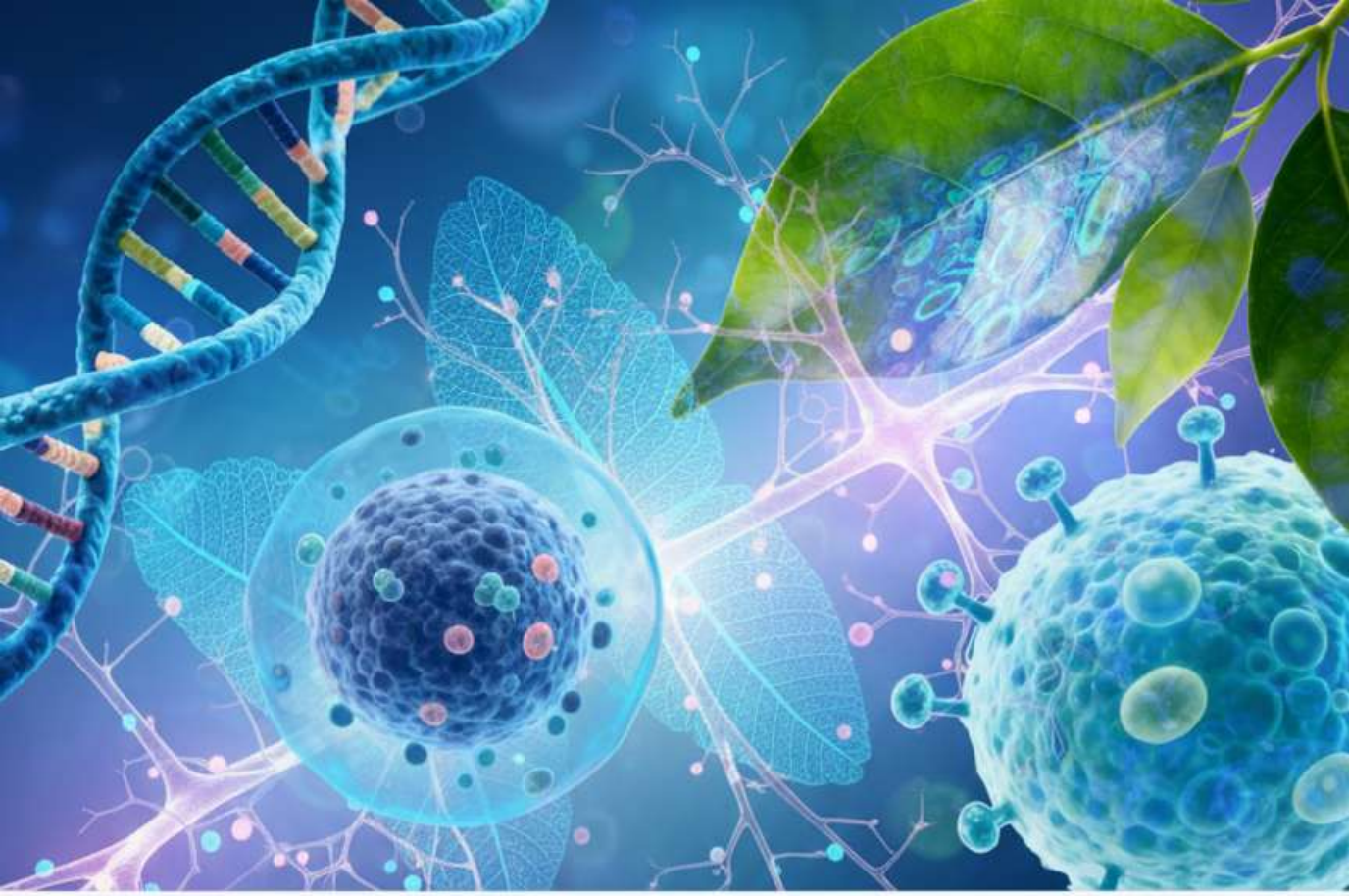

# *Advances in Life Science:*
## *Concepts, Trends, and Applications*


**Editors**
**Dr. Kalpana Singh**
**Dr. Anand Konkala**
**Ms. Anu Chaudhary**
**Mr. Anup Kumar Verma**



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# Advances in Life Science: Concepts, Trends and Applications

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# *Preface*

*The life sciences have witnessed unprecedented growth in recent decades, propelled by rapid technological innovation, interdisciplinary convergence, and an increasing focus on sustainability, health, and environmental resilience. Contemporary research in this domain is no longer confined to traditional disciplinary boundaries but increasingly integrates concepts from biotechnology, environmental science, computational biology, material science, and engineering. In this context, the present edited volume, Advances in Life Science: Concepts, Trends and Applications, seeks to provide a comprehensive and scholarly platform that reflects current developments and emerging research directions in the life sciences.*

*This volume comprises a carefully selected collection of original research articles and review chapters contributed by academicians and researchers from diverse institutions. The chapters collectively address a wide spectrum of themes, including sustainable aquaculture practices, emerging technologies for agriculture and food security, bio-energy systems, natural product applications, environmental biotechnology, bioremediation, genetic disorders and therapeutics, RNA biology, additive manufacturing in biopharmaceutics, and biophysical approaches to plant transport systems. Together, these contributions underscore the expanding scope and application-oriented nature of modern life science research.*

*Particular emphasis has been placed on scientific rigor, methodological clarity, and relevance to contemporary global challenges such as environmental degradation, climate change, resource sustainability, and public health. The contributors have not only synthesized existing knowledge but also highlighted future prospects, limitations, and research gaps, thereby offering valuable insights for both early-career researchers and established scientists.*

*This book is primarily intended for postgraduate and doctoral students, researchers, educators, and professionals engaged in life sciences and allied*

*disciplines. It is expected to serve as a reliable reference source, support advanced teaching and learning, and stimulate further interdisciplinary research and innovation.*

*The editors express their sincere appreciation to all contributing authors for their scholarly contributions and commitment to maintaining high academic standards. We also extend our gratitude to the reviewers for their critical evaluations and to the publisher for their professional support throughout the publication process. We hope that this volume will contribute meaningfully to the advancement of life science research and inspire future scientific endeavors.*

***Editors***

# Advances in Life Science: Concepts, Trends and Applications

## *Table of Content*

# Ornamental Fish Aquaculture: Practices, Management, and Sustainable Development

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## Abstract

Ornamental fish aquaculture has grown into a dynamic and commercially valuable branch of modern aquaculture, focusing on the production of visually appealing fish species for the aquarium trade. The sector incorporates a wide spectrum of activities including brood stock development, induced and natural breeding, larval and juvenile rearing, nutritional planning, water quality regulation, and comprehensive health care. Effective management practices, coupled with strong biosecurity measures, ensure high survival rates and reduce dependence on wild-harvested stocks. Sustainable development in this field emphasizes responsible breeding of native and exotic species, adoption of environmentally safe culture methods, and protection of aquatic biodiversity. With rising global demand, technological progress, and increasing involvement of small-scale farmers, ornamental fish aquaculture offers significant opportunities for livelihood enhancement, entrepreneurship, and sustainable utilization of aquatic resources.

***Keywords:*** Ornamental fish, Aquarium species, Breeding and rearing, Water quality, Fish health, Sustainable aquaculture

## Introduction

Ornamental fish aquaculture involves the scientific breeding, rearing, and maintenance of attractive fish species intended for decorative, hobby, and display purposes. Unlike food fish aquaculture, the main goal is to produce fish with desirable colour patterns, fin shapes, and aesthetic appeal. The ornamental fish trade is expanding globally and has become an important source of income, employment, and export revenue (Livengood & Chapman, 2012).

More than 2,000 species of ornamental fish are traded worldwide. Freshwater varieties dominate global markets because they are easier to breed and maintain compared to marine species (FAO, 2019). Singapore, Thailand, Indonesia, Sri

Lanka, and India are among the major exporters, while the United States, European Union, and Japan remain the major importers (Raja et al., 2014). India's large freshwater biodiversity, favourable climate, and increasing domestic demand offer considerable scope for industry growth (Ghosh et al., 2003).

Adopting scientific techniques in ornamental fish farming can significantly reduce pressure on natural populations and promote sustainable exploitation of aquatic resources (Tlusty, 2002). This chapter outlines key aspects of ornamental fish aquaculture including culture systems, broodstock and breeding management, nutrition, water quality control, health care, and sustainable practices.

## Methodology

- **Literature Review**

A detailed literature survey was conducted through Science Direct, PubMed, FAO databases, and fisheries research repositories using keywords such as "ornamental fish farming," "RAS," "biofloc systems," "fish nutrition," and "disease management."

- **Data Collection**

Secondary information was compiled from FAO aquaculture production reports (FAO, 2023), scientific journals, government documents, and institutional publications.

## Scope and Importance of Ornamental Fish Aquaculture

- **Economic Importance**

Ornamental fish farming is a low-cost, high-profit venture suitable for smallholders, women, and youth entrepreneurs. Due to the high value of ornamental species, even small-scale culture units can generate significant income (Ghosh et al., 2003).

- **Ecological and Conservation Importance**

Captive breeding minimizes the need for harvesting ornamental species from natural habitats, thereby aiding conservation of threatened and endemic species (Andrews, 1990).

- **Social and Therapeutic Importance**

Aquariums are widely known to reduce stress, enhance relaxation, and serve therapeutic functions, which increases the demand for ornamental species globally (Livengood & Chapman, 2012).

## Classification of Ornamental Fish

### Freshwater Ornamental Fishes

Freshwater species dominate global trade because they are hardy, easily bred, and

adaptable. Examples include goldfish, guppy, molly, angelfish, barbs, gouramis, and tetras (FAO, 2019). Table-1 provides representative freshwater ornamental fish species.

***Table-1. Some common freshwater ornamental fish species***

| **Scientific Name** | **Common Name** | **Order** | **Native Place / Origin** |
|---|---|---|---|
| *Carassius auratus* | Goldfish | Cypriniformes | China |
| *Cyprinus carpio (ornamental)* | Koi carp | Cypriniformes | Japan, China |
| *Poecilia reticulata* | Guppy | Cyprinodontiformes | South America |
| *Poecilia sphenops* | Molly | Cyprinodontiformes | Central America |
| *Xiphophorus hellerii* | Swordtail | Cyprinodontiformes | Mexico |
| *Xiphophorus maculatus* | Platy | Cyprinodontiformes | Central America |
| *Pterophyllum scalare* | Angelfish | Cichliformes | Amazon basin |
| *Symphysodon discus* | Discus fish | Cichliformes | Amazon basin |
| *Astronotus ocellatus* | Oscar fish | Cichliformes | South America |
| *Paracheirodon innesi* | Neon tetra | Characiformes | Peru, Brazil |
| *Hyphessobrycon rosaceus* | Rosy tetra | Characiformes | South America |
| *Danio rerio* | Zebra fish | Cypriniformes | India, Nepal |
| *Puntius conchonius* | Rosy barb | Cypriniformes | India |
| *Pethia ticto* | Ticto barb | Cypriniformes | India |
| *Trichogaster lalius* | Dwarf gourami | Anabantiformes | India, Bangladesh |
| *Betta splendens* | Siamese fighting fish | Anabantiformes | Thailand |
| *Badis badis* | Blue perch | Anabantiformes | India |
| *Channa gachua* | Dwarf snakehead | Anabantiformes | India |
| *Corydoras aeneus* | Cory catfish | Siluriformes | South America |
| *Ancistrus sp.* | Bristlenose pleco | Siluriformes | South America |

**Marine Ornamental Fishes**

Marine ornamental species such as clownfish, angelfish, butterflyfish, and seahorses are highly prized but require specialized systems and higher management standards (Tlusty, 2002). Table-2 lists commonly cultured marine ornamental fishes.

***Table-2. Some common marine ornamental fish species***

| Fish Species | Common Name | Order | Native Place |
|---|---|---|---|
| *Amphiprion ocellaris* | Clownfish | Perciformes | Indo-Pacific |
| *Zebrasoma flavescens* | Yellow Tang | Acanthuriformes | Hawaii, Central Pacific |
| *Pterapogon kauderni* | Banggai Cardinalfish | Perciformes | Banggai Islands, Indonesia |
| *Synchiropus splendidus* | Mandarin Fish | Perciformes | Western Pacific |
| *Chelmon rostratus* | Copperband Butterflyfish | Perciformes | Indo-Pacific |
| *Paracanthurus hepatus* | Blue Tang | Acanthuriformes | Indo-Pacific |
| *Centropyge loricula* | Flame Angelfish | Perciformes | Central Pacific |
| *Chaetodon auriga* | Threadfin Butterflyfish | Perciformes | Indo-Pacific |
| *Gramma loreto* | Royal Gramma | Perciformes | Caribbean Sea |
| *Hippocampus kuda* | Yellow Seahorse | Syngnathiformes | Indo-Pacific |

**Indian Native Ornamental Fishes**

India has rich biodiversity of fish about 2500 species, of which more than 300 fish species are reported ornamental. See Table-3 for Indian native species.

***Table 3. Indian native ornamental fish species***

| **Scientific Name** | **Common Name** | **Order** | **Native Place / Distribution in India** |
|---|---|---|---|
| *Danio rerio* | Zebra fish | Cypriniformes | Ganga–Brahmaputra basin |
| *Danio dangila* | Moustache danio | Cypriniformes | Northeast India |
| *Devario aequipinnatus* | Giant danio | Cypriniformes | Himalayan foothills |
| *Puntius sophore* | Pool barb | Cypriniformes | Throughout India |
| *Puntius conchonius* | Rosy barb | Cypriniformes | North India |
| *Pethia ticto* | Ticto barb | Cypriniformes | Gangetic plains |
| *Botia dario* | Queen loach | Cypriniformes | Northeastern India |
| *Lepidocephalichthys guntea* | Guntea loach | Cypriniformes | Northern India |
| *Badis badis* | Blue perch | Anabantiformes | Eastern and Northeast India |
| *Badis tuivaiei* | Scarlet badis | Anabantiformes | Northeast India |
| *Channa bleheri* | Rainbow snakehead | Anabantiformes | Assam (Brahmaputra basin) |
| *Channa gachua* | Dwarf snakehead | Anabantiformes | Peninsular India |
| *Aplocheilus lineatus* | Striped panchax | Cyprinodontiformes | Western Ghats |
| *Aplocheilus panchax* | Blue panchax | Cyprinodontiformes | Eastern India |
| *Etroplus suratensis* | Green chromide | Cichliformes | Kerala and Tamil Nadu |
| *Etroplus maculatus* | Orange chromide | Cichliformes | Western Ghats |
| *Trichogaster lalius* | Dwarf gourami | Anabantiformes | Northern and Eastern India |
| *Colisa fasciata* | Banded gourami | Anabantiformes | Eastern India |
| *Garra mullya* | Sucker fish | Cypriniformes | Western Ghats |
| *Nandus nandus* | Leaf fish | Anabantiformes | Northeastern and Eastern India |

**Exotic Ornamental Fish Species**

Exotic fishes are non-native species introduced for aquarium use. They form a major portion of India's ornamental fish market (Sinha et al., 2017). Representative exotic species are listed in Table-4.

***Table 4. Exotic Ornamental Fish Species***

| **Scientific Name** | **Common Name** | **Order** | **Native Place / Origin** |
|---|---|---|---|
| *Carassius auratus* | Goldfish | Cypriniformes | China |
| *Cyprinus carpio (ornamental strain)* | Koi carp | Cypriniformes | Japan, China |
| *Poecilia reticulata* | Guppy | Cyprinodontiformes | South America |
| *Poecilia sphenops* | Molly | Cyprinodontiformes | Central America |
| *Xiphophorus hellerii* | Swordtail | Cyprinodontiformes | Mexico, Central America |
| *Xiphophorus maculatus* | Platy | Cyprinodontiformes | Central America |
| *Pterophyllum scalare* | Angelfish | Cichliformes | Amazon River basin |
| *Paracheirodon innesi* | Neon tetra | Characiformes | Peru, Brazil |
| *Symphysodon discus* | Discus fish | Cichliformes | Amazon River basin |
| *Astronotus ocellatus* | Oscar fish | Cichliformes | South America |
| *Betta splendens* | Siamese fighting fish | Anabantiformes | Thailand, Cambodia |
| *Trichogaster trichopterus* | Three-spot gourami | Anabantiformes | Southeast Asia |
| *Hyphessobrycon serpae* | Serpae tetra | Characiformes | South America |
| Corydoras aeneus | Cory catfish | Siluriformes | South America |
| *Ancistrus sp.* | Bristlenose pleco | Siluriformes | South America |
| *Amphiprion ocellaris* | Clownfish | Perciformes | Indo-Pacific |
| *Zebrasoma flavescens* | Yellow tang | Acanthuriformes | Pacific Ocean |

**Principles of Ornamental Fish Culture**

Successful ornamental fish culture requires optimal water quality, high-quality broodstock, minimal stress, balanced feeding, and effective disease prevention (Pillay & Kutty, 2005). Cosmetic traits such as coloration, finnage, and body symmetry determine market value.

**Systems of Ornamental Fish Aquaculture**

**Aquarium Systems**

Glass and acrylic aquaria are ideal for breeding, rearing, and display because they allow fine control of environmental conditions (Livengood & Chapman, 2012).

**Cement and Fiberglass Tanks**

These tanks are commonly used for brood stock and mass seed production due to their durability and ease of disinfection (FAO, 2019).

**Pond Culture**

Hardy species like koi carp and goldfish are raised in outdoor ponds where natural food and sunlight support growth (Raja et al., 2014).

**Recirculatory Aquaculture Systems (RAS)**

RAS enables high-density, indoor culture with strong biosecurity and efficient water use (Tlusty, 2002).

**Water Quality Management**

Ornamental fish require stable water parameters for optimal health. Temperature, pH, dissolved oxygen, ammonia, and nitrite must be monitored regularly (Boyd, 2015). Table-5 describes major water quality parameters and management guidelines.

***Table-5. Water quality parameters and their management in ornamental fish aquaculture***

| Water Quality Parameter | Optimal Range | Importance in Ornamental Fish Culture | Causes of Deviation | Management Practices |
|---|---|---|---|---|
| Temperature | 24–28°C (species-specific) | Regulates metabolism, immunity, growth, breeding | Seasonal variation, heater failure | Use thermostatic heaters, cooling systems, keep tanks away from sunlight |
| pH | 6.5–7.5 | Affects physiological | Overfeeding, poor | Use buffers, frequent |

| | | | | |
|---|---|---|---|---|
| | | functions and enzyme activity | buffering, substrate type | monitoring, partial water changes |
| Dissolved Oxygen (DO) | > 5 mg/L | Essential for respiration and microbial balance | Overcrowding, poor aeration, organic waste | Provide aeration, avoid overstocking, remove organic debris |
| Ammonia ($NH_3$) | 0 mg/L | Highly toxic; damages gills and reduces immunity | Overfeeding, poor filtration, decomposing waste | Improve biofiltration, reduce feeding, regular water changes |
| Nitrite ($NO_2^-$) | < 0.1 mg/L | Causes brown blood disease | Insufficient nitrifying bacteria | Add nitrifying bacteria, maintain biological filter, salt addition (0.5–1 g/L) |
| Nitrate ($NO_3^-$) | < 40 mg/L | High levels stress fish and promote algae | Accumulation from nitrogen cycle | Regular water changes, algae control, live plants |
| Total Hardness | 50–150 mg/L $CaCO_3$ | Enhances buffering capacity; required for osmoregulation | Hard/soft water sources | Use RO water to dilute hardness or mineral additives |
| Alkalinity | 80–120 mg/L $CaCO_3$ | Stabilizes pH and prevents fluctuations | Excess $CO_2$, mineral imbalance | Use commercial buffers, crushed coral; remove excess $CO_2$ |

| | | | | |
|---|---|---|---|---|
| Carbon Dioxide ($CO_2$) | < 10 mg/L | Reduces pH and oxygen availability | Overcrowding, inadequate aeration | Increase aeration, reduce stocking density |
| Salinity | 28–35 ppt (marine); 5–15 ppt (brackish) | Osmoregulation | Improper salt mixing, evaporation | Use marine salt mixes, monitor salinity |
| Turbidity | Low | Ensures visibility, reduces stress | Suspended particles, bacterial bloom | Filtration, settling, avoid overfeeding |
| Total Dissolved Solids (TDS) | 150–300 ppm | Mineral balance | Poor water quality | Activated carbon, RO treatment |
| Chlorine/Chloramine | 0 mg/L | Toxic | Tap water treatment | Use dechlorinators or age water |

**Species Selection and Broodstock Management**

Species choice is based on market value, breeding ability, coloration, and disease resistance (Raja et al., 2014). Broodstock should be healthy, active, and well-fed. Proper nutrition and stress-free environments significantly improve reproductive performance (James & Sampath, 2003).

**Breeding and Seed Production**

- **Livebearing Fishes**

Guppy, molly, platy, and swordtail are common livebearers that undergo internal fertilization and produce free-swimming young (FAO, 2019).

- **Egg-Laying Fishes**

Goldfish, tetras, barbs, and angelfish lay eggs using diverse spawning behaviors including substrate attachment, bubble nests, and open-water scattering (Livengood & Chapman, 2012).

- **Induced Breeding**

Hormonal induction is occasionally used for species that do not breed readily under captive conditions (Pillay & Kutty, 2005).

**Larval Rearing and Juvenile Management**

Larvae initially depend on microscopic live feeds—infusoria, rotifers, and Artemia nauplii. Gradual weaning to formulated feeds ensures better growth and survival. Grading helps eliminate competition and maintain quality (FAO, 2019).

**Nutrition and Feeding Practices**

Nutritional balance influences growth, survival, immune function, and coloration. Feeds should contain 30–45% protein, essential fatty acids, vitamins, and minerals. Live feeds and high-quality formulated diets are commonly used (James & Sampath, 2003).

**Colour Enhancement and Quality Management**

Colour development in ornamental fish can be enhanced by carotenoid-rich feeds such as spirulina and astaxanthin. Good lighting, stress-free handling, and clean water further improve colour intensity (James & Sampath, 2003).

**Health Management and Biosecurity**

Ornamental fish are prone to parasitic, fungal, bacterial, viral, and environmental diseases due to handling and water quality stress. Preventive measures, quarantine, and timely treatment are crucial for maintaining stock health. Table-6 lists common diseases and their management.

***Table -6. Lists of common fish diseases and their Management***

| Disease Type | Disease / Pathogen | Common Symptoms | Treatment / Management |
|---|---|---|---|
| Parasitic | Ichthyophthiriasis (White Spot Disease) Ichthyophthirius multifiliis | White cyst-like spots, scratching, rapid breathing | Raise temperature to 28–30°C; Salt bath (2–3 g/L); Methylene blue or malachite green |
| Parasitic | Costiasis Ichthyobodo necator | Grayish-blue patches; excess mucus; lethargy | Salt treatment (1–2 g/L); Formalin bath (15–25 mg/L) |

| | | | |
|---|---|---|---|
| Parasitic | Fluke Infection (Gyrodactylus / Dactylogyrus) | Clamped fins; gill irritation; ulcers | Praziquantel (2 mg/L); KMnO4 dip; Improve aeration |
| Fungal | Saprolegniasis Saprolegnia spp. | Cotton-like white/gray growth on skin or eggs | Methylene blue; Salt dip; Potassium permanganate |
| Bacterial | Fin Rot / Tail Rot Aeromonas, Pseudomonas | Frayed fins, redness, inflamed edges | Water quality correction; Oxytetracycline; Salt (1–3 g/L) |
| Bacterial | Dropsy (Aeromonas hydrophila) | Swollen abdomen; pinecone scales | Broad-spectrum antibiotics; Salt; Isolation |
| Bacterial | Columnaris Disease Flavobacterium columnare | Cotton-like lesions; mouth erosion; ulcers | Formalin bath; KMnO4; Improve aeration |
| Viral | Koi Herpesvirus Disease (KHV) | Gill necrosis; lethargy; sudden mortality | No cure; strict biosecurity; quarantine |
| Viral | Lymphocystis Disease (Iridovirus) | Cauliflower-like nodules on fins and body | Supportive care; stress reduction; water quality control |
| Nutritional / Environmental | Swim Bladder Disorder | Abnormal buoyancy; sinking or floating | Fasting; boiled peas; improved diet |
| Environmental | Ammonia Toxicity | Gasping; red/inflamed gills | Water change; ammonia binders; improved filtration |

| | | | |
|---|---|---|---|
| Environmental | Nitrite Toxicity (Brown Blood Disease) | Brown gills; lethargy; surface breathing | Water change; salt addition (1–3 g/L); improved biofiltration |

**Packaging, Transportation, and Marketing**

Fish are packed in oxygen-filled polythene bags with stress-relieving additives and temperature control. Careful handling during transport reduces stress and mortality (Raja et al., 2014).

**Environmental and Ethical Considerations**

Sustainable ornamental fish culture discourages wild collection, promotes captive breeding, and advocates responsible trade to protect natural ecosystems (Andrews, 1990).

**Emerging Trends and Future Prospects**

Advances include genetic enhancement for colour traits, application of biofloc technology, digital monitoring tools, and automation of aquarium systems (FAO, 2024). India's expansive native fish diversity provides great opportunities for future development.

**Conclusion**

Ornamental fish aquaculture is a rapidly expanding sector offering economic, ecological, and societal benefits. Implementing scientific culture practices, effective health management, and environmentally responsible approaches will help ensure sustainable growth while supporting biodiversity conservation.

***References***

1. Andrews, C. (1990). The ornamental fish trade and fish conservation. Journal of Fish Biology, 37(Suppl. A), 53–59. https://doi.org/10.1111/j.1095-8649.1990.tb05016.x
2. Boyd, C. E. (2015). Water quality: An introduction. Springer.
3. FAO. (2019). Ornamental fish farming. Food and Agriculture Organization of the United Nations.
4. FAO. (2024). The state of world fisheries and aquaculture 2024. Food and Agriculture Organization.
5. Ghosh, A., Mahapatra, B. K., & Datta, N. C. (2003). Ornamental fish farming—A successful small-scale aqua-business in India. Aquaculture Asia, 8(3), 14–16.
6. Ghosh, A., & Mahapatra, B. K. (2019). Economics of ornamental fish farming. Aquaculture Economics & Management, 23(4), 412–425.
7. James, R., & Sampath, K. (2003). Effect of dietary carotenoids on

pigmentation of ornamental fish. Indian Journal of Fisheries, 50(4), 427–432.
8. Livengood, E. J., & Chapman, F. A. (2012). The ornamental fish trade: An introduction. University of Florida IFAS Extension.
9. Pillay, T. V. R., & Kutty, M. N. (2005). Aquaculture: Principles and practices (2nd ed.). Blackwell Publishing.
10. Prasad, G., Nair, R., & Pillai, A. (2018). Biology and management of exotic ornamental fishes. Indian Journal of Aquaculture, 26(2), 112–129.
11. Raja, K., Aanand, P., & Padmavathy, S. (2014). Present and future market trends of ornamental fish trade in India. International Journal of Fisheries and Aquatic Studies, 1(4), 245–251.
12. Sarkar, U. K., Lakra, W. S., & Gupta, S. (2016). Diversity of exotic ornamental fishes and their ecological risks. NBFGR Bulletin, 4(1), 1–56. National Bureau of Fish Genetic Resources (NBFGR), Lucknow.
13. Sinha, A. K., Das, P., & Mandal, S. (2017). Popular exotic ornamental fish species and their management in India. In ICAR Training Manual on Ornamental Aquaculture (pp. 22–36). ICAR–Central Institute of Freshwater Aquaculture (CIFA).
14. Sinha, A (2017). Evolution, trend and status of ornamental fisheries in India and their commercialization In book: Social Entrepreneurship in Aquaculture (pp.225-240), Editors: V.R.P. Sinha, Gopal Krishna, P. Keshavanath and N.R. Kumar Publisher: Narendra Publishing House, Delhi, India
15. Swain, S. K., Dash, P., & Chandra, G. (2019). Exotic ornamental fish farming and trade: Current status and challenges. Aquaculture Spectrum, 7(4), 18–27
16. Tlusty, M. F. (2002). The benefits and risks of aquacultural production for the aquarium trade. Aquaculture, 205(3–4), 203–219. https://doi.org/10.1016/S0044-8486(01)00711-9

# Emerging Life Science Technologies for Sustainable Agriculture and Food Security

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## Abstract

Sustainable agriculture and global food security are among the most pressing priorities of modern life sciences. Rapid population growth, climate change, soil degradation, and shrinking natural resources have intensified the need for innovative, science-based agricultural solutions. Recent advances in life sciences have introduced powerful technologies that enhance crop productivity, resilience, and environmental sustainability. This chapter presents an in-depth discussion of emerging life science technologies including molecular breeding, genome editing, plant–microbe interactions, biofertilizers, biopesticides, omics-based approaches, and nanobiotechnology. Emphasis is placed on their concepts, mechanisms, and applications in sustainable agriculture and food production systems. The chapter also highlights policy relevance, socio-economic impact, and future research prospects, underscoring the transformative role of life sciences in achieving long-term food security.

***Keywords:*** Sustainable agriculture, Life science innovations, Food security, Genome editing, Biofertilizers, Omics technologies

## Introduction

Agriculture is undergoing a major transformation driven by scientific innovation and the urgent need for sustainability. Traditional farming practices, largely dependent on chemical inputs and intensive resource use, have contributed to environmental degradation, reduced soil fertility, and ecological imbalance. At the same time, global food demand continues to rise, placing immense pressure on agricultural systems.

Advances in life sciences have provided new insights into plant biology, soil ecosystems, and genetic mechanisms, enabling the development of sustainable agricultural technologies. By integrating molecular biology, biotechnology, microbiology, and systems biology, life sciences offer holistic solutions that enhance productivity while preserving natural resources. This chapter examines the major life science technologies shaping sustainable agriculture and their contribution to food security.

## Sustainable Agriculture as a Life Science Concept

Sustainable agriculture is rooted in biological efficiency, ecological harmony, and long-term productivity. From a life science perspective, sustainability emphasizes the optimization of natural biological processes such as nutrient cycling, plant defense mechanisms, and microbial symbiosis.

Life science research has demonstrated that biologically driven agricultural systems improve soil structure, enhance biodiversity, and increase resilience to climatic stress. Understanding plant physiology, genetic diversity, and ecosystem interactions forms the scientific foundation of sustainable agricultural practices.

## Molecular Breeding for Enhanced Crop Performance

Molecular breeding integrates classical breeding methods with modern molecular tools to accelerate crop improvement. DNA markers linked to desirable traits enable precise selection of plants with superior yield, quality, and stress tolerance.

Marker-assisted selection and genomic selection have significantly reduced the time required to develop improved crop varieties. These approaches are particularly valuable in developing climate-resilient crops capable of sustaining productivity under adverse environmental conditions.

## Genome Editing as a Precision Tool in Agriculture

Genome editing technologies such as CRISPR-Cas systems have revolutionized agricultural life sciences by allowing targeted genetic modifications. These technologies enable the enhancement of agronomically important traits without altering the overall genetic makeup of plants.

Applications of genome editing include improvement of drought and salinity tolerance, resistance to pests and diseases, enhancement of nutritional content,

and reduction of post-harvest losses. Genome editing thus represents a major advancement in precision agriculture and sustainable crop development.

**Plant–Microbe Interactions and Rhizosphere Dynamics**

Plants exist in close association with diverse microbial communities that influence their growth, nutrition, and stress responses. The rhizosphere plays a crucial role in nutrient availability and plant health through complex biochemical signaling.

Life science research has unraveled the molecular basis of plant–microbe interactions, enabling the development of microbial inoculants that enhance crop productivity naturally. Harnessing beneficial microbes offers a sustainable alternative to chemical inputs in agriculture.

**Biofertilizers and Biopesticides in Sustainable Farming Systems**

Biofertilizers and biopesticides are biologically derived inputs that improve soil fertility and crop protection. Biofertilizers enhance nutrient availability by nitrogen fixation, phosphate solubilization, and stimulation of root growth.

Biopesticides control pests and diseases through biological mechanisms such as antagonism, parasitism, and induced resistance. Their use promotes environmentally safe agriculture while maintaining crop yield and quality.

**Omics Technologies and Systems Biology in Agriculture**

Omics technologies provide comprehensive insights into plant biological systems at molecular, cellular, and metabolic levels. Genomics, transcriptomics, proteomics, and metabolomics collectively reveal how plants respond to environmental stress and management practices.

Systems biology integrates omics data to understand complex biological networks, supporting precision breeding and crop management. These approaches enable data-driven decision-making in sustainable agricultural systems.

**Nanobiotechnology and Smart Agricultural Innovations**

Nanobiotechnology introduces nanoscale materials and tools for improved agricultural efficiency. Nano-fertilizers and nano-pesticides ensure targeted delivery, reduced dosage, and minimal environmental impact.

Smart agriculture combines nanotechnology with biosensors, automation, and digital tools to optimize resource use. Life science-driven smart technologies contribute to climate-resilient and precision-based farming practices.

**Socio-Economic and Policy Dimensions of Life Science Innovations**

Life science technologies influence agricultural productivity, rural livelihoods, and national food security. Their adoption promotes income generation, employment opportunities, and value addition in agricultural sectors.

Policy frameworks play a vital role in supporting research, innovation, and technology transfer. Collaboration among scientists, policymakers, farmers, and industry stakeholders is essential for the successful implementation of life science-based agricultural solutions.

**Conclusion**

Emerging life science technologies have redefined the scope of sustainable agriculture and food security. By leveraging molecular breeding, genome editing, microbial interactions, omics sciences, and nanobiotechnology, agriculture can achieve enhanced productivity with minimal environmental impact. The integration of scientific innovation with sustainable practices ensures resilient food systems capable of meeting future global demands. Continued advancement and application of life science research will remain central to achieving sustainable and secure food production.

***References***

1. Altieri, M. A. (2018). Agroecology: The science of sustainable agriculture. CRC Press.
2. FAO. (2021). The state of food security and nutrition in the world. FAO.
3. Godfray, H. C. J., et al. (2010). Food security and sustainable intensification. Science, 327(5967), 812–818.
4. Gupta, V. V. S. R., & Sharma, A. (2020). Plant–microbe interactions. Applied Soil Ecology, 147, 103368.
5. Jaganathan, D., et al. (2018). CRISPR for crop improvement. Plant Cell Reports, 37(12), 175–185.
6. Kumar, S., & Singh, R. (2021). Marker-assisted breeding. Plant Science Today, 8(2), 215–223.
7. Mishra, D. J., et al. (2018). Biofertilizers in agriculture. Journal of Cleaner Production, 173, 300–317.
8. OECD. (2020). Innovation and sustainability in agriculture. OECD Publishing.
9. Ricroch, A. E., et al. (2017). Genome editing in plants. Plant Biotechnology Journal, 15(3), 295–302.
10. Rockström, J., et al. (2009). Planetary boundaries. Nature, 461(7263), 472–475.
11. Sharma, P., & Singh, B. (2020). Biopesticides. Indian Journal of Agricultural Sciences, 90(4), 645–652.
12. Singh, J. S., et al. (2016). Microbial contributions to sustainability. Ecological Indicators, 67, 465–473.
13. Tilman, D., et al. (2011). Sustainable food production. PNAS, 108(50), 20260–20264.

14. Tripathi, L., et al. (2019). Biotechnology and food security. Plant Cell Reports, 38(9), 1013–1024.
15. Zhang, Y., et al. (2019). Omics technologies in agriculture. Trends in Plant Science, 24(6), 531–544.

# Powering the Future with Biology: Systematic Review of Bio-Battery

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**Abstract**

Bio-batteries have emerged as a promising class of sustainable energy devices that generate electricity using biological materials. Unlike conventional chemical batteries, they operate through natural biochemical reactions and offer an environmentally friendly alternative for low-power applications. In this review, we examine the fundamental concept of bio-batteries, outlining how they function and the principles behind their operation. We discuss the major types of bio-batteries, including enzymatic systems, microbial fuel cells, photosynthetic models, and plant- or fruit-based devices. The paper also highlights their applications in medical devices, wearable electronics, environmental monitoring, and emerging green technologies. In addition, we analyse the advantages that make bio batteries a promising option, as well as the limitations that currently restrict their large-scale use, such as low power output and stability issues. Illustrative diagrams and comparative explanations are included to support conceptual understanding. Overall, this review aims to present a clear and comprehensive overview of bio-battery technology while emphasizing its potential for future sustainable energy solutions.

***Keywords:*** Bio-batteries, biochemical reactions, enzymatic system, microbial fuel cells, sustainable energy.

## Introduction

One kind of energy storage system that uses organic chemicals is a bio-battery. It generates electricity from renewable fuels including fructose, glucose, and sucrose, providing a reliable and portable power source whenever needed. Enzymes in living things break down glucose and liberate protons and electrons. The same natural process is used by bio-batteries. They absorb the produced energy and store it for later use by using certain enzymes to break down glucose.

This operational approach is quite similar to how living things acquire energy to stay alive. The process of converting chemical energy into electrical energy in bio-batteries involves the use of biocatalysts, which can be enzymes or even entire microbes, to oxidize materials obtained from biomass. Glucose, the same sugar that is present in our blood, is the most frequent organic substance used to power bio-batteries. Enzymes in the body release protons and electrons when they break down glucose. Bio-batteries store and generate electricity by directly tapping into this energy source using enzymes that are comparable to these. Natural electricity production is also possible in certain microorganisms. For instance, when proteins in the cell membrane of the marine bacterium Shewanella incidences come into contact with mineral surfaces, an electric current is produced. These proteins have the ability to transfer electrons across the membrane quickly enough to give the bacteria the energy they require to survive. Researchers can create bio-batteries that power tiny sensors in difficult or distant situations by having a better understanding of this process. Due to its natural availability and lack of hazardous waste, human blood sugar is regarded as a desirable and clean energy source. It's interesting to note that tiny bio-batteries powered by human pee have also been produced by scientists. These batteries can be used in inexpensive, disposable diagnostic kits and are roughly the size of a credit card. The technology is straightforward and useful because the battery and testing components are consolidated into a single, disposable chip (Siddiqui and Pathrikar, 2013).

Adopting energy solutions has various advantages, both societal and economic, as it improves how we create and consume energy. Choosing green and sustainable energy sources provides several significant advantages. To begin with, it reduces greenhouse gas emissions, which is critical for environmental protection. Switching to renewable energy sources like solar or wind lessens our reliance on fossil fuels, resulting in long-term cost savings. Communities gain as well, as renewable energy projects frequently create new jobs and enhance local infrastructure. Reducing dependence on foreign fuels improves a country's energy security and stability. The demand for clean energy also encourages new technologies and ideas, which improve future systems' efficiency. A significant benefit is improved public health: cleaner energy equals fewer toxins in the air, which decreases health issues associated with bad air quality. As the renewable energy business expands, it opens up a variety of job prospects for those interested in sustainability and green technologies (Pathak, 2024).

Electric vehicles and mobile phones alike have relied on traditional lithium-ion batteries for their power needs. However, there are a few drawbacks to be aware of, even if they are widely used. Their efficiency decreases with time due to constant charging and discharging, which manifests as a gradual reduction in their power storage capacity with age (Ritchie, 2004).

Liquid electrolytes could pose a number of dangers. Some of these risks include electrolyte leakage and the gradual formation of dendrites, which are little structures made of lithium (Chen et al., 2020). The electrolyte can react with substances it shouldn't with a leakage from the battery. This could damage adjacent components and impact the overall functionality of the device (Zhang et al., 2024). When tiny, needle-shaped structures begin to form within the battery, this process is called dendrite growth. These microscopic protrusions have the potential to rip through the battery's inner layers, leading to overheating, short circuits, and, in the worst-case scenario, an explosion (Whittingham, 2004). Nevertheless, dendrite development remains a potential failure mode in lithium-ion batteries (Shah et al., 2024). More and more portable devices, medical implants, and tiny sensors are being produced, which is increasing the demand for small and dependable energy sources. These devices need a secure and effective power source because they cannot rely on bulky batteries.

Considered crucial for an extended period. Patients who depend on implants for everyday functioning must have an energy system that is biocompatible so it does not damage the surrounding tissues. Reducing the number of surgical procedures required to replace old batteries is another major benefit of such low-power systems. Devices that can self-sustain for several months or even years are safer and more convenient for patients. Devices powered by these sources can be downsized to a more discreet size, making them easier to wear or implant. This is why scientists are attempting to create autonomous power systems that can supply constant energy with minimal input from humans once implanted in a person or integrated into a gadget (Shuvo et al., 2022: Sohail et al., 2024).

In general, there is a lot of hope for the future of bioenergy, which is still in its early stages of development. Bio-batteries provide a safer and cleaner alternative to conventional power sources for numerous low-power devices, although they have not yet completely replaced them. Their compatibility with live tissues and ease of working with natural materials make them ideal for application in medical and wearable devices. Ongoing research is consistently enhancing their performance, despite obstacles like as low output and stability difficulties. With the ongoing development of these technologies, bio-batteries have the potential to play a significant role in future sustainable energy solutions.

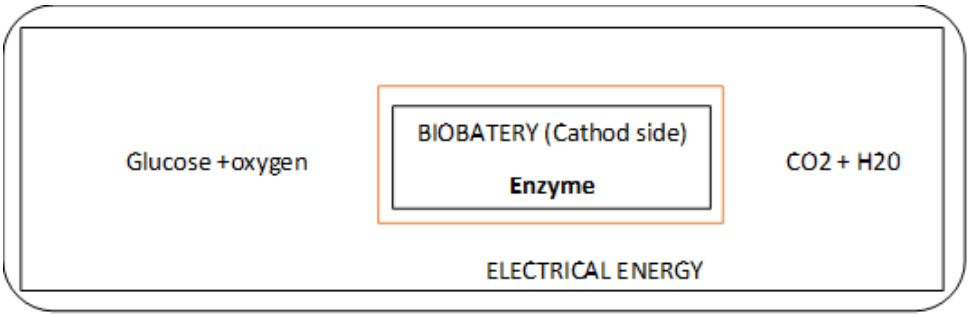


*Image credit: https://www.rfwireless-world.com/tutorials/bio-battery-construction working-advantages disadvantages#: ~:text=Water%20Production: %20Enzymes%20at%20the, %E2%86%922H 2O*

The bio-battery's operation is depicted in the graphic above. Electricity is created by the arrangement, which allows protons and electrons to travel freely. An electric current is created when protons begin to move due to the driving force inside the cell. While conventional current is thought to flow in the opposite direction, electrons in this arrangement move from the anode to the cathode. The following is a basic description of the procedure:

**Glucose and Enzyme Placement:** Glucose is present at the anode, and the enzyme used for the reaction is located at the cathode.

**Breakdown of Glucose:** The glucose molecules split into electrons and protons during the reaction. Movement of particles: The protons pass through the separator to reach the cathode, while the electrons take a different path through a mediator and eventually reach the cathode as well.

**Water Formation:** At the cathode, the enzyme uses the incoming electrons and protons along with oxygen to form water. This happens through an oxygen-reduction process.

$$O2 + 4H+ + 4e- \rightarrow 2H2O$$

**Energy Production:** As these reactions take place, electrons and protons are released in the system, and their movement is what produces the electrical energy.

**Need**

The most pressing issue is energy conservation, which bio-battery has the potential to address. The electrical definition of a battery is a device that can store chemical energy and then release it as electricity. A wide variety of electrical devices make use of several types of batteries. Furthermore, these batteries are harmful to the environment due to the hazardous compounds they contain, such

as lead and mercury. Chemical batteries also have the potential to explode, leak, or fail multiple times. Problems like these do not arise with bio-batteries. Due to its safety and environmentally favorable characteristics, bio-batteries show great potential as alternatives to conventional chemical batteries in the future. One renewable and environmentally friendly energy source is bio-batteries (Amogha, 2020).

**Types of Bio-Batteries**

1. **Enzymatic Bio-Battery:** In enzyme biobatteries, redox enzymes catalyze reactions and create electricity. Researchers discovered that the enzymes laccase and glucose oxidase are employed to convert glucose and oxygen into energy. Because bio-batteries are biocompatible, they can be used to power medical implants and tiny devices (Nelson et al. 2018).
2. **Algae Based Bio-Battery:** Besides conducting photosynthesis and generating biomass, algae have been explored as bioelectricity sources because to their ability to produce accessible, utilizable electrons for bio-battery systems (Gogoi and Dutta, 2025).
3. **Microbe Based Bio-Battery:** One form of bio-electrochemical system that utilizes the power of microbes to produce energy is known as a microbial fuel cell (MFC). Bacteria engage in metabolic processes that release electrons when they decompose organic or reduced chemicals at the anode in this configuration. Rather than being wasted, these electrons are directed toward the cathode via an external circuit, where they react with an oxidizing agent—typically oxygen—to produce light. An electric current is generated as electrons move through the circuit. A potential clean-energy technology, MFCs convert metabolic activities performed by bacteria into useful electricity (Logan et al., 2006; Beylier et al., 2011).

**Comparison of Bio Batteries and Lithium Batteries:**

| Batteries | Lithium batteries | Bio-bateries |
|---|---|---|
| Properties | This material is the primary constituent of electrolyte characterised by a very low standard reduction potential (-3.05v) and high capacity (3860mah/g) | Moderate to high (depands on the bio-fuel and catalyst used) Generally lower than conventional Lithium batteries. Typically, ambient to slightly elevated (20-40$^0$C); some designs tolerate wider range. |

| | | |
|---|---|---|
| Benefits | It enables the creation of high energy density. Lightweight batteries with excellent electrochemical performance, fascinating high-capacity power sources. | They are sustainable using organic waste as fuel and minimizing pollution. Their non-toxic properties allow for safe use in medical implants and wearable devices. |
| Limitation | The material is highly reactive and require protections from air and water. Consequently, it necessitates secure and tamper resistant sealing for safe use. | Biobatteries have low power density and efficiency. Means they cannot generate as much electricity per unit of volume or time compared to conventional batteries. |

*Table: Properties, benefits and limitations of batteries (Nzereogu et al., 2022; Varzi et al., 2020; Mandal and Das et al., 2022).*

**Advantages**

Biobatteries, also known as biological fuel cells, offer several advantages compared to conventional batteries. (GirishKumar et al.,2010; Mink et al.,2016).

- **Renewable and Sustainable:** Biobatteries use organic substances like glucose from renewable sources like biomass or garbage, making them a more sustainable option.
- **Environmental Friendliness:** They produce minimum harmful emissions or waste during operation, relying on natural metabolic processes to lessen environmental effect compared to traditional batteries that contain hazardous ingredients.
- **Scalability and Flexibility:** These batteries may be used in a variety of applications, including portable devices and grid-scale energy storage systems.
- **Self-Sustaining Systems:** Some designs use microorganisms to manufacture their own fuel, allowing for autonomous functioning without external fuel supply.
- **Biochemical Diversity:** Using a variety of organic substrates, including waste materials, can help treat waste by converting it into electricity.

Biobatteries have promising benefits, but they also have disadvantages, such as poor power density, short lifespans, and the need to improve electrode materials and microbial strains. Ongoing research aims to address these difficulties and

improve the performance and market viability of biobatteries.

- Enzymes work quickly, allowing biobatteries to charge gadgets much faster than conventional batteries.
- Because glucose or sugar is consistently available, bio-batteries do not require a separate power source.
- High-energy-density bio-batteries are readily accessible and can be conveniently used at room temperature.
- Biobatteries produce no harmful by products and can be recharged without limit.
- Biobatteries can be used confidently because they do not leak or explode like chemical batteries sometimes do.

**Application**

Soil batteries possess potential applications across diverse domains, including agriculture, environmental monitoring, and renewable energy storage. Here are several potential applications of soil batteries Li et al. (2014).

- **Agriculture:** Utilizing sensors to monitor soil parameters (moisture, temperature, nutrients), hence enhancing crop management and optimizing irrigation and fertilization efficiency.
- **Environmental Monitoring:** Supplying energy to sensors that monitor environmental parameters such as air and water quality, climatic variations, and biodiversity to inform policy and management decisions.
- **Remote Power:** Producing electricity in remote areas devoid of traditional power sources, beneficial for communication equipment and monitoring stations.
- **Renewable Energy Storage:** Capturing energy from variable sources such as solar and wind to guarantee a reliable power supply despite adverse weather conditions.
- **Disaster Relief:** Soil batteries provide a solution for emergency electricity in disaster-stricken areas. They can provide electricity for vital systems such as communication devices, illumination, and other fundamental infrastructure.

**Overall Potential**

Soil batteries have a wide range of potential applications. The further development of these batteries has the potential to make them a reliable and eco-friendly energy source, an asset for environmental monitoring, and a boon to agriculture.

**Future Prospect**

Bio batteries exhibit promising potential in specialized markets such as low-power electronics (toys, sensors, medical implants) owing to their sustainability (biodegradable, non-toxic, renewable fuels like sugar/starch) and environmental

benefits, with companies like Sony aiming for initial commercialization in small devices. Despite existing challenges in energy density, power output, and scalability for high-demand applications such as smartphones, continuous research in bioengineering and materials science is enhancing performance, potentially positioning them as next-generation solutions for remote sensors, wearables, and self-powered biomedical devices by utilizing natural processes for clean, sustainable energy.

## Conclusion

Bio-batteries provide an eco-friendly substitute for traditional chemical batteries; nevertheless, this technology is still in its early stages of development and has great promise. In order to produce electricity, they imitate the energy conversion processes in living things by using biocatalysts, which are enzymes or microbes, and organic substances, such as glucose or waste products.

## *References*

1. Amogha, A., K. (2020). Bio-Batteries. International Research Journal of Innovations in Engineering and Technology, 4(5), 1-4.
2. Beylier, M. R., Balaguer, M., Coprime, J., Pellicer-Nacher, C., Ni, B., Smets, B., Sun, S., & Wang, R. (2011). Biological nitrogen removal from domestic wastewater. In Elsevier eBooks (pp. 285-296) https://doi.org/10.1016/b978-0-444-64046-8.00360-8
3. Chen, R., Nolan, A M., Lu, J., Wang, J., Yu, X., Mo, Y., Chen, L., Huang, X., & Li, H. (2020). The Thermal Stability of Lithium Solid Electrolytes with Metallic Lithium. Joule, 4(4), 812-821.
4. GirishKumar, B., Mccloskey, A., C. Luntz, S., Swanson and Wickel, W. (2010). "Lithium – air battery: promise and challenges. Journal of physical chemistry, 1(40),2193-2203.
5. Gogoi, B., & Dutta, P. (2025). Bio-Batteries: An Overview of Recent Advances and Future Directions. International Journal of Scientific Research in Multidisciplinary Studies, 11(3), 54-58.
6. Logan, B. E., Hamelers, B., Rozendal, R., Schröder, U, Keller, J., Freguia, S., Aelterman, P., Verstraete, W., & Rabaey, K. (2006). Microbial Fuel Cells: Methodology and technology. Environmental Science & Technology, 40(17), 5181 5192.
7. Li, W., W., Yu, H., Q., & He, Z. (2014). Towards sustainable wastewater treatment by using microbial fuel cells-centered technologies. Energy and environmental science, 7(3),911-924.
8. Mandal, A., Das, H., T. (2022). Energy storage batteries: basic feature and applications K P Misra, R.D.K. Misra (Eds.), Elsevier Series on Advanced Ceramic Materials, Ceramic Science and Engineering, Elsevier.,323-351.

9. Mink, J., E., and Muller, F. A. (2016). Biofuel cells—Recent advances and applications. Biosensors and Bioelectronics, (76), 225-231.
10. Nzereogu,P., U., Omaha, A., D., Ezema, F., I., Iwuoha, E., I., Nwanya, A., C. (2022). Anode materials for lithium-ion batteries: a review. Appl.surface sci.Adv.,9.
11. Pathak, S. (2024). Sustainable Energy Powering a Greener Future. Hero Future Energies www.herofutureenergies.com
12. Ritchie, A. (2004). Recent developments and likely advances in lithium rechargeable batteries. Journal of Power Sources, 136(2), 285-289.
13. Shah, R., Mittal, V., & Precilla, A M. (2024). Challenges and advancements in All Solid-State Battery Technology for electric vehicles. J-Multidisciplinary Scientific Journal, 7(3), 204-217.
14. Shuvo, M. M. H., Titirsha, T., Amin, N., & Islam, S. K. (2022). Energy harvesting in implantable and wearable medical devices for enduring precision healthcare. Energies, 15(20), 7495.
15. Siddiqui, U, Z., & Pathrikar, A., K. (2013). THE FUTURE OF ENERGY BIO BATTERY. International Journal of Research in Engineering and Technology, 02(11), 99-111.
16. Sohail, A, Ali, A, Shaukat, H., Bhatti, F. M., Ali, S., Kouritem, S. A, Noori, M., & