microorganisms to detoxify or transform pollutants into less harmful byproducts.

- **Intrinsic Bioremediation:** Enhancement of native microbial communities under natural conditions.
- **Bioaugmentation:** Introduction of specialized strains capable of degrading recalcitrant compounds (e.g., polychlorinated biphenyls, aromatic hydrocarbons).
- **Biostimulation:** Optimizing environmental conditions (nutrients, electron donors/acceptors) to stimulate microbial activity.

Molecular diagnostics (e.g., qPCR and metagenomics) are now used to monitor microbial populations and pathways during bioremediation.

Phytoremediation

Phytoremediation employs plants to extract, stabilize, or degrade contaminants from soil and water.

- **Phytoextraction:** uptake of metals into harvestable plant tissues.
- **Phytodegradation:** plant enzymatic transformation of organic pollutants.
- **Rhizodegradation:** stimulation of microbial breakdown in the rhizosphere.

Plants such as *Populus spp*. and *Brassica spp*. have demonstrated efficacy in heavy metal cleanup.

Bioenergy and Biofuels

- **Bioethanol and Biodiesel**

Biofuels derived from biomass present alternatives to fossil fuels. Bioethanol is produced by fermenting sugars from crops or lignocellulosic biomass, while biodiesel results from transesterification of vegetable oils or waste fats.
Genetically engineered yeasts and enzymes have improved conversion efficiencies and broadened feedstock utilization.

- **Biogas and Algal Biofuels**

Anaerobic digestion of organic waste generates biogas (primarily methane), which can replace natural gas for heat and power. Microalgae are another promising bioenergy source due to high lipid content and rapid growth rates.

Bioproducts and Industrial Biotechnology
Environmental biotechnology contributes to sustainable industrial processes.

- Bioplastics such as polyhydroxyalkanoates (PHAs), produced by microbial fermentation.
- Enzymatic processes replacing chemical catalysts in textile, detergent, and paper industries, reducing energy and water consumption.
- Valorisation of waste into chemicals, fertilizers, and feed stocks.

Sustainable Agriculture and Green Biotechnology

Applications in agriculture

- **Biofertilizers:** Beneficial microbes (e.g., Rhizobium, Azospirillum) that enhance nutrient availability.
- **Biopesticides:** Biological agents (e.g., Bacillus thuringiensis) that control pests with reduced non-target toxicity.
- Transgenic crops with traits for improved stress tolerance and reduced input requirements.

Integration with precision agriculture tools (sensors, drones, data analytics) enhances resource efficiency.

Tools and Technologies in Environmental Biotechnology

Omics and Systems Biology

- Metagenomics reveals the composition and functional potential of complex microbial communities.
- Metatranscriptomics and metaproteomics track active genes and proteins, improving understanding of biodegradation pathways.

- Systems biology models integrate multi-omics data to predict responses and optimize biotechnological processes.

Synthetic Biology and CRISPR Technologies

Synthetic biology enables design of tailored organisms or pathways for pollutant degradation, nutrient cycling, and sustainable production. CRISPR-based editing accelerates development of strains with enhanced functions.

Environmental Monitoring and Biosensors

Biosensors employ biological recognition elements to detect contaminants (e.g., heavy metals, pesticides) in real time. Advances in nanomaterials and microfabrication have improved sensitivity and portability.

Examples include

- Enzyme-based electrochemical sensors
- DNA aptamer sensors
- Whole-cell bioreporters responding to toxicants

Regulatory, Ethical, and Risk Considerations

Use of genetically modified organisms (GMOs) in open environments raises concerns about horizontal gene transfer, ecosystem impacts, and public acceptance. Regulatory frameworks (e.g., Cartagena Protocol on Biosafety) govern release and monitoring. Ethical considerations focus on equitable access to technologies and protection of indigenous knowledge.

Future Prospects and Challenges

Key drivers of future research

- Integration of artificial intelligence (AI) for process optimization and predictive environmental modelling.
- Development of robust microbial consortia for complex pollutant degradation.
- Expansion of circular bioeconomy pathways linking waste streams to value-added products.
- Enhanced risk assessment tools to balance innovation with safety.

Challenges include scalability, public perception, and harmonizing global regulatory standards.

Conclusion

Environmental and green biotechnology transform how society addresses environmental degradation and resource limitations. From microbial remediation and bioenergy production to sustainable agriculture and bioproducts, these technologies align with principles of sustainability and ecological stewardship. Continued interdisciplinary research integrating molecular biology, engineering, and digital technologies will further enhance our capacity to protect ecosystems while meeting human needs. Responsible deployment, guided by ethical and regulatory frameworks, will be essential to maximize benefits and minimize risks.

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DNA-Based Molecular Approaches for Parasite Identification and Characterization

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Abstract

Reliable identification of parasites is a cornerstone of parasitology, directly influencing diagnosis, epidemiology, taxonomy, and control measures. Traditional identification methods based on morphology and life-cycle traits are often inadequate due to cryptic species, phenotypic variation, damaged specimens, and the absence of diagnostic stages. In recent years, DNA-based molecular techniques have transformed parasite identification by offering greater accuracy and reproducibility. This chapter explains the role of DNA as a molecular tool in parasite identification and outlines the major methodological steps involved, including sample collection, DNA extraction, selection of genetic markers, polymerase chain reaction (PCR), gel electrophoresis, and DNA sequencing. Advanced approaches such as DNA barcoding, next-generation sequencing, and metabarcoding are discussed for their applications in clinical, veterinary, wildlife, and forensic parasitology. Overall, molecular identification using DNA provides sensitive and precise tools for resolving taxonomic ambiguities and enhancing modern parasitological research and diagnostics.

Keywords: Molecular parasitology, DNA-based identification, Polymerase chain reaction (PCR) DNA sequencing, Genetic markers

Introduction

Parasites constitute a diverse group of organisms that infect humans, animals, and plants, causing a wide range of diseases with significant medical, veterinary, agricultural, and ecological consequences. Accurate identification of parasites is fundamental to parasitology, as it underpins disease diagnosis, epidemiological surveillance, taxonomy, phylogenetic analysis, and the development of effective prevention and control strategies. Traditionally, parasite identification has relied on morphological characters, host specificity, life-cycle patterns, and staining techniques. Although these methods have served as the foundation of classical parasitology, they often suffer from serious limitations.

Morphological identification becomes challenging when closely related species exhibit overlapping diagnostic characters, when parasites display polymorphism, or when only immature, damaged, or cryptic life stages are available for examination. In addition, mixed infections and phenotypic plasticity further complicate accurate diagnosis. These challenges have resulted in frequent misidentification and underestimation of parasite diversity (Soulsby, 1982; Bowman, 2014).

The advent of molecular biology has revolutionized parasite identification by introducing DNA-based techniques that overcome the limitations of morphology-based approaches. DNA acts as a stable, universal, and highly informative molecular marker that allows parasites to be identified at species, strain, and population levels, irrespective of developmental stage. Molecular identification has therefore become an indispensable tool in modern parasitology, with applications extending from clinical diagnostics to biodiversity assessment and forensic investigations (Gasser, 2006).

History of Molecular Approaches in Parasite Identification

Early parasite identification relied mainly on morphology, life-cycle characteristics, host specificity, and microscopic examination, methods that were widely used until the mid-20th century but were limited by phenotypic variation and cryptic species (Nadler & Pérez-Ponce de León, 2011). The late 1970s marked the introduction of biochemical techniques such as isoenzyme electrophoresis for strain differentiation. A major breakthrough occurred in the mid-1980s with the advent of recombinant DNA technology and the polymerase chain reaction (PCR), enabling sensitive detection of parasite DNA (Snounou et al., 1993). During the 1990s, ribosomal DNA (18S rRNA, ITS) and mitochondrial markers became standard tools for species identification and epidemiological studies globally and in India, particularly for parasites such as Plasmodium and Leishmania. In the early 2000s, DNA sequencing and DNA barcoding using the COI gene enhanced species-level resolution (Hebert et al., 2003). More recently, real-time PCR and next-generation sequencing have enabled rapid diagnosis, drug-resistance monitoring, and population-level analyses, making molecular approaches integral to modern parasitology (Verweij & Stensvold, 2014). The historical development and evolution of parasite identification from traditional to molecular approaches and its timeline briefly presented in table-1.

Table 1. Timeline of Molecular Approaches in Parasite Identification

Period / Year	Milestone	Key Developments	India-specific Examples
Pre-1970s	Classical era	Parasite identification based on morphology, host specificity, and life cycles	Extensive morphological surveys of human and livestock parasites
1970–1980	Biochemical phase	Isoenzyme electrophoresis introduced for strain differentiation	Early strain studies in *Leishmania donovani*
1983–1985	Molecular revolution	Development of recombinant DNA technology and PCR	Initial adoption of molecular biology techniques in medical institutes
1990–1995	PCR era	PCR used for parasite detection and species identification	PCR diagnosis of *Plasmodium falciparum* and *P. vivax*
1995–2000	Marker-based identification	Use of 18S rRNA, ITS regions, mitochondrial genes	Molecular typing of *Entamoeba histolytica*
2000–2005	DNA sequencing	Sanger sequencing for taxonomic confirmation	Phylogenetic studies on *Leishmania* and helminths
2003–2010	DNA barcoding	COI gene used for species-level identification	Barcoding of trematodes and cestodes
2010–2015	Real-time PCR	Quantitative detection and drug-resistance monitoring	qPCR studies on malaria and kala-azar
2015–Present	NGS & metabarcoding	Whole-genome sequencing, community analysis	Helminth diversity studies and drug-resistance research

Principle of DNA-Based Parasite Identification

DNA-based parasite identification is based on the fundamental biological principle that every organism possesses a unique genetic composition encoded within its DNA. Although many genes are conserved across taxa due to shared evolutionary ancestry, specific nucleotide sequence variations distinguish one species from another. These variations serve as molecular signatures that can be exploited for accurate identification (Avise, 2004).

Certain genomic regions evolve slowly and remain highly conserved, making them suitable for universal primer design, while other regions evolve rapidly and provide sufficient variability for species-level discrimination. By isolating parasite DNA, amplifying target gene regions, and analyzing nucleotide sequence differences, parasites can be reliably identified and classified. This approach provides high sensitivity and specificity, even when parasite material is scarce or degraded.

Steps Involved in DNA-Based Identification of Parasites

- **Collection of Parasitic Material**

Parasitic DNA can be obtained from a wide variety of biological sources depending on the parasite group and study objective. These include whole parasites such as protozoa, helminths, and arthropods; developmental stages such as eggs, larvae, cysts, and oocysts; and host-derived clinical samples including blood, stool, urine, sputum, and tissue biopsies. DNA may also be isolated from vectors or environmental samples such as soil and water, particularly in ecological and epidemiological studies (Thompson, 2013).

- **Isolation of Parasite DNA**

DNA isolation is a critical prerequisite for molecular identification. The objective is to obtain pure, intact DNA free from proteins, lipids, polysaccharides, and other inhibitors that may interfere with enzymatic reactions. DNA extraction generally involves cell lysis, protein removal, and DNA precipitation using standardized molecular protocols (Sambrook & Russell, 2001). The complete process of DNA isolation briefly describes in figure-1.

Biological Sample
↓
Sample Preparation
(washing / grinding)
↓
Cell Lysis
(detergent + buffer + EDTA)
↓
Protein Removal
(Proteinase K / Phenol–chloroform)
↓
RNA Removal (optional)
(RNase treatment)
↓
DNA Precipitation
(ethanol / isopropanol + salt)
↓
DNA Washing (70% ethanol)
↓
DNA Solubilization
(TE buffer / water)
↓
Purified DNA

Figure 1. Flow Diagram of DNA Isolation Process

High-quality DNA is essential for successful amplification, sequencing, and downstream molecular analyses.

Selection of Molecular Markers

The choice of molecular markers depends on the parasite group and the taxonomic resolution required. Commonly used genetic markers include ribosomal RNA genes, internal transcribed spacer (ITS) regions, and mitochondrial genes.

- 18S rRNA gene is widely used for broad detection of protozoa and helminths due to its conserved nature.
- ITS-1 and ITS-2 regions provide species-level resolution because of their high variability.
- 28S rRNA gene is useful for phylogenetic and evolutionary studies.
- Mitochondrial genes, such as cytochrome c oxidase subunit I (COI) and cox1, are extensively used for DNA barcoding and population genetics.
- Microsatellite markers are employed for strain differentiation and epidemiological investigations (Gasser et al., 2012).

DNA Amplification by Polymerase Chain Reaction (PCR)

The polymerase chain reaction (PCR) is a cornerstone technique in molecular parasitology. Developed by Kary Mullis in 1983, PCR enables exponential amplification of specific DNA fragments from minute quantities of template DNA. PCR relies on cyclic repetition of denaturation, annealing, and extension steps, resulting in rapid and highly specific DNA amplification (Mullis et al., 1986). The outline of amplification of target DNA mentioned below in figure-2.

Double-stranded DNA

↓ (94–95°C)

Denaturation

↓ (50–65°C)

Primer Annealing

↓ (72°C)

Extension by DNA Polymerase

↓

One PCR Cycle

↓

Repeated 25–35 Cycles

↓

Exponential DNA Amplification (2^n copies)

Figure 2. Principle of PCR Amplification

Variants such as nested PCR, multiplex PCR, and quantitative PCR enhance sensitivity and allow detection of mixed infections.

Agarose Gel Electrophoresis

Following PCR amplification, agarose gel electrophoresis is used to separate and visualize DNA fragments based on size. The appearance of a DNA band of expected size provides preliminary confirmation of parasite identity and amplification success (Brown, 2016).

DNA Sequencing as a Tool for Parasite Identification

DNA sequencing involves determination of the precise order of nucleotides within a DNA fragment. Sequence data act as molecular fingerprints that enable discrimination of closely related parasite species and strains. DNA sequencing has revolutionized parasitology by providing high-resolution taxonomic and phylogenetic information (Blaxter, 2004). The molecular techniques involved in DNA sequencing are-

Sanger (Dideoxy) Sequencing

The chain-termination sequencing method was developed by Fred Sanger and colleagues in 1977. This method uses dideoxynucleotides that terminate DNA synthesis at specific positions, generating fragments of varying lengths that are resolved by capillary electrophoresis. Despite the emergence of high-throughput sequencing technologies, Sanger sequencing remains the gold standard for accurate sequencing of individual parasite genes (Sanger et al., 1977). The flowchart of Sanger sequencing mentioned in figure-3.

PCR-amplified DNA
↓
Sequencing Reaction
(dNTPs + fluorescent ddNTPs)
↓
Chain Termination
↓
Capillary Electrophoresis
↓
Fluorescence Detection
↓
DNA Sequence Output

Figure 3. Sanger Sequencing flow chart

Maxam–Gilbert Sequencing

This chemical cleavage-based sequencing method was developed by Allan Maxam and Walter Gilbert. Although historically significant, it is rarely used today due to technical complexity and safety concerns (Maxam & Gilbert, 1977).

Next-Generation Sequencing (NGS)

Next-generation sequencing technologies allow massively parallel sequencing of millions of DNA fragments simultaneously. NGS platforms enable detection of mixed parasite communities, whole-genome sequencing, and identification of drug resistance genes. These technologies have transformed epidemiological and ecological parasitology (Goodwin et al., 2016). The process of Next-generation sequencing (NGS) of DNA given below in figure-4.

DNA Fragmentation

↓

Adapter Ligation

↓

Library Amplification

↓

Massively Parallel Sequencing

↓

Bioinformatic Analysis

↓

Parasite Identification

Figure 4. NGS-Based Parasite Identification

DNA Barcoding and Metabarcoding

DNA barcoding uses standardized gene regions, commonly mitochondrial COI, to identify parasite species. Metabarcoding combines DNA barcoding with NGS, enabling simultaneous identification of multiple parasites from mixed samples. These approaches are particularly useful in biodiversity assessment and environmental parasitology (Hebert et al., 2003; Taberlet et al., 2012).

Bioinformatics and Reference Databases

Sequence comparison is performed using curated biological databases such as NCBI and GenBank. Analytical tools such as BLAST and phylogenetic tree construction algorithms facilitate accurate parasite identification and evolutionary analysis (Altschul et al., 1990). The whole process of bioinformatics involved in parasite identification mention in figure-5.

Sample Collection

↓

DNA Isolation

↓

PCR Amplification

↓

DNA Sequencing

↓
Database Comparison
↓
Phylogenetic Analysis
↓
Accurate Parasite Identification

Figure 5. DNA Sequencing-Based Parasite Identification

Applications of DNA-Based Parasite Identification

DNA-based molecular techniques are widely applied in clinical diagnosis, epidemiology, veterinary medicine, wildlife conservation, forensic investigations, and biodiversity assessment. They enable identification of cryptic species, larval stages, mixed infections, and drug-resistant strains. The molecular techniques involved in different groups of parasites briefly listed in table-2.

Table 2. Molecular Techniques Used in Parasite Identification

Molecular Technique	**Target Gene / Marker**	**Parasite(s) Identified (Examples)**	**Application**	**Selected References**
Polymerase Chain Reaction (PCR)	18S rRNA, ITS, COI	*Plasmodium spp.*, *Entamoeba histolytica*, *Giardia lamblia*	Species identification, diagnosis	Singh et al. (1999); Verweij et al. (2004)
Nested PCR	18S rRNA	*Plasmodium falciparum*, *Plasmodium vivax*	Detection of low-level infections	Snounou et al. (1993)
Real-time PCR (qPCR)	ITS, 18S rRNA	*Leishmania spp.*, *Toxoplasma gondii*	Quantification and rapid diagnosis	Mary et al. (2004); Lin et al. (2000)
Restriction Fragment Length Polymorphism (PCR-RFLP)	ITS, COI	*Echinococcus granulosus*, *Taenia spp.*	Strain and genotype differentiation	Bowles & McManus (1993)
DNA Sequencing (Sanger)	COI, cox1, 18S rRNA	*Schistosoma spp.*, *Fasciola spp.*	Accurate species confirmation	Blair et al. (2001)
DNA Barcoding	COI	*Strongyloides*	Species-level	Hebert et al.

		spp., *Anisakis spp.*	identification	(2003); Nadler & Pérez-Ponce de León (2011)
Next-Generation Sequencing (NGS)	Whole genome / targeted genes	*Plasmodium spp.*, *Trypanosoma spp.*	Genomics, drug resistance studies	Carlton et al. (2008)
Metabarcoding	COI, 18S rRNA	Mixed helminth and protozoan communities	Community-level parasite detection	Pompanon et al. (2012)
Loop-Mediated Isothermal Amplification (LAMP)	18S rRNA	*Trypanosoma spp.*, *Plasmodium spp.*	Field-based rapid detection	Notomi et al. (2000)
RAPD-PCR	Random primers	*Leishmania donovani*, *Schistosoma spp.*	Genetic diversity analysis	Williams et al. (1990)

Advantages and Limitations

Advantages

- High sensitivity and specificity
- Independent of morphology
- Applicable to all life stages
- Detects mixed infections
- Highly reproducible

Limitations

- High cost and infrastructure requirements
- Risk of contamination
- Dependence on reference databases
- Need for trained personnel

Conclusion

DNA-based molecular tools have fundamentally transformed parasite identification by overcoming the inherent limitations of traditional morphological approaches. Techniques such as PCR, DNA sequencing, and metabarcoding enable accurate, reliable, and reproducible identification of parasites at species and strain levels. Continued advancements in sequencing technologies and bioinformatics will further enhance the role of molecular parasitology in disease control, biodiversity conservation, and evolutionary research.

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Waste-to-Energy (WtE): Sustainable Approach of Waste Management

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Abstract

The rapid growth of the global population and corporate interests has exacerbated issues related to rising energy consumption, inadequate waste management, and environmental degradation. The 5Rs (Reduce, Reuse, Repair, Recover, and Recycle) form the basis of contemporary methods that effectively address these challenges. There is a practical solution for materials that cannot be recycled: transforming waste into energy (WtE). In WtE, the system processes include biological conversion (anaerobic digestion, fermentation), thermal conversion (incineration, gasification, and pyrolysis), and physicochemical conversion (transesterification). While the ecological processes involved in WtE include microbial decomposition (the breakdown of organic waste by microorganisms), thermophilic digestion (high-temperature decomposition of organic waste), and photodegradation (the breakdown of organic waste by light). Therefore, this chapter systematically evaluates various waste-to-energy processes, emphasizing their capacity to reduce waste.

Keywords: Waste-to-Energy, 5Rs, Microbial Decomposition, Thermophilic Digestion

Introduction

Waste is anything discarded by an individual, household or organisation. Waste produces a complex mixture of substances, only some of which are intrinsically hazardous to health. The collection, processing, transport, and disposal of waste constitute the Waste Management (WM) process and are important for both public health and aesthetic and environmental reasons. Managing solid waste is a significant challenge worldwide in urban areas. Without a comprehensive, efficient solid-waste management program, waste generated by numerous human activities can pose health risks and harm the environment (Tanveer et al., 2022) There are many types of waste: Municipal (household, commercial and demolition waste), hazardous (industrial), biomedical, electronic (e-waste), radioactive, etc. Waste management includes the collection, transport, valorization and disposal of this waste. More broadly, it includes any activity involved in the organization of waste management from production to final treatment. Sustainable waste management is a key concept of the circular economy and offers many opportunities. The main advantage of sustainable waste management is to lessen the environmental impact by improving air and water quality and reducing greenhouse gas emissions (Titto & Savino, 2019). Besides, reducing food waste also helps reduce the high environmental cost of producing more. Waste management involves collecting, sorting, treating, recycling, and, when properly facilitated, providing a source of energy and resources. Therefore, it has a huge economic potential that needs to be leveraged by public and private entities. Besides creating jobs, improved waste management enhances the quality of life for local populations by improving hygiene conditions and reducing health risks associated with illegal dumping and inadequate garbage collection.

Waste Management Methods

The important techniques used for waste management include:

- **Waste Collection:** the collection of household waste is usually done by means of garbage trucks, which go to each point of garbage production to collect garbage. There are also collection systems where a network centralizes waste, such as automated vacuum collection.
- **Landfills:** A landfill, sometimes referred to as a garbage dump, is a place where waste is generally stored on the ground. Landfills are the most common and cheapest method of waste disposal worldwide.
- **Incinerator:** Incineration of waste is a technique of transformation by the action of fire. Waste combustion can generate electricity and heat, but it is also a significant source of air pollution.
- **Recycling:** recycling is the physical reprocessing of old materials such as metals, plastics and e-waste, industrial or household, into new products. The types of materials collected for recycling can vary by city and country.
- **Biological Reprocessing:** Composting is a process of conversion and recovery of organic matter into a stabilized, hygienic, soil-like product rich in humic compounds: the compost. Anaerobic digestion is a process similar to composting that treats organic waste and sludge through fermentation in the absence of oxygen.
- **Waste-to-Energy:** Energy recovery from waste, often called waste-to-energy, is the process of converting non-recyclable waste materials into usable heat, fuel or electricity through combustion, anaerobic digestion, gasification, pyrolysis, etc.

Green Technology of Waste Management

Green technology refers to the development and use of technologies that minimize the negative impact of human activities on the environment and society. It encompasses a wide range of products, services and practices that support a more sustainable future (Reddy et al., 2025). Green technology solutions are meant to be sustainable; they are designed to meet the needs of the present without compromising future generations' ability to meet their own needs.

- **AI and Robotics for Sorting:** Robots with AI, like AMP Clarity™, identify and sort diverse waste materials (plastics, metals) on conveyor belts, improving recycling efficiency.
- **Waste-to-Energy (WtE) System:** Controlled incineration or advanced thermal processes convert non-recyclable waste into electricity or heat, reducing landfill volume.
- **Bioremediation & Phytoremediation:** Using plants and microbes to absorb, stabilize, or transform pollutants like heavy metals and organic waste into less harmful forms.
- **Advanced Recycling:** Technologies that break down complex waste into valuable raw materials or new products, often using chemical processes or smart sorting.
- **Bioproducts and Biofuels:** Converting organic waste into valuable items like biopolymers, fertilizers, and biofuels, creating added value from waste.
- **Smart Waste Collection:** Route optimization software and sensors in bins predict fill levels, creating efficient collection routes to save fuel, reduce emissions, and manage resources better.

Green technology in waste management offers multiple environmental, economic, and social benefits by promoting efficient and sustainable handling of waste materials. One of the most significant advantages is the reduction of the environmental footprint (Orazalin et al., 2025). By minimizing reliance on landfills, green technologies help decrease soil and groundwater contamination, reduce air pollution from open dumping and burning, and significantly lower greenhouse gas emissions such as methane. Techniques like composting, recycling, anaerobic digestion, and waste-to-energy systems contribute to cleaner ecosystems and improved public health (Brunner & Morf, 2025). Green technologies encourage responsible consumption patterns, reduce resource depletion, and minimise waste generation at every stage of the product lifecycle. This approach promotes long-term ecological balance and intergenerational equity.

Waste-to-Energy

Waste-to-Energy (WtE) refers to a group of waste treatment technologies that convert solid waste into usable forms of energy, primarily through thermal processes such as incineration. It is regarded as a controlled waste management strategy that complements conventional approaches like landfilling and recycling (Ogidi & Izah, 2025). As global waste generation continues to increase due to rapid urbanization, industrialization, and population growth, WtE has emerged as a practical option for reducing the volume of municipal solid waste (MSW) while simultaneously recovering energy.

The operational mechanism of large-scale WtE facilities generally involves controlled high-temperature incineration. Waste is first transported to the plant, where recyclable materials are removed to optimize energy recovery and reduce environmental impact. The remaining waste is then mixed and fed into a combustion chamber, where it is burned at temperatures typically ranging between 850°C and 1,450°C. The heat generated from combustion is used to convert water into high-pressure steam, which drives a turbine connected to a generator to produce electricity (Rogoff & Screve, 2011). The energy output depends on the calorific value of the waste, with materials such as plastics yielding higher energy than low-calorific organic matter. Overall, Waste-to-Energy technology represents an integrated approach to waste management and energy recovery. While it offers significant advantages in reducing landfill dependency and generating electricity, its environmental performance and sustainability require careful evaluation within the broader context of circular economy and climate goals (Boloy et al., 2021).

Waste-to-Energy Technologies

- **Incineration:** Direct combustion of waste at high temperatures is the most common WtE technology and the most commercially viable.
- **Anaerobic Digestion (AD):** Anaerobic digestion is a controlled, oxygen-free process that promotes the decomposition of organic solid waste using microorganisms. While it can occur naturally, AD is also used in residential and industrial settings to produce biogas as a fuel. Biogas, which mainly consists of methane and carbon dioxide, is considered a renewable energy source.
- **Pyrolysis:** In a thermochemical treatment, pyrolysis exposes organic waste to high temperatures in the absence of oxygen. This process initiates the decomposition and disintegration of the material. The byproducts are commonly carbon-rich char (biochar) and combustible gases. Some of these gases can then be condensed into a combustible liquid called bio-oil or bio-crude.
- **Landfill Gas (LFG) Recovery:** The decomposition of organic material in landfills creates a natural byproduct called landfill gas. LFG consists of methane, carbon dioxide and a small percentage of non-methane compounds. It can be collected, treated, and used as fuel for industrial applications, vehicles, and more. LFG recovery is one method for reducing landfill methane emissions.
- **Gasification:** Gasification is also a thermochemical process that converts organic waste (biomass) into a combustible gas by using high temperatures and

a controlled amount of oxygen, steam, or both. The result is a combustible natural gas called syngas or producer gas used to make ammonia and methyl alcohol (methanol). It can also replace gasoline as a biofuel alternative.

Waste-to-Energy Benefits

Waste-to-energy has many benefits when compared to traditional waste management systems:

- **Reduced Waste Volume:** Yearly, the world generates more than 2 billion tonnes of MSW. The growing volume of waste and the pollution it generates are inherently linked to climate change, while the best way to reduce waste is to produce less of it. WtE offers a provisional solution to reduce the waste volume by around 87%.
- **Cleaner Than Landfills:** WtE plants emit less greenhouse gases and pollutants than landfills and the open burning of waste because WtE processes are significantly more controlled and monitored (Quicker et al.,2020). Most modern WtE plants are held to strict emissions standards across pollutants, including heavy metals and dioxins.
- **Resource Recovery:** WtE processes offer greater opportunities for resource recovery than landfilling, particularly for the metals left behind after incineration (Morf et al., 2013). It aligns with circular economy principles focused on keeping materials in a closed-loop system and reducing waste.

Conclusion

WtE is the leading MSW management method, treating about 40 million tonnes. Today, WtE is one of the best investigated and optimized technologies in waste management. It enables the recovery of energy as heat and electric power, and facilitates the ‘cleaning’ of cycles by destroying hazardous organic substances. A thorough review of technological developments has highlighted the following environmental impacts of waste-to-energy transformations: a shift towards more sustainable energy sources, reduced landfill waste, and lower greenhouse gas emissions. The results show how important it is to generate Waste-to-Energy (WtE) to enhance energy efficiency, reduce the environmental impact of manufacturing, and advance the circular economy (Van Caneghem et al., 2019). This chapter concludes by proposing directions for additional research and media focus that may enhance the ecological benefits of waste-to-energy (WtE) projects.

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Phthalate Esters in Aquatic Environments: Occurrence, Environmental Fate and Ecotoxicological Effects

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Abstract

The prevalence of plasticizers in aquatic environments and their ecotoxicological effects are examined in this review. Synthetic organic compounds known as plasticizers, especially phthalate acid esters (PAEs), are widely used in polyvinyl chloride (PVC) to increase its durability and flexibility. They are constantly released into the environment through industrial effluents, municipal wastewater, and the breakdown of plastic waste because they are not chemically bound to polymer matrices. Anthropogenic activities continue to be the main source of contamination, despite the fact that small amounts of natural production by cyanobacteria and algae have been documented. Plasticizers persist in aquatic systems and easily accumulate in organisms because of their low volatility, limited water solubility, and high lipophilicity. Globally, there has been notable spatial variation in contamination, with developing nations like China, India, and Brazil typically reporting higher concentrations, sometimes surpassing regulatory limits. Plasticizers undergo hydrolysis, photodegradation, and mostly microbial degradation in aquatic environments; however, transformation rates are frequently slow, leading to extended environmental persistence. Endocrine disruption, immunotoxicity, neurotoxicity, genotoxicity, metabolic disorders, developmental abnormalities, and reproductive impairment are examples of ecotoxicological effects. In aquatic species, exposure, both acute and chronic, has been connected to

mortality, organ damage, and deformities. Their lipophilic nature encourages trophic transfer and bioaccumulation in fatty tissues, posing risks to the environment and human health and emphasizing the need for ongoing research and monitoring.

Keywords: Aquatic Environments, Ecotoxicological Impacts, Plasticizers, Bioaccumulation, Endocrine Disruption.

Introduction

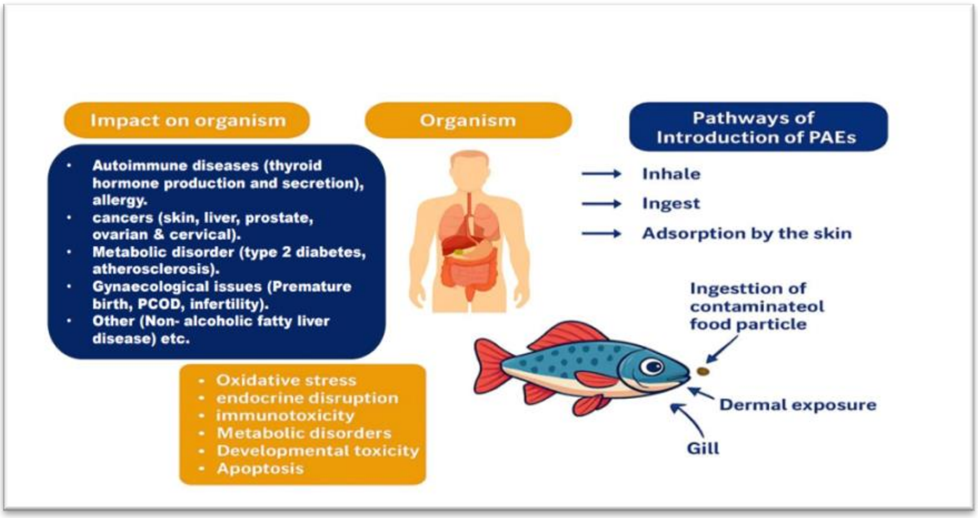
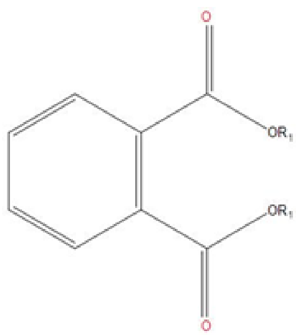


Fig.1. Different pathway and toxicity of PAEs on Human and Fish based on (based on Khoshmanesh et al., 2024)

Plasticizers, crucial additives for improving the characteristics of plastics, have become major environmental and health issues because of their durability and extensive application (Manatunga et al., 2024). Plasticizers, particularly phthalates, have significant adverse effects on the health of aquatic organisms. Plasticizers, as additives in microplastics, can adversely affect aquatic organisms by increasing toxicity and bioavailability of hazardous compounds. phthalic acid esters, have gained widespread use as plasticizers in various products due to their capability to enhance the flexibility, softness, reliability, and durability of polymers (Salazar-Beltran et al., 2018; Vinas et al., 2015).Phthalate esters (PEs) are a class of industrial organic compounds characterized by a common chemical structure dialkyl or alkyl/aryl esters of 1,2-benzenedicarboxylic acid (Annamalai & Vasudevan, 2020; Giuliani et al., 2020). These esters are synthesized through the reaction of phthalic anhydride with various alcohols (Annamalai & Vasudevan, 2020). The polar carboxyl groups have minimal influence on the physical properties of PEs, except when the alkyl chains (R and R') are very short, such as methyl or ethyl groups. Phthalates, or phthalate acid esters (PAEs), are coluorless, low-volatility, and poorly water-soluble synthetic organic compounds (Jiménez-Skrzypek et al., 2020). Due to their ability to enhance transparency, flexibility, strength, and durability, PAEs are widely used as plasticizers in a broad range of products, including plastics, fertilizers, pesticides, toys, cosmetics, and other consumer goods (Zhang et al., 2020; Tao et al., 2020). Global industrial usage of phthalate derivatives exceeds 3 million metric tons annually (Golestanzadeh et al., 2019). PAEs are easy to dissolve, migrate, and volatilize from plastic products. PAEs are ubiquitous in the atmosphere, water, sediment, soil, and even organisms, and humans (Kaewlaoyoong et al., 2018; Borges Ramirez et al., 2019;). Over 25 types of PAEs are utilized in commercial applications, each contributing distinct functional properties to the products they are incorporated into (Wang et al., 2019). The 11 most commonly used PAEs in consumer products include dimethyl phthalate (DMP), diethyl phthalate (DEP), dibutyl phthalate (DBP), benzyl butyl phthalate (BzBP), dicyclohexyl phthalate (DCHP), di-2-ethylhexyl phthalate (DEHP), diisobutyl phthalate (DiBP), diisononyl phthalate (DiNP), diisodecyl phthalate (DiDP), di-n-hexyl phthalate (DnHP), and di-n-octyl phthalate (DnOP) (Dutta et al., 2020). In most commercial applications, PAEs act as additives and are not chemically bonded to the polymer matrix, making them prone to environmental release through evaporation, leaching, and abrasion (Jiménez-Skrzypek et al., 2020). Studies have shown that certain freshwater algae and cyanobacteria can naturally produce compounds such as monoethylhexyl phthalate (MEHP) and DBP, which may be released into aquatic ecosystems. Nevertheless, the primary source of PAE contamination in water bodies is the discharge of industrial and municipal wastewater, as well as the breakdown of plastic waste containing synthetic PAEs. Due to their high n-octanol–water partition coefficients (Kow) and low vapor pressures, PAEs have low volatility in aquatic environments and tend to persist and migrate within water systems (Cao et al., 2018). Consequently, PAEs can be readily absorbed by aquatic organisms. In various aquatic species, PAEs have been associated with a wide range of toxic effects, including immunotoxicity, endocrine disruption, neurotoxicity, metabolic disturbances, genotoxicity, and developmental toxicity (Yu et al., 2019; Li et al., 2020a; Xu et al., 2020; Mondal & Mukherjee, 2020). Recognizing these potential risks, international bodies such as the United Nations Environment Programme (UNEP) and the International Maritime Organization are actively assessing the environmental and public health implications of PAEs. Regulatory measures have also been implemented in several countries; for instance, the use of BBP, DEHP, DBP, and DiBP has been restricted or banned in children’s toys and related products under the U.S. Consumer Product Safety Improvement Act (CPSIA, 2008) and Canada’s Dangerous Products Act (2010) (Zhang et al., 2020). The production of phthalate all over the world has grown from 2.7 to approximately 6 million tons per annum from 2007 to 2017(Gao et al. 2018). Because phthalates cannot be removed by traditional wastewater treatment, they end up in nearby lakes and rivers where they accumulate in the ecosystem and fish and other aquatic species systems before entering the food chain and having harmful effects on people and other biota (Kasonga et al. 2021). Like other synthetic compounds, PAEs infiltrate aquatic ecosystems via multiple routes, including direct or indirect discharge from wastewater, surface runoff, and atmospheric deposition (Zhang et al., 2018; Hajiouni et al., 2022; Gholaminejad et al., 2024). In addition, the review of 39 studies concerning water contamination from 2022 to 2023, only 22 studies could be analyzed due to a lack of sufficient information regarding the numerical values of plasticizer concentrations. In a similar vein, the research on soil and sediment contamination was limited, with merely 11 studies concentrating on sediments. These investigations indicate that elevated plasticizer concentrations, particularly in industrial and urban regions, frequently surpass recommended environmental thresholds, thereby threatening ecological health and human well-being through bioaccumulation. The accumulation of these substances in soil and water can adversely impact microbial communities, disrupt nutrient cycling, and destabilize the overall ecological balance (Manatunga et al., 2024)

Structure of Phthalate esters

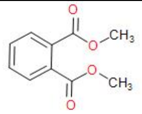
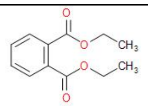
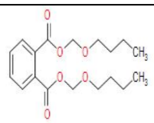
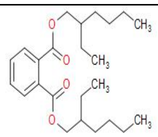
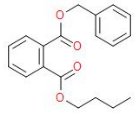
Structure of ortho phthalate

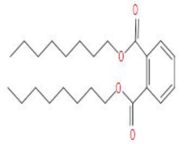


Types of Phthalate Esters

The diesters of 1,2-benzenedicarboxylic acid are commonly referred to as phthalic acid esters (PAEs), or phthalates. Several toxic PAEs—such as dimethyl phthalate (DMP), diethyl phthalate (DEP), di-isobutyl phthalate (DIBP), dibutyl phthalate (DnBP), butyl benzyl phthalate (BBP), and di-(2-ethylhexyl) phthalate (DEHP)—are frequently detected as environmental contaminants. Phthalic acid exists in three isomeric forms—ortho, meta, and para—based on the relative positions of the carboxylic acid groups on the benzene ring. Among these, the ortho-isomer is predominantly used in industrial processes. When ortho-phthalic acid reacts with specific alcohols, it forms esters that are widely used as plasticizers. These compounds constitute the majority of global phthalate ester production due to their effective plasticizing properties. PAE's are extensively employed in the manufacture of plastic products, particularly polyvinyl chloride (PVC) and other

Table 1. Overview of key toxic phthalates, including their molecular weights, chemical structures, toxicological effects, and industrial uses.

Phthalate name	**Cas no.**	**Molecular formulae**	**Chemical structure**	**Molecular weight type (g·mol⁻¹)**	**Example of uses**	**References**
DMP	131-11-3	$C_{10}H_{10}O_4$		259.17	Insect repellent, plastic, additives In cosmetics, household products.	USEPA (2018); Wang Y. et al. 2019)
DEP	84-66-2	$C_{12}H_{14}O_4$		222.24	Shampoo, scents, soap, lotion, cosmetics, industrial solvent, pharmaceutical coatings, additives cosmetics, fragranced products	Wang X. et al. (2019)
DBP	84-74-2	$C_{19}H_{20}O_4$		278.348	Adhesives, caulk, cosmetics, industrial solvent, pharmaceutical coatings, plasticisers in polymers, such as PVC, fillers, putties, plasters, modelling clay, inks and dyes. electronics (e.g. sewing machine, lamp Vinyl flooring. adhesives, scalants. industrial solvent. automotive trim. food conveyor belts. and artificial leather	Wang Y. et al. (2019)
DEHP	117-81-7	$C_{24}H_{38}O_4$		390.564	Plasticisers in polymers, such as PVC, soft plastic, tubing, toys, home products. electronics, lamps. food containers. food packaging. medical devices. such as plastic tubing used for catheters and intravenous drug and fluid delivery. personal protective equipment - Popples	USEPA (2018); Wang Y. et al. (2019)
BBP	85-68-7	$C_{19}H_{20}O_4$		312.365	Perfumes, hair Sprays, adhesives and glues, automotive products, Vinyl floor.	Wang Y. et al. (2019)

DnOP		$C_{24}H_{38}O_4$		390.56	Medical tubing, and blood storage bags, wire and cable, floor tiles and adhesive.	Rudel and Perovich (2009); Olujimi et al. (2010).

polymers, where they enhance flexibility, durability, and lifespan (Yang et al., 2018). Currently, nearly 60 different phthalate esters and their derivatives are utilized across diverse industries, including cosmetics, adhesives, lubricants, packaging materials, paints, insecticides, and other additives (Eichler et al., 2019).

Table 1 provides an overview of key toxic phthalates, including their molecular weights, chemical structures, toxicological effects, and industrial uses. Among them, DMP, DEP, and DnBP are classified as low-molecular-weight (LMW) PAEs, commonly found in industrial solvents, personal care and cosmetic products, pharmaceuticals, fragrances, insecticides, adhesives, waxes, and inks (Koniecki et al., 2011). In particular, DMP and DEP are used in perfumes to slow down fragrance evaporation, thereby extending scent longevity. DnBP is added in small quantities to nail polish formulations to improve chip resistance.

In contrast, DEHP is a high-molecular-weight (HMW) PAEs, predominantly used in the PVC industry. Approximately 80% of long-chain alkyl phthalates are employed as plasticizers in products such as flooring, wall coverings, and other flexible materials. However, the weak physical binding between PAEs and the polymer matrix results in their gradual release into the environment as they leach during product use, weathering, and disposal (Net et al., 2015).

Due to their widespread industrial application and ease of release, phthalate esters have become ubiquitous environmental pollutants. In personal care products, DEP is the most frequently used phthalate, followed by DBP and DEHP. DEP exposure typically occurs through fragrances, lotions, and deodorants, while DnBP exposure is mainly associated with nail polishes (Koniecki et al., 2011).

Most PAEs are colourless liquids with low volatility, poor water solubility, and good solubility in oils and organic solvents. They have also been used as plasticizers in elastomers and cellulosic materials. Given their pervasive use and environmental mobility, there is an urgent need to monitor the sources, toxicity, and ecological risks associated with PAEs, especially in urban regions with concentrated phthalate-based industries (Zhang et al., 2022).

Properties, Production, and Usage of PAEs

PAEs are incorporated into plastics to enhance their flexibility and strength. A comprehensive list of the most frequently utilized PAEs, detailing their chemical names, abbreviations, CAS numbers, chemical structures, molecular weights, and Log Kow values. PAEs are categorized into low-molecular-weight (LMW) PAEs, which consist of 3–6 carbon atoms in their backbone, and high-molecular-weight (HMW) PAEs, which contain 7–13 carbon atoms in their backbone (Weng et al., 2023). LMW PAEs are primarily used in medical devices, adhesives, inks, and paints. HMW PAEs are predominantly employed in the manufacture of flooring, wires, coated fabrics, and applications in the automotive sector (Hidalgo-Serrano et al., 2022). The length of the alkyl chains present in PAEs significantly influences their structure-activity relationships. Consequently, the length of the alkyl chains impacts the biological effects of PAEs. Since the 1930s, PAEs have been extensively utilized in various products including plastics, paints, lubricants, adhesives, insecticides, packaging, and cosmetics DEP and DMP found extensive applications in everyday items (such as perfumes, pesticides, pharmaceuticals, and inks) as well as in personal care products like cosmetics. Their presence was linked to the various industries that traversed the basin within river sediments. It has been noted that DEP and DMP serve as fundamental components in personal care items, cosmetics, and pharmaceuticals. Auxiliary products for textile printing and dyeing include DBP and BBP, with BBP being the predominant element. DBP is frequently utilized in the manufacturing of plastic and rubber goods. (He et al., 2019, Paluselli and Kim, 2020). The global annual production of PAEs is estimated to be around 6–8 million tons, with global consumption from 2017 to 2022 experiencing an anticipated growth rate of 1.3% (Ghosh & Sahu, 2022) (Luo et al., 2018). Among the frequently used PAEs, di-(2-ethylhexyl) phthalate (DEHP) and di-n-butyl phthalate (DBP) are the most prevalent congeners. PAEs are incorporated into polymer matrices through weak intermolecular forces instead of chemical covalent bonds, which facilitates their easy release into the environment during manufacturing, storage, usage, and disposal (Gao et al., 2018). Beyond industrial production, some PAEs can be synthesized de novo by algae and cyanobacteria in aquatic ecosystems. Due to their controversial reputation, the use of PAEs has been subjected to regulations by governments. For instance, DEHP has been included in the blacklist of priority water pollutants in both the USA and China. The majority of PAEs exhibit low volatility, low water solubility, and high lipophilicity. Therefore, most PAEs maintain stable chemical properties in aquatic environments. The hydrolysis rates of PAEs are considerably influenced by their structural characteristics and the surrounding environment. The calculated hydrolysis half-life of dimethyl phthalate (DMP) is 4 months, whereas that of DEHP exceeds 100 years. (Gao et al., 2018).

Fate and Transformation of PAEs in Aquatic Environments

PAEs typically undergo transformation through hydrolysis, photodegradation, and microbial degradation (Shen et al., 2019a). The photolysis of PAEs in aqueous environments primarily occurs via the absorption of incident ultraviolet light within the range of 290 to 400 nm. As UV light penetrates the water, its energy diminishes considerably, resulting in a lower degree of complete mineralization of PAEs; hence, photolysis of PAEs is more likely to take place in a gaseous environment.

The presence of longer alkyl chains leads to a slower hydrolysis of PAEs, and due to the steric hindrance posed by alcohols, the hydrolysis process cannot be fully completed (Cao et al., 2018). The rate of abiotic degradation in the environment is relatively slow, and microbial degradation is deemed the primary mechanism for transforming PAEs in aquatic settings (Yu et al., 2020). Microbial degradation is often influenced by external environmental factors, and the aquatic environment frequently lacks particular degrading bacteria. Given these constraints, PAEs are challenging to degrade in natural settings, with a half-life that is exceedingly long, typically spanning from several years to several hundred years (Fang et al., 2018). The PAEs in the body are easy to degrade and metabolize, which mainly includes two metabolic phases. In phase I (hydrolysis), PAE is hydrolyzed into the main metabolite MPE. In phase II (combination), the formed monoester is converted by P450 enzymes into its oxidative metabolites, namely hydrophilic glucuronide conjugates. It has been reported that the toxicity of these metabolites is lower than that of PAEs themselves. However, most of these metabolites are fat-soluble and can easily be stored in the fat tissues of animals for a long time (Dutta et al., 2020), and the detection rate in organisms is higher than that of PAEs. Studies have demonstrated that MPE can accumulate in biological tissues for up to 6 months and is also toxic to the living organisms (Jiao et al., 2020). Consequently, their toxicity evaluation also cannot be ignored.

Prevalence and Distribution Patterns of Phthalate Acid Esters in Aquatic Ecosystems

The pollution levels of PAEs in the aquatic environments of various nations reveal that China experiences the highest overall pollution of PAEs. This is likely due to China being the leading global producer of PAEs, coupled with insufficient measures for managing and mitigating PAE pollution. Notably, the situation in Brazil and India is also quite severe. In India, the concentration of DBP in groundwater reaches a staggering 207.781 μg / L, while in Brazil's Lake Paranoa, the DEP concentration is recorded at 248.65 μg / L. This situation arises because regulatory measures have resulted in a slowdown of PAE production in Europe and North America. Consequently, most production is now being directed towards developing nations such as Brazil, China, and India (Gao et al., 2018). Additionally, the levels of DEHP in the aquatic environments of Korea, America, France, India, Israel, Spain, Kuwait, Iran, and the Netherlands have all surpassed 1.3 μg / L, exceeding the EU's environmental quality standards for priority pollutants as outlined in the water framework (Zhou et al., 2019).In freshwater ecosystems, the levels of individual PAEs found in various fish species can differ significantly, ranging from undetectable amounts to several hundred μg/g of fish tissue (Liu et al. (2024) identified di-(2-ethylhexyl) phthalate and di-n-butyl phthalate as the primary PAEs present in organisms from both marine (Bohai Bay, BHB) and freshwater

(Songhua River, SHR) habitats (Liu et al., 2024). Sun et al. (2021) discovered that dimethyl phthalate (DMP), di-butyl phthalate (DBP), di-isobutyl phthalate (DIBP), and diethylhexyl phthalate (DEHP) were the main PAEs found in both water and marine organisms in the Bohai. Due to their extensive utilization in both domestic and industrial contexts, it is unsurprising that the prevalence of polyethylene (PE) contaminants is observed across various compartments of aquatic ecosystems, including marine waters, sediments, rivers, lakes, wetlands, and effluents from wastewater treatment facilities (Gao and Wen, 2016; Al-Saleh et al., 2017; Wang et al., 2017; Salaudeen et al., 2018; Arfaeinia et al., 2019; Zhu Q. et al., 2019; Cheng et al., 2019; Zacharia, 2019; WHO, 2021). Polyethylene contaminants are readily emitted during the processes of manufacturing, utilization, and disposal, and possess the propensity to leach into aquatic environments with relative ease (Salaudeen et al., 2018; Henkel et al., 2019). Specifically, their environmental release is facilitated by the absence of covalent bonding between polyethylene and the polymers in which they are incorporated (Gao L. et al., 2019). Furthermore, as plastics age and undergo degradation, the liberation of polyethylene contaminants into the environment is significantly expedited. Given that polyethylene plasticizers are not chemically tethered to polyvinyl chloride (PVC), they also readily infiltrate aquatic ecosystems, among other environments (Olkowska et al., 2017; Chen et al., 2018; Kashyap and Agarwal, 2018). Polyethylene contaminants ingress into the environment via multiple pathways, which include losses incurred during manufacturing, as well as leaching or volatilization from the final products (Kashyap and Agarwal, 2018; Das et al., 2021). Various aquatic ecosystems, such as lakes (Lee Y.-M. et al., 2019; Gao X. et al., 2019), wetlands (Gao and Wen, 2016; Wang et al., 2017), marine environments, and rivers (Arfaeinia et al., 2019; Zacharia, 2019; Ai et al., 2021; WHO, 2021), have been identified as significantly contaminated with polyethylene contaminants, among other organic pollutants. In consideration of the aforementioned, a substantial body of scholarly work has examined the temporal and spatial distribution of these compounds across various aquatic ecosystems to elucidate the presence and dissemination of these substances in diverse nations worldwide (Paluselli et al., 2018; Abtahi et al., 2019; Mi et al., 2019; He et al., 2020; Ai et al., 2021). In light of the points mentioned earlier, a substantial amount of literature has examined the temporal and spatial distribution of these compounds across diverse aquatic ecosystems, underscoring their presence and distribution in various countries globally (Paluselli et al., 2018; Abtahi et al., 2019; Mi et al., 2019; He et al., 2020; Ai et al., 2021). DMP, DEP, DBP, BBP, and DEHP rank as the most prevalent PEs found in both aquatic sediments and waters, with DEHP concentrations often surpassing the annual average environmental water quality standard of 1.3 µg/L (freshwater and marine) as established by EU directive 2008/105/EC (UNEP, 2020). The significant number of recent studies on PEs and the detection rates in Asia may be linked to the higher quantities of PEs produced and utilized in these regions, largely due to the lack of stringent regulations governing the use of PEs identified as priority pollutants, unlike the regulations in developed countries like those in Europe and the United States of America (UNEP, 2020; Das et al., 2021; Li et al., 2021).Albeit, biodegradation is the dominant route for PEs degradation in the environment (Xu et al., 2020; Das et al., 2021).

Interestingly, recent research has validated the capacity of Bacillus mojavensis B1811 (Zhang et al., 2018a) and Paracoccus kondratievae BJQ0001 (Xu et al., 2020) to effectively degrade PEs with remarkable efficiency. The findings from Zhang et al. (2018a) indicated that at a concentration of 0.5 μg/L, DEHP, DBP, BBP, and DPP were almost entirely degraded by strain B1811 within a span of 4 days in a mineral salt medium under shaking conditions, whereas only 5.9% of DMP and 42.9% of DEP with shorter alkyl chains were broken down by strain B1811 under identical conditions, resulting in DMP and DEP concentrations dropping to 0.471 and 0.285 μg/L, respectively.) also discovered that strain BJQ0001 can effectively degrade the substrates DMP, DEP, DBP, DIBP, and DEHP simultaneously when present together in the fermentation system at a concentration of 0.2 μg/L of the PEs mixture, albeit with slightly diminished degradation rates (Xu et al. 2020). The examined the bioaccumulation of PEs in the muscle tissues of domestic livestock (such as pigs, cattle, and chickens) as well as fish in China. In their findings, the authors noted that most PEs congeners are unlikely to accumulate in organisms, given their bioaccumulation factors are ≤2, with the exception of DEP, DMP, and BBP, which showed BAF values of ≥2, revealing that pigs have a greater capacity to accumulate PEs than the tissues of the other animals. This phenomenon could be attributed to the higher fat content typically found in pigs compared to both cattle and chickens, as PEs are lipophilic in nature (He et al. 2020).

Impacts on Aquatic Ecosystems

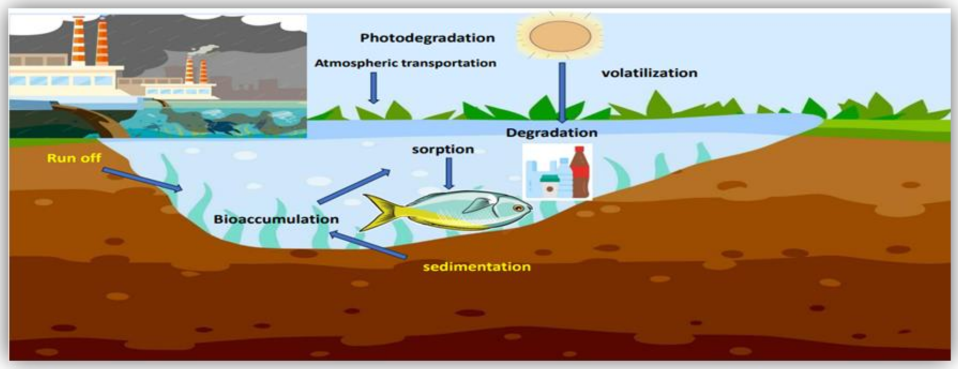


Figure 2. Different processes Affecting plasticizers in aquatic ecosystem.

Plasticizers are subject to degradation via both biotic (microbial) and abiotic (chemical) mechanisms (figure 2). Studies indicate that biotic degradation can reach an impressive efficiency of over 94%, while abiotic processes also play a crucial role (Panthi et al., 2024).

Plasticizers cause immunotoxicity, neurotoxicity, genotoxicity, and endocrine, metabolic, and developmental toxicities in fish and other aquatic organisms, negatively impacting their health and disrupting their biological functions. This exposure poses significant risks to aquatic ecosystems and biodiversity. (Bansal, 2024) (Petrova et al., 2023). Plasticizers, particularly phthalates like Di(2-Propylheptyl) Phthalate (DPHP), can be hazardous to aquatic organisms, causing increased mortality and potential reproductive issues, as demonstrated in studies with Daphnia magna, where DPHP showed higher toxicity compared to the previously dominant DEHP (Petrova et al., 2023). Plasticizers can accumulate in the tissues of aquatic organisms, leading to higher concentrations in predators, which can disrupt entire food webs (Genuchten,2023; Naz et al., 2024). exposure to plasticizers can lead to genotoxic effects, causing DNA damage in aquatic organisms (Bansal, 2024). Endocrine disruption is a significant concern, as plasticizers can interfere with hormone regulation, affecting growth, reproduction, and development in aquatic species (Bansal, 2024) (Orose et al., 2023). Aquatic ecosystems, including rivers, dams, lakes, and wetlands, that are situated within or traverse agricultural, industrial, residential, and urban areas are subjected to various types of PEs from numerous anthropogenic sources (Salaudeen et al., 2018; Ai et al., 2021; Li et al., 2021). While both cyanobacteria and freshwater algae have the ability to generate DBP or monoethylhexyl phthalate (MEHP) under natural conditions, which can subsequently be released into the aquatic ecosystem, pollutants from human activities such as agricultural pesticides, industrial discharges, commercial wastewater, landfills (Zhang et al., 2021), and household electronic and solid waste (Zhang et al., 2019) serve as the primary pathways for PEs entering these aquatic systems. Due to their high octanol-water partition coefficients (Kow/logKow25) (1.61–9.46) and low vapor pressures (Pa25) (1.84×10^{-6} –0.263), the majority of PEs that enter the aquatic environment exhibit extremely low volatility, allowing them to migrate easily into various water bodies and infiltrate aquatic organisms (Das et al., 2021; Zhang et al., 2021). As the value of KOW rises with increasing alkyl chain length, this leads to heightened hydrophobicity for higher molecular weight PEs, thereby resulting in their increased sorption to organic matter (Das et al., 2021; Zhang et al., 2021). Upon entering the aquatic ecosystem, PEs immediately interact with aquatic organisms. PEs can be absorbed by high-nutrient aquatic organisms through consumption and subsequently move up the food chain (Zhang et al., 2021). DMP, DEP, DAP, DBP, DIBP, and BBP exposure was identified to have both acute and chronic impacts on algae, invertebrates, and fish species. A recent review conducted also highlighted the presence of acute and chronic toxic effects on aquatic organisms, manifesting symptoms such as deformed tails, tissue necrosis, cardiac swelling, unresponsiveness to touch, and mortality in aquatic animal embryos (Zhang et al. 2021), meanwhile, exposure to PEs in adult organisms could result in negative reproductive outcomes, and harm to the liver, kidneys, and other vital organs (Gao et al., 2018). The toxicity levels of chemical compounds to aquatic life are assessed using the median lethal concentration (LC50) (Huang et al., 2020; Zhang et al., 2021). Research suggests that the majority of PEs (with short carbon chains) can be broken down in the environment; however, a few are known to accumulate in biological samples (He et al., 2020; UNEP, 2020). This emphasizes the critical need for augmented and persistent surveillance of PEs in aquatic habitats, especially those utilized by communities for indispensable activities such as fishing, irrigation, drinking, and bathing. This is supported by a recent investigation conducted by in a rural agricultural region of western China, where local populations rely on river water for domestic purposes and found that samples of meat from pigs, cattle, and chickens all contained PEs (He et al. 2020). A notable correlation was also identified between the biological samples and river water samples, suggesting that the river water significantly impacted the uptake and accumulation of PEs in the biological specimens (He et al., 2020). PEs, especially those with high molecular weights, possess a remarkable capacity to accumulate in sediments; where their sorption to particles, combined with low-oxygen conditions, hinders the degradation process of PEs by decreasing their degradation rates (UNEP, 2020). A growing body of research has demonstrated that PAEs result in both acute and chronic toxic effects on aquatic creatures. Common indicators of PAE toxicity in the embryos of aquatic animals encompass mortality, deformed tails, tissue death, cardiac swelling, and an absence of tactile response. In mature organisms, exposure to PAEs produces negative impacts on reproductive health, along with detrimental effects on the liver, kidneys, and other organs (Gao et al., 2018).

Table 2. Toxic effects of some Plasticizers on aquatic organisms

Organisms	**PEs**	**Test concentration levels**	**Toxicity**	**References**
Common carp (Cyprinus common *carpio L.*)	DEHP	40 μmol / L and 200 μmol / L, 2 h	DEHP could affect the phagocytic ability of neutrophils by regulating the expression of inflammatory cytokines (Interleukin-6, Interleukin-8, transforming growth factor, tumor necrosis factor (TNF-α, TNF-R1, TNF-T1, IFN-2a, IFN-g2b, IFN-g1, IL-1β and IL-10) and disrupting cytochrome P450 homeostasis (CYP-1A, CYP-1B1, CYP-C1, CYP-2 K,	Wang et al., 2020b

			CYP-3A), which caused the immunosuppression in common carp.	
Gilthead seabream (*Sparus aurata*)	DiNP	15, 1500 μg / kg bw / day via the diet, 21 days	The mRNA levels of genes involved in the immune response, such as pla2, 5-lox, tnfa and cox2, were upregulated significantly only by the high dose of DiNP, while il1 mRNA increased in both doses	Carnevali et al., 2019
Nile tilapia (*Oreochromis niloticus*)	DEHP	50 mg / kg, 7 days	Gene ontology (GO) classification system and Kyoto Encyclopedia of Genes and Genomes (KEGG) database analysis demonstrated that DEHP significantly disturbed the expression level of genes associated with immunity.	Zhang et al., 2019b
Daphnia magna	DEP, DBP, DEHP	1 and 10 μmol/L, 14 days.	Enhanced fat accumulation upon exposure to all the tested PEs, analysis of genes involved in fat metabolism suggests the increase in fat content could be due to inhibition of absorption and catabolism of fatty acids, reduced body size. While DEP and DBP decrease lifespan, DEHP results in increased reproduction output	Seyoum and Pradhan (2019)
Zebrafish embryos (*Danio rerio*)	DBP	50 μg / L, 4 hpf-96 hpf	Proteomics revealed abnormal development and metabolism of zebrafish embryos after exposure to DBP.	Dong et al., 2018

Discussion

The present review provides a comprehensive synthesis of current scientific knowledge on the occurrence, environmental fate, bioaccumulation, and ecotoxicological impacts of phthalate acid esters (PAEs) in aquatic ecosystems. By integrating findings from multiple regions and scientific disciplines, the study emphasizes the environmental, ecological, and public health relevance of plasticizer contamination and highlights its increasing global significance.

From an environmental perspective, PAEs are continuously introduced into aquatic systems due to their large-scale industrial production and widespread use. Since these compounds are not chemically bound to polymer matrices, they are easily released through industrial effluents, municipal wastewater, plastic degradation, and surface runoff. Their physicochemical characteristics particularly high lipophilicity, low water solubility, and strong environmental persistence favor their accumulation in water bodies and sediments. Evidence compiled in this review indicates that PAE contamination is globally distributed, with particularly elevated concentrations reported in rapidly industrializing regions where regulatory enforcement may be limited. This geographic variability underscores the urgent need for strengthened monitoring programs and improved wastewater treatment strategies. Ecologically, the review demonstrates that aquatic ecosystems are highly vulnerable to PAE exposure. Documented toxic effects include endocrine disruption, immunotoxicity, neurotoxicity, developmental abnormalities, and reproductive impairment. Such impacts have been observed in key aquatic species, including Danio rerio, Cyprinus carpio, Oreochromis niloticus, and Daphnia magna. These findings suggest that PAE contamination may extend beyond individual toxicity to influence population stability and disrupt aquatic food webs, posing risks to biodiversity and fisheries sustainability. The study also highlights potential human health concerns. Due to their lipophilic nature, PAEs bioaccumulate in aquatic organisms and may enter the human food chain through contaminated fish and livestock. Communities dependent on polluted water bodies for drinking, irrigation, and fishing may face chronic exposure risks. Furthermore, the review stresses the need for strengthened environmental regulations, standardized monitoring approaches, and the development of safer alternatives to conventional plasticizers. By identifying knowledge gaps such as mixture toxicity and long-term ecological effects the study provides a foundation for future research and supports global efforts toward sustainable water management and pollution control.

Conclusion

This review highlights that phthalate acid esters (PAEs) are pervasive and persistent contaminants in aquatic environments, primarily originating from extensive industrial production and widespread use in plastic and consumer products. Due to their weak physical binding within polymer matrices, PAEs are continuously released into water bodies through industrial effluents, municipal wastewater, plastic degradation, and surface runoff. Their physicochemical characteristics such as low water solubility, high lipophilicity, and resistance to degradation facilitate long-term persistence, sediment accumulation, and bioavailability in aquatic ecosystems. Evidence compiled in this study demonstrates significant spatial variation in PAE contamination, with higher concentrations frequently reported in rapidly industrializing regions. In many cases, detected levels exceed established environmental quality standards, indicating gaps in regulatory control and wastewater treatment efficiency. Although hydrolysis, photodegradation, and microbial degradation contribute to PAE transformation, these processes are often slow under natural conditions, allowing prolonged environmental residence times.

Ecotoxicological findings confirm that PAEs exert both acute and chronic toxic effects on aquatic organisms. Documented impacts include endocrine disruption, immunotoxicity, neurotoxicity, genotoxicity, metabolic disorders, developmental abnormalities, and reproductive impairment. These adverse outcomes have been observed in ecologically and economically important species such as Danio rerio, Cyprinus carpio, Oreochromis niloticus, and Daphnia magna. Such biological disturbances may extend beyond individual organisms to influence population stability, trophic interactions, and overall ecosystem functioning. The lipophilic nature of PAEs promotes bioaccumulation in aquatic organisms and facilitates trophic transfer through food webs, raising concerns about indirect human exposure via contaminated fish and livestock. Consequently, PAE pollution represents not only an ecological issue but also a potential public health risk, particularly in communities dependent on contaminated water resources. Overall, this review underscores the urgent need for comprehensive monitoring programs, stricter regulatory frameworks, improved wastewater treatment technologies, and the development of safer alternatives to conventional phthalates. Future research should focus on mixture toxicity, long-term ecological effects, transgenerational impacts, and effective bioremediation strategies. Addressing these challenges is essential for protecting aquatic ecosystems, ensuring sustainable resource use, and minimizing risks to human health.

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Conflict of Interest

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Biotechnological Interventions for Enhancing Stress Tolerance in Paddy (*Oryza sativa* L.)

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Abstract

Rice (*Oryza sativa* L.) is one of the most important staple crops feeding more than half of the world's population. However, rice cultivation is severely affected by abiotic stresses such as drought, salinity, submergence, and temperature extremes, as well as biotic stresses caused by pathogens and pests. Conventional breeding approaches have contributed significantly to rice improvement, but the process is often slow and limited by the availability of genetic variability. Modern biotechnology provides powerful tools to enhance stress tolerance in rice through molecular breeding, genetic engineering, tissue culture, and genome editing techniques. Marker-assisted selection, transgenic approaches, and CRISPR-based gene editing have enabled the development of stress-resilient rice varieties with improved productivity and adaptability. Additionally, the use of plant growth–promoting microorganisms and biofertilizers contributes to sustainable rice cultivation by improving plant health and stress tolerance. This chapter discusses various biotechnological strategies used to improve stress tolerance in paddy and

highlights their significance in ensuring food security and sustainable agriculture under changing climatic conditions.

Keywords: Rice biotechnology, Stress tolerance, Genetic engineering, Marker-assisted selection

Introduction

Rice is cultivated across diverse agroclimatic regions and plays a crucial role in global food security. In many developing countries, rice serves as the primary source of calories and nutrition. However, rice production is increasingly threatened by environmental stresses, including drought, salinity, flooding, and extreme temperatures. Biotic stresses such as bacterial blight, blast disease, and insect pests also cause significant yield losses. Climate change has further intensified these challenges, making it necessary to develop stress-tolerant rice varieties.

Traditional plant breeding has been effective in improving yield and resistance to certain stresses, but the process is time-consuming and often limited by genetic barriers. Biotechnology offers innovative tools that enable precise manipulation of genes, rapid multiplication of elite varieties, and improved understanding of stress-response mechanisms. Advances in molecular biology, genomics, and bioinformatics have facilitated the identification of stress-responsive genes and their incorporation into cultivated varieties. As a result, biotechnological interventions have become essential components of modern rice improvement programs.

Abiotic Stress in Paddy and Its Impact

Abiotic stresses such as drought, salinity, submergence, and temperature extremes adversely affect rice growth, development, and yield. Drought stress reduces water availability, leading to decreased photosynthesis and impaired nutrient uptake. Salinity stress causes ionic imbalance and osmotic stress, which inhibit root growth and seedling establishment. Submergence, particularly in flood-prone regions, restricts oxygen availability and disrupts normal metabolic processes. Temperature extremes, both high and low, affect enzyme activity, pollen viability, and grain filling. These stresses collectively reduce crop productivity and threaten food security, emphasizing the need for developing stress-tolerant rice varieties through advanced techniques.

Molecular Breeding and Marker-Assisted Selection

Molecular breeding has revolutionized crop improvement by enabling the identification and transfer of specific genes associated with stress tolerance. Marker-assisted selection allows breeders to track desirable traits at the DNA level, thereby accelerating the breeding process and increasing selection accuracy. In rice, quantitative trait loci associated with drought tolerance, submergence tolerance, and salinity resistance have been successfully identified and introgressed into high-yielding varieties. The development of submergence-tolerant rice varieties containing the SUB1 gene is a notable example demonstrating the effectiveness of molecular breeding in enhancing stress tolerance.

Genetic Engineering for Stress Tolerance

Genetic engineering enables the direct introduction of stress-responsive genes into rice plants to improve tolerance to adverse conditions. Genes involved in osmoprotection, antioxidant defense, and stress signaling pathways have been transferred into rice to enhance tolerance to drought, salinity, and temperature stress. Transgenic rice varieties expressing genes such as DREB and HVA1 have shown improved stress tolerance under controlled and field conditions. This approach provides greater precision compared to conventional breeding and allows the incorporation of genes from diverse organisms, thereby expanding the genetic base of rice.

Genome Editing and CRISPR Technology

Genome editing technologies, particularly CRISPR-Cas systems, have emerged as powerful tools for precise modification of plant genomes. In rice, genome editing has been used to alter genes associated with stress response, plant architecture, and yield. Unlike transgenic approaches, genome editing enables targeted gene modification without introducing foreign DNA, making it a promising technique for developing improved crop varieties. The application of CRISPR technology in rice has accelerated the identification of gene functions and facilitated the development of stress-resilient cultivars in a shorter time frame.

Plant Tissue Culture and Micropropagation

Plant tissue culture plays a significant role in rice biotechnology by enabling rapid multiplication of elite and stress-tolerant varieties. Techniques such as somatic embryogenesis and anther culture are widely used for producing haploids and doubled haploids, which accelerate breeding programs. Tissue culture also facilitates the development of transgenic plants and the conservation of valuable germplasm. The ability to regenerate plants under controlled conditions ensures uniformity and enhances the efficiency of genetic improvement programs.

Role of Microbial Biotechnology in Stress Management

Microbial biotechnology contributes to stress tolerance in rice through the use of plant growth–promoting rhizobacteria, mycorrhizal fungi, and biofertilizers. These beneficial microorganisms enhance nutrient uptake, improve soil fertility, and induce systemic resistance against pathogens. Certain microbial strains help plants withstand drought and salinity by producing phytohormones, osmoprotectants, and stress-related enzymes. The integration of microbial biotechnology with crop management practices supports sustainable rice cultivation and reduces dependence on chemical inputs.

Future Prospects of Rice Biotechnology

Advances in genomics, transcriptomics, proteomics, and metabolomics are providing deeper insights into the molecular mechanisms of stress tolerance in rice. The integration of these approaches with artificial intelligence and precision agriculture is expected to further enhance crop improvement strategies. The development of climate-resilient rice varieties will play a critical role in ensuring food security in the face of global climate change. Continued research and collaboration among scientists, breeders, and policymakers are essential to harness the full potential of biotechnology in rice cultivation.

Conclusion

Biotechnological interventions have significantly contributed to improving stress tolerance in rice, enabling the development of resilient varieties capable of withstanding adverse environmental conditions. Molecular breeding, genetic engineering, genome editing, tissue culture, and microbial biotechnology collectively provide effective solutions for enhancing productivity and sustainability in rice cultivation. As climate change continues to pose challenges to agriculture, the adoption of advanced biotechnological tools will be crucial for ensuring stable rice production and global food security. Future research focusing on integrated approaches and farmer-friendly technologies will further strengthen the role of biotechnology in paddy cultivation.

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Biomass Energy: Some Useful Conversion

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Abstract

India has many energy resources which known as renewable energy resources like wind, solar, hydropower, biomass etc. But biomass energy is one of the maximum produces from the India after solar energy. Biomass energy comes from organic matter (plants, animal waste, crops). Biomass energy can be harvest by various ways and presently we are using many applications of biomass energy. Biomass energy also can help to reduce the organic waste volume and helps to provide solid liquid and gaseous fuels. Biomass energy helps to clean environment comparatively using fossil-based energy produces. It can help to gain the rural economy.

Keywords: Energy, resources, biomass, fuel, applications.

Introduction

India is a land of village where the energy required for domestic purpose such as a cooking is met from dried woods, twigs and leaves of plants and other dried organic matter such as cow dung. This organic matter called as biomass is available freely as waste. Biomass consists from two words Bio means living (Plants and animals) and mass means material. It contains stored energy from the sun. The biomass is fast renewable forms of energy and available freely as waste and discarded matters. Biomass energy generates the energy from various ways.

Applications of Biomass Energy

Many applications have been using from biomass presently. Apart from their use in homes for heating or cooking purposes the energy is using for large power plants used by centralized utilities to produce electricity in industrial sector. Industry and commercial use biomass for several purposes including space heating, hot water heating etc. Wood is the most common source of fuel, although many different materials are used. New designs for woodstoves can improve the efficiency of the cooking or heating system, decreasing the amount of fuel that is needed. Many industries uses naturally produce organic waste.

Biomass energy can be can be generate by various methods:

- Thermo chemical Process: In this process biomass can burn into useful fuel or can convert electricity like direct burning, pyrolysis and gasification.
- Biochemical Process: In this process with the help of microorganisms biomass can produce gas or liquid fuel or alcohol like biogas, hydrogen and alcohol by fermentation.
- Agrochemical Process: In agrochemical process biomass can be converting into diesel like fuel which can use after processing in vehicles.

Pyrolysis process produces gas (mixture of CO, H2 and CH4) and liquid products and leaves a solid residue richer in content. This process occurs in the absence of O2 at under high pressure and temperature. Various types of fuel from biomass can be used as applications mainly in three different forms like:

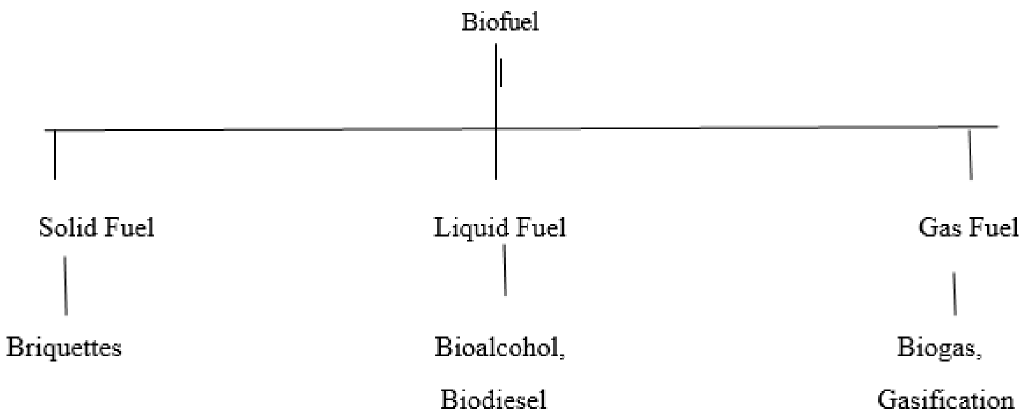

Figure: 2.1

1. Thermo Chemical Process

Thermo chemical conversion means biomass structure degradation with oxygenic or an oxygenic atmosphere at high temperature. It includes three kinds of technology, namely

a. Direct burning
b. Biomass gasification
c. Biomass pyrolysis
d. Biomass liquification

- **Direct Burning**

Direct combustion or burning is the most common method for converting biomass to useful energy. All biomass can be burned directly for heating buildings and water, for providing industrial process heat, and for generating electricity in steam turbines.

- **Biomass Gasification**

Gasification is a chemical reaction process that reacts with gasifying agent (air, oxygen, and water) at high temperatures in gasifiers. The main problem of biomass gasification technology is that the tar obtained in the gasification of gas is difficult to purify, which has become the main factor restricting the biomass gasification technology.

Biomass can also be directly converted to energy through gasification. During the gasification process, a biomass feedstock (usually MSW) is heated to more than 700° C (1,300° F) with a controlled amount of oxygen. The molecules break down, and produce syngas and slag.

- **Pyrolysis**

Pyrolysisis a thermal process in which the organic polymer molecules in the biomass are quickly broken into short chain molecules, coke, bio-oil and non-condensable gas in the absence of oxygen or a small amount of oxygen under high temperatures.

Pyrolysis is a related method of heating biomass. During pyrolysis, biomass is heated to 200° to 300° C (390° to 570° F) without the presence of oxygen. This keeps it from combusting and causes the biomass to be chemically altered.

Pyrolysis produces dark liquid called pyrolysis oil, a synthetic gas called syngas, and a solid residue called biochar. All of these components can be used for energy.

Pyrolysis oil, sometimes called bio-oil or biocrude, is a type of tar. It can be combusted to generate electricity and is also used as a component in other fuels and plastics. Scientists and engineers are studying pyrolysis oil as a possible alternative to petroleum.

Biochar, produced during pyrolysis, is valuable in agricultural and environmental use.

- **Black Liquor**

When wood is processed into paper, it produces a high-energy, toxic substance called black liquor.

However, black liquor retains more than 50 percent of the wood's biomass energy. Recently an experimented has been done for gasifying black liquor to produce syngas, which can then be used to generate electricity.

- **Biomass Liquefaction**

Biomass liquid fuel could provide an alternative to petroleum up to a certain extent. After some modification, industrial oil-fired boilers and internal combustion engines can use bio-oil as fuel directly.

- **Hydrogen Fuel Cells**

Biomass is rich in hydrogen, which can be chemically extracted and used to generate power and to fuel vehicles. Stationary fuel cells are used to generate electricity in remote locations, such as spacecraft and wilderness areas. Hydrogen fuel cells may hold even more potential as an alternative energy source for vehicles.

- **Biochar**

When biomass rots or burns (naturally or by human activity), it releases high amounts of methane and carbon dioxide into the atmosphere. However, when biomass is charred, it sequesters, or stores, its carbon content. When biochar is added back to the soil, it can continue to absorb carbon and form large underground stores of sequestered carbon—carbon sinks—that can lead to negative carbon emissions and healthier soil.

Biochar also helps enrich the soil. It is porous. When added back to the soil, biochar absorbs and retains water and nutrients.

2. Bio Chemical Processes

The biochemical process is based on breaking down the cellulosic part of the organic fraction of the waste stream. This would include certain foods (e.g., vegetables, fruits), paper products, and yard vegetation. Bio solids can also be added as a waste material.

Biochemical conversion processes include anaerobic digestion or decomposition and anaerobic fermentation. Fermentation is an anaerobic process that breaks down the glucose within organic materials. It is a series of chemical reactions that convert sugars to alcohol or acid. Yeast or bacteria are added to the biomass material, which feed on the sugars to produce ethanol and carbon dioxide. Through anaerobic digestion biogas or methane gas may produce in digester tank. Biophotolysis is the production of hydrogen from water by sunlight energy using biological systems.

- **Anaerobic Decomposition**

Anaerobic decomposition is the process where microorganisms, usually bacteria, break down material in the absence of oxygen. Anaerobic decomposition is an important process in landfills, where biomass is crushed and compressed, creating an anaerobic (or oxygen-poor) environment.

- **Agrochemical Process**

In physical method of conversion, biomass is densified into solid briquettes while in agrochemical route of conversion, fuel is extracted from freshly cut plants. Thermo chemical process of conversion consists of combustion, pyrolysis, gasification and anaerobic digestion to methane.

Syngas can be converted into fuel (such as synthetic natural gas). It can also be converted into methane and used as a replacement for natural gas.

- **Biofuel**

Biomass is the only renewable energy source that can be converted into liquid biofuels such as ethanol and biodiesel. Ethanol is made by fermenting biomass that is high in carbohydrates, such as sugarcane, wheat, or corn. Biodiesel is made from combining ethanol with animal fat, recycled cooking fat, or vegetable oil. Biofuels do not operate as efficiently as gasoline. However, they can be blended with gasoline to efficiently power vehicles and machinery, and do not release the emissions associated with fossil fuels.

Growing enough corn for ethanol also creates a strain on the environment because of the lack of variation in planting, and the high use of pesticides. Ethanol has become a popular substitute for wood in residential fireplaces. When it is burned, it gives off heat in the form of flames, and water vapor instead of smoke.

Some of the advantages of biomass energy are:

1. Biomass is always and widely available as a renewable source of energy.

The organic materials used to produce biomass are infinite, since our society consistently produces waste such as garbage, wood and manure.

2. It is carbon neutral.

As a natural part of photosynthesis, biomass fuels only release the same amount of carbon into the atmosphere as was absorbed by plants in the course of their life cycle.

3. It reduces the overreliance of fossil fuels.

Not only is there is a limited supply of fossil fuels, but fossil fuels come with environmental baggage, including the release of large amounts of carbon dioxide into the atmosphere and the pollutants that result from removal, transportation and production.

4. It is less expensive than fossil fuels.

While fossil fuel production requires a heavy outlay of capital, such as oil drills, gas pipelines and fuel collection, biomass technology is much cheaper. Manufacturers and producers are able to generate higher profits from a lower output.

5. Biomass production adds a revenue source for manufacturers.

Producers of waste can add value by channeling their garbage to create a more profitable use in the form biomass energy.

6. Less garbage in landfills.

By burning solid waste, the amount of garbage dumped in landfills is reduced by 60 to 90 percent, and reduces the cost of landfill disposal and amount of land required for landfill.

While the advantages of biomass energy are plenty, there are also some shortcomings, including:

- **Biomass energy is not as efficient as fossil fuels**

Some biofuels like Ethanol is relatively inefficient as compared to gasoline. In fact, it has to be fortified with fossil fuels to increase its efficiency.

- **It is not entirely clean**

While biomass is carbon neutral, the use of animal and human waste escalates the amount of methane gases, which are also damaging to the environment. Additionally, the pollution created from burning wood and other natural materials can be considered just as bad as that resulting from burning coal and other types of energy resources.

- **Can Lead to Deforestation.**

Since wood is one of the most used sources of biomass energy, vast amounts of wood and other waste products have to be burned to produce the desired amount of power. While currently there is enough wood waste already, there is a risk of deforestation in the future.

- **Biomass Plants Require a Lot of Space.**

It's difficult to find a plant that is in a convenient place in an urban area, while there are some downsides to biomass energy, more research and innovation is continuing to be devoted to the field as a more widely available, cheaper alternate and valuable substitute for traditional electricity and other energy sources.

Currently, hydrogen fuel cells are used to power buses, forklifts, boats, and submarines, and are being tested on airplanes and other vehicles.

Disadvantages of Biomass Energy

However, there is a debate as to whether this technology will become sustainable or economically possible. The energy that it takes to isolate, compress, package, and transport the hydrogen does not leave a high quantity of energy for practical use.

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Transformative Advances in Biotechnology and Biological Research

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Abstract

New discoveries in the area of biotechnology have shaped the area of biological studies and translational uses swiftly, making it possible to comprehend and control living systems never previously. Such improvements are noteworthy since they are enhancing the speed at which precision medicine can be created, increase agricultural productivity, industries can operate in a sustainable manner and offer innovative solutions to global health issues. The major technology such as CRISPR-based genome editing, mRNA therapeutics, artificial intelligence, and big data analytics, and stem cell and organoid technologies, are driving these advances. All these techniques have facilitated more specific, efficient, and high throughput methods to investigate and interact with genes, proteins, cells and tissues. The chapter covers the most significant achievements in biotechnology and biological research, and it deals with four key fields, namely: (1) CRISPR and next-generation gene editing technologies, (2) mRNA-based therapeutics and vaccine innovations, (3) the convergence of artificial intelligence and big data in biological research, and (4) stem cells, organoids, and regenerative medicine. In some cases, the chapter

gives the core principles, the latest advancements in the methodology, its major uses, and future prospects to be made in each area. All of these issues collectively demonstrate how the state of science, medicine, and industry providing a framework for understanding current and emerging trends in the field.

Keywords: Cas proteins, T-cell immunity, embryonic stem cells, double-strand breaks

Introduction

Biotechnology and biological research have embarked upon a period of fast and radical innovation, which has altered the very of the nature in which scientists investigate life at molecular, cellular and systems levels. The innovations are necessary for solving the significant global issues, such as the invention of targeted therapies, enhancement of agricultural productivity, and designing a sustainable solution to industries. Current technology enables researchers to now pinpoint, examine and analyze biological systems with unmatched accuracy, making discoveries that were unimaginable before possible. Among the most vivid instances of such advancements is genome editing technology particularly CRISPR-based systems including base editing and prime editing that allow the precise editing of nucleotides without creating harmful double-strand breaks (DSBs). Base editors, that is, catalytically inactive Cas proteins that have been conjugated with deaminase enzymes, have dramatically improved the specificity of base editing of point mutations of interest in human disease and agricultural phenotype (Komor et al., 2016; review in Frontiers in Genome Editing, 2025). A more recent strategy called prime editing combines an engineered reverse transcriptase to a nickase Cas9 and relies on longer guide RNAs to search and replace genomic sequences- without DSBs or donor templates- and hence extends the range of therapeutic applications. At the same time, integration of artificial intelligence (AI) and machine learning (ML) with genome editing platforms has increased the design, prediction, and optimization of the editing results, leading to a greater efficiency as well as specificity. Models based on AI are currently being used to forecast the best guide RNA sequences, foresee off target effects, and utilize protein engineering to develop better-performing next-generation editors. Concurrently, omics technologies, such as genomics, transcriptomics, epigenomics, proteomics and spatial multi-omics, offer detailed, multi-layered cellular function and heterogeneity maps. Spatial multi-omics technologies capture the physical structure of cells in tissues and measure numerous modalities of cells and microenvironment interactions, allowing accurate characterization of cellular networks and microenvironment interactions in development, disease, and therapy response (Liu et al., 2024; spatial multi-omics overview in Seminars in Cancer Biology, 2026).

Three-dimensional tissue cultures (organoid models) have also revolutionized biological studies by recapitulating important details of the structure and function of human organs in culture that are made of pluripotent or adult stem cells. These mini-organs build on the traditional cell cultures and animal models, they offer more physiologically relevant platforms for disease modeling, high-throughput drug screening, toxicity testing, and precision medicine applications (Yao et al., 2024; Singh et al., 2025). Genome editing and multi-omics profiling in combination with organoid technology enable systematic interrogation of genetic perturbation and therapeutic targets in multicellular human tissue settings. Concurrently, the mRNA therapeutic, boosted to the mainstream due to the success of COVID-19 vaccines, has rapidly diversified into protein replacement therapies, cancer immunotherapy, and rare genetic disease treatment. The advances in the mRNA design and delivery systems such as lipid nanoparticles, and a different computational optimization of the untranslated regions and coding sequence have contributed to the improvement of the stability, translational efficiency and clinical performance of these new applications. Biological studies have also developed an interest in AI and big data analytics in all these fields, which support sequence and guide RNA optimization, multi-omics integration, predictive phenotyping, and dynamic modeling of biological processes. Drug discovery, synthetic biology and personalized medicine are also being discovered using computational tools, which allow one to find statistically significant patterns in intricate data sets in a manner that can reduce the cost of experimentation and the time to discovery. Together, interdisciplinary breakthroughs in genome editing, organoid modeling, multi-omics modeling, AI integration and mRNA therapeutics are redefining the scientific research paradigm and expanding its limits into practice in health, agriculture and industry.

Clustered Regular Interspaced Short Palindromic Repeats and Next-Generation Gene Editing Technologies.

CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) and the associated Cas nucleases have revolutionized the biotechnology and biological science in general in that they have provided a highly specific, programmable, and efficient system of genome editing. CRISPR-CAS9 relies on a bacterial adaptive immune system in which the system is capable of specifically cleaving DNA with a customizable guide RNA and permits scientists to disrupt, insert, or alter genes with ease, like never previously feasible using technologies such as zinc-finger nucleases and TALENs. This has enabled easy access to genome editing by labs worldwide and accelerated functional genomics studies, disease modeling and drug discovery.

In the medical field, CRISPR has been used to develop gene therapies against hereditary diseases, create immune cells to treat cancer through immunotherapy, and enhance antiviral therapy plans. It has enabled quick creating of crops with a high yield, improved nutritional content and stress tolerance in agriculture, as well as enabling the engineering of strain-resistant microbes in the production of biofuel and bioproducts in industrial biotechnology. CRISPR combined with high-throughput sequencing and computational biology has also solidified systems-level insights into gene regulation and cellular networks, transforming research paradigms throughout the life sciences (Doudna & Charpentier, 2014; Pickar-Oliver and Gersbach, 2019).

The latest developments in the next generation gene editing technologies have enhanced the specificity, safety, and flexibility of the CRISPR-based systems to a great extent. Base editing, which is accomplished by catalytically inactive Cas proteins fused with nucleotide deaminases, enables direct conversion of single bases on DNA without creating a double-strand break and prevents creating additional insertions or deletions, and improves therapeutic applications (Komor et al., 2016). Prime editing extends editing to even more powerful editing capabilities, providing the ability to simply make targeted insertions, deletions, and all possible base replacements directly without the need to use donor DNA templates by combining a Cas9 nick with reverse transcriptase with a prime editing guide RNA (pegRNA) (Anzalone et al., 2019). Further applications of Cas variants in engineered versions with reduced off-target activity and higher fidelity and other RNA-targeting systems such as Cas13 have extended the applications of CRISPR to transcriptome editing and molecular diagnostics. Together, these innovations indicate a transition to a more precise search-and-replace form of genome engineering as opposed to simply the process of gene interference and place CRISPR technologies at the heart of translational medicine, synthetic biology and precision biotechnology.

mRNA Therapeutics and Vaccines

The therapeutics of messenger RNA (mRNA) started as experimental platforms and has quickly developed into clinically viable technologies that have greatly enhanced the current biotechnology and translational medicine. The effectiveness of mRNA vaccines against COVID-19, especially BNT162b2 and mRNA-1273, established that synthetic mRNA was safe to be used as a way of vaccination strategy in a humans being and induce both humoral and cellular responses (Baden et al., 2021; Polack et al., 2020). The paradigm shift in the field of vaccinology was these landmark studies that demonstrated that it is feasible to design vaccines rapidly using only the genomic sequences of pathogens. Other mRNA applications are in oncology, where personalized cancer vaccines encode tumor-specific neoantigens to elicit directed T-cell immunity. Sahin et al. (2017) presented initial clinical data suggesting that customized mRNA cancer vaccines have the potential to trigger effective immune responses in patients with melanoma, which leads to further phase II and III studies. In addition, mRNA therapeutics are also being explored in protein replacement approaches in rare genetic disorders, and provides transient expression without the potential risks of genomic insertion of virus-based gene therapy. All these occurrences put mRNA as a versatile and adaptable curative form that can be customized to diverse illness conditions.

The recent research has been geared towards improving the stability of mRNAs, translation efficiency, delivery system, and safety to increase clinical utility. Other chemical alterations such as incorporation of N1-methylpseudouridine has the potential to significantly reduce the immune recognition potential of natural immunity and augment protein translation, a finding on which the modern models of vaccination are formed (Kariko et al., 2008). Technology of lipid nanoparticles (LNP) has facilitated the process of tissue targeting, biodistribution and intracellular release and made systemic delivery more efficient and less toxic (Hou et al., 2021). Self-amplifying mRNA (saRNA) systems are also able to amplify antigen expression with even less doses, making them more scalable and less expensive to make (Blakney et al., 2021). Circular RNA (circRNA) platforms are newer platforms with a higher level of stability and longer protein expression than linear mRNA constructs (Qu et al., 2022). Collectively, these technologies constitute a second wave of RNA biotechnology--not emergency vaccine making, but long-lasting, precision-based therapeutics of cancer, infectious diseases, autoimmune diseases, and regenerative medicine.

Biological Research Artificial Intelligence

The impact of artificial intelligence (AI) on biotechnology has become a groundbreaking technology because it facilitates the interpretation and analysis of large volumes of biological data in a manner that was not practical before using their traditional computing capabilities. Chen, Engkvist, Wang, Olivecrona, and Blaschke were among the earliest to find that deep learning models can be used to predict molecular properties, get chemical designs optimal, and fast track the drug discovery process, and this method saves both time and cost compared to traditional methods (Chen et al., 2018). Ching et al. emphasized the ability of deep neural networks to combine heterogeneous biological data including genomic, proteomic, and chemical data to produce predictive models that can guide experimental choices with great precision, and transform the scientific fields of structural biology and functional genomics (Ching et al., 2018). Such AI systems have been useful in finding more intricate patterns in high-dimensional data and in protein structure and interaction folding, and systems-level modeling of cell behaviors, extending the potential of biotechnology beyond classical empirical methods and into predictive and data-driven studies.

Further developments, based on these developments, more recent studies have used machine learning to directly enhance bioengineering practice and therapeutic discovery. Yang, Wu, and Arnold have given strong arguments to the fact that directed evolution guided by AI can effectively search over extensive protein sequence space to find enzymes and biologics with improved function (Yang et al., 2019). Similarly, Zou and co-authors found that the trained deep learning models based on genomes of large scale could recognize regulatory genomic components and make accurate predictions of genotype-phenotype associations, which have greatly improved the functional annotation of biological discoveries and accelerated the discovery process (Zou et al., 2019). These applications in practice are ideas that optimize the guide RNA of CRISPR, with AI minimizing off-target effects and maximizing the specificity of editing, and in the development of diagnostics, AIs are making biomarkers more sensitive and diseases more identifiable. The net effect of these innovations is that today AI is central to the precision biotechnology whereby it is possible to predict in scale, generate hypotheses automatically and translate biological findings into practical solutions in a short time.

Stem Cells, Organoids and Regenerative Medicine

Stem cells have long been considered a foundation of regenerative medicine since they possess the only known property of self-renewal and differentiation into various types of cells giving them unparalleled potential at repairing or replacing damaged tissues. Embryonic stem cells (ESC) and induced pluripotent stem cells (iPSC) are the pluripotent stem cells (PSCs) that enable scientists to produce hundreds of cell lineages in culture, to form versatile models of disease biology and pharmaceutical testing. The introduction of organoid technology, where stem cells are used to create three-dimensional mini organs, has changed the way scientists examine the development and organogenesis of tissues and the pathophysiology of diseases. iPSC-derived or adult stem cell-derived organoids recapitulate important structural and functional aspects of organs and thus can be used to model complex diseases such as neurodegenerative conditions, liver fibrosis and intestinal inflammatory diseases. This technology can not only be used to study mechanistic studies that could not be previously in traditional two-dimensional cultures but also to test therapeutic interventions specific to patients in a personalized platform (Lancaster et al., 2013; Clevers, 2016).

Regenerative medicine has used the developments in the fields of stem cell and organoids to create new treatments to restore tissue and organ functionality. In clinical practice, hematopoietic stem cell transplantation of blood diseases, iPSC derived retinal pigment epithelium for macular degeneration, and bioengineered skin or cartilage to repair injury have all been clinically applied. In addition, platforms based on organoids are becoming valuable in high-throughput drug screening, precision medicine, and modeling rare genetic diseases, which is making therapeutic discovery much faster. Recent studies focus on combining stem cells and organoid with biomaterials, gene editing, and bioprinting to further maturation, vascularization, and functional integration of tissues using transplantation. These convergent innovations underscore the fact that a rapidly growing area is experiencing the integration of stem cell biology, organoid systems, and regenerative approaches to the creation of personalized, effective, and scalable therapeutic solutions (Huch et al., 2015; Takahashi and Yamanaka, 2016). Together as a whole, these strategies will transform regenerative medicine, tying the basic biological discovery to clinical translation in order to cure conditions otherwise untreatable.

Future Trends

Stem cell, organoids, and regenerative medicine are moving into a period of high innovation with emerging technologies coming together to overcome the existing limitations and create new clinical opportunities. One major direction here is to combine gene editing technologies, such as CRISPR, with stem cell and organoid models, to enable not only correction of genetic error but also custom regenerative therapeutic approaches. The creation of 3D bioprinting and tissue engineering is improving the vascularization of organoids, complexity of their structure and functional maturation and nowadays it is possible to create tissues that can be transplanted and, in the future, whole organs. Besides, AI and machine learning is applied to optimise the state of culture, distinguish predictions, and high-throughput drug testing on an organoid model. These advancements enhance reproducibility of the innovations, shorten development cycles and make the translation of bench-to-clinic findings.

Conclusion

In conclusion, it is possible to mention that the regenerative medicine due to the stem cells and technologies related to organoids has been radically changed and is very important to study the human development, the disease pathways, and the result of the therapy. Development of personalized and precision medicine The ongoing convergence of cellular biology, genetic engineering, biofabrication, and computational modeling is expanding the range of possibilities of personalized and precision medicine. These methods are still in development stages and are soon to leave the experimental phase and move on to clinically applicable regenerative therapies and offer a regenerative curative to hitherto incurable diseases.

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Image-Based Phenotyping and Computer Vision in Agriculture

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Abstract

Phenotyping has long been recognized as a major bottleneck in crop improvement, limiting the effective translation of genomic advances into genetic gain. Traditional phenotyping methods relied heavily on manual measurements and visual scoring, which were labor intensive, and time-consuming. The integration of image-based phenotyping with computer vision (CV), machine learning (ML), and deep learning (DL) technologies has enabled high-throughput, non-destructive, objective, and scalable trait assessment across both the controlled and field environments. This chapter provides a comprehensive overview of imaging platforms including greenhouse-based systems, unmanned aerial vehicles (UAVs), tractor-mounted sensors, and ground phenocarts and imaging modalities such as RGB, hyperspectral, thermal, and LiDAR-based 3D systems. It describes the evolution of computational approaches from classical image processing to modern deep learning architectures such as Faster R-CNN, U-Net, and YOLO. Crop-specific applications in rice, wheat, maize, sorghum, soybean, barley, potato, tomato, cotton, and grapevine are discussed, highlighting advances in stress detection, yield prediction, organ counting, disease diagnosis, and structural trait extraction. The chapter further outlines standard phenotyping workflows, advantages for breeding programs, limitations, and emerging trends such as multimodal data fusion and AI-driven digital breeding systems.

Keywords: Phenotyping; Computer vision; Deep learning; Machine learning; imaging; Stress; Crop improvement; Precision agriculture

Introduction

Phenotyping, the measurement of observable plant traits, has historically been a major bottleneck in crop improvement. While genotyping costs have declined dramatically with advances in sequencing technologies, phenotypic data collection remains labor-intensive, time-consuming, and often subjective. Traditional phenotyping relied on manual measurements such as plant height using rulers, disease scoring using visual scales, and biomass estimation through destructive sampling. These methods limited throughput, reduced reproducibility, and constrained breeding progress.

The emergence of digital imaging and computational analysis has transformed phenotyping. Image-based phenotyping integrates imaging sensors with computer vision (CV) algorithms to extract quantitative traits automatically (Fahlgren et al., 2015). With the adoption of machine learning (ML) and deep learning (DL), particularly convolutional neural networks (CNNs), phenotyping is transitioning from manual scoring to AI-assisted precision analysis (Singh et al., 2018; Kamilaris & Prenafeta-Boldú, 2018; Li et al., 2021).

In the past, phenotyping was manual and subjective. The present era is characterized by automated greenhouse and field platforms using RGB, hyperspectral, thermal, and LiDAR sensors. The future will likely involve real-time autonomous phenotyping integrated with genomics, multi-omics datasets, robotics, and edge computing systems, enabling predictive breeding and climate-resilient agriculture (Li et al., 2021).

This chapter addresses the importance of image-based phenotyping, discusses imaging platforms and computational techniques, presents crop-specific applications, and outlines future prospects in AI-driven agriculture.

Imaging Platforms and Modalities

Controlled Environment Platforms

Growth chambers and greenhouse-based phenotyping systems equipped with RGB, thermal, and hyperspectral cameras allow time-series trait measurement under controlled stress treatments (Fahlgren et al., 2015). These platforms are valuable for studying genotype × environment interactions.

Major Crop Examples

- **Rice (*Oryza sativa*):** Biomass and drought response monitoring (Yang et al., 2020)
- **Wheat (*Triticum aestivum*):** Canopy temperature and drought screening (Fahlgren et al., 2015)
- **Barley (*Hordeum vulgare*):** Salinity stress detection (Neumann et al., 2015)
- **Maize (*Zea mays*):** Leaf counting and growth tracking (Li et al., 2021)
- ***Arabidopsis thaliana:*** Model species for automated segmentation validation (Minervini et al., 2015)

Field-Based Platforms

Field phenotyping uses UAVs, tractor-mounted systems, and ground-based phenocarts for large-scale trait assessment (Araus & Cairns, 2014).

Major Crop Examples

- **Wheat:** Hyperspectral-based yield prediction (Zhang et al., 2019)
- **Maize:** LiDAR-based canopy structure analysis (Paulus, 2019)
- **Rice:** Multispectral drought response mapping (Araus & Cairns, 2014)
- **Sorghum:** UAV-based panicle detection (Ghosal et al., 2019)
- **Soybean:** Canopy coverage assessment (Falk et al., 2020)
- **Cotton:** Boll detection via multispectral imaging (Singh et al., 2018)

Imaging Modalities

Imaging Type	Application	Crop Examples	Reference
RGB Imaging	Morphological traits	Rice, Wheat, Maize, Tomato	Li et al., 2021
Hyperspectral Imaging	Physiological stress detection	Wheat, Barley	Zhang et al., 2019
Thermal Imaging	Drought and heat stress	Wheat, Rice	Araus & Cairns, 2014
3D Imaging (LiDAR)	Structural traits	Maize, Sorghum	Paulus, 2019

Computer Vision Techniques in Phenotyping

Image Processing and Segmentation

Early approaches used thresholding, edge detection, and morphological operations for plant-background separation (Minervini et al., 2015). These methods were effective in controlled environments but struggled under field variability.

Examples

- **Arabidopsis:** Rosette area segmentation (Minervini et al., 2015)
- **Wheat:** Projected shoot area estimation (Fahlgren et al., 2015)
- **Rice:** Leaf rolling quantification (Yang et al., 2020)

Recent updates include adaptive thresholding and hybrid preprocessing techniques.

Machine Learning Approaches

Machine learning methods such as SVM, Random Forest, and k-NN improved classification accuracy (Pound et al., 2017).

Examples

- **Potato:** Late blight detection using SVM (Polder et al., 2019)
- **Grapevine:** Disease classification using Random Forest (Singh et al., 2018)
- **Wheat:** Yield prediction via vegetation indices (Zhang et al., 2019)

Modern updates include ensemble learning (XGBoost) and automated feature selection.

Deep Learning Revolution

CNN-based architectures such as Faster R-CNN, YOLO, U-Net, and Mask R-CNN significantly improved detection and segmentation accuracy (Kamilaris & Prenafeta-Boldú, 2018).

Examples

- **Sorghum:** Panicle detection (Ghosal et al., 2019)
- **Tomato:** Fruit counting under occlusion (Rahnemoonfar & Sheppard, 2017)
- **Maize:** Automated leaf counting (Li et al., 2021)
- **Wheat:** Spike segmentation (Zhou et al., 2021)

Recent advancements include Vision Transformers, multimodal deep learning, transfer learning, and edge deployment for real-time field phenotyping.

Applications in Major Crops

Crop	Objective	Method	Reference
Rice	Drought screening	RGB + CNN	Yang et al., 2020
Wheat	Yield prediction	UAV hyperspectral + ML	Zhang et al., 2019
Maize	Leaf counting	Deep CNN	Li et al., 2021
Sorghum	Panicle detection	Faster R-CNN	Ghosal et al., 2019
Soybean	Root analysis	CV + ML	Falk et al., 2020
Barley	Salinity stress	RGB imaging	Neumann et al., 2015
Potato	Late blight detection	CNN	Polder et al., 2019
Tomato	Fruit counting	Deep learning	Rahnemoonfar & Sheppard, 2017

Image-Based Phenotyping Workflow

Image-based phenotyping follows a systematic pipeline that converts raw images into biologically meaningful traits. This workflow integrates sensor technology, computer vision, and machine learning algorithms to generate accurate, reproducible, and scalable phenotypic measurements.

Image Acquisition

Image acquisition is the first and most critical step in digital phenotyping. It involves capturing plant images using sensors such as RGB, hyperspectral, thermal, or LiDAR cameras mounted on controlled-environment platforms or field-based systems like UAVs and tractor-mounted rigs (Araus & Cairns, 2014). In crops such as wheat and rice, UAV-mounted multispectral cameras are commonly used for canopy monitoring, while greenhouse systems equipped with RGB and depth cameras are used for maize and barley growth tracking (Fahlgren et al., 2015). Proper calibration, standardized lighting, camera positioning, and temporal scheduling are essential to ensure data consistency and comparability.

Preprocessing

Raw images often contain noise, shadows, background clutter, and illumination variation. Preprocessing improves image quality before analysis. Common preprocessing steps include:

- Image resizing and normalization
- Noise filtering (Gaussian, median filters)
- Color space transformation (RGB to HSV or LAB)
- Illumination Correction and Background Removal

Adaptive histogram equalization improves contrast in rice canopy images, while radiometric correction is essential for hyperspectral data in wheat stress studies (Zhang et al., 2019). Effective preprocessing enhances downstream segmentation accuracy.

Segmentation

Segmentation separates plant pixels from background and isolates plant organs such as leaves, spikes, fruits, or roots. Early methods relied on thresholding and morphological operations (Minervini et al., 2015). However, these approaches struggled in complex field backgrounds. Modern approaches use deep learning models such as U-Net and Mask R-CNN for precise organ-level segmentation (Kamilaris & Prenafeta-Boldú, 2018).

Examples include:

- Wheat spike segmentation using CNN models
- Tomato fruit detection under occlusion
- Sorghum panicle extraction from UAV imagery
- Accurate segmentation is fundamental for reliable trait extraction.

Feature Extraction

Feature extraction transforms segmented images into quantitative traits. These features can be morphological features like leaf area, plant height, canopy cover and fruit size; color features like greenness indices and chlorophyll-related metrics;

texture features like disease lesion patterns and leaf surface characteristics; spectral features like vegetation indices (NDVI, PRI) and stress-related reflectance bands

Model Training

Extracted features are used to train predictive or classification models. Depending on the objective, different approaches are used like regression models for yield prediction, classification models for disease detection and object detection models for fruit or spike counting

Machine learning algorithms such as Random Forests and Support Vector Machines were widely used initially (Pound et al., 2017). Today, deep learning architectures such as YOLO and Faster R-CNN dominate plant organ detection and stress classification tasks (Kamilaris & Prenafeta-Boldú, 2018). Transfer learning using pretrained CNN models has significantly reduced training data requirements in crops like rice and maize.

Validation and Deployment

Model validation ensures accuracy, robustness, and generalizability. Common evaluation metrics include accuracy, precision and recall, F1-score and Root Mean Square Error (RMSE). Cross-validation and independent test datasets are used to avoid overfitting. After validation, models can be deployed: On cloud-based phenotyping platforms, on mobile applications for farmers and on edge devices integrated with UAVs. Recent developments emphasize real-time deployment in field conditions using lightweight AI models.

Advantages of Image-Based Phenotyping

Image-based phenotyping offers transformative benefits for crop improvement programs:

- **High Throughput**

Thousands of plots can be evaluated rapidly using UAVs or automated greenhouse systems, significantly accelerating breeding cycles.

- **Non-Destructive Monitoring**

Traits such as biomass accumulation, stress response, and growth rate can be monitored over time without damaging plants.

- **Objective Quantification**

Digital imaging eliminates subjectivity associated with visual scoring, increasing reproducibility across locations and seasons.

- **Early Stress Detection**

Thermal and hyperspectral imaging detect physiological stress before visible symptoms appear, enabling early intervention (Araus & Cairns, 2014).

- **Integration with Genomic Selection**

High-dimensional phenotypic data improve genomic prediction accuracy and enable more precise selection decisions.

Challenges

Despite major progress, several limitations remain:

- **Lighting Variability**

Changing sunlight conditions affect image quality in field environments, complicating segmentation and classification.

- **Limited Annotated Datasets**

Deep learning models require large labeled datasets, which are labor-intensive to generate.

- **Model Transferability**

Models trained in one environment may not generalize well across different locations, crop varieties, or seasons.

- **High Computational Demand**

Training deep neural networks requires powerful GPUs and large storage infrastructure.

Addressing these challenges requires standardized datasets, collaborative data sharing, and domain adaptation techniques.

Future Perspectives

The next generation of phenotyping systems will integrate artificial intelligence, robotics, and omics data for predictive breeding.

- **Integration with Genomic Prediction**

Combining high-throughput phenomics with genomic data will enhance breeding value estimation and accelerate genetic gain.

- **Real-Time Edge Computing**

Deployment of lightweight AI models on UAVs and mobile devices will enable in-field decision-making without cloud dependency.

- **Multimodal Sensor Fusion**

Combining RGB, hyperspectral, thermal, and LiDAR data will provide a comprehensive view of plant structure and physiology.

- **Open-Access Agricultural Image Databases**

Large annotated datasets will improve model training, benchmarking, and reproducibility across research groups.

- **AI-Driven Digital Breeding Systems**

Fully automated breeding pipelines integrating phenotyping, genotyping, and predictive analytics will redefine crop improvement under climate change scenarios.

Conclusion

Image-based phenotyping and computer vision have revolutionized trait measurement in agriculture. By integrating advanced imaging systems with deep learning algorithms, breeders can now perform high-throughput, accurate, and scalable phenotyping across crops. Continued advances in AI, robotics, and multi-omics integration will further accelerate precision breeding and sustainable agriculture.

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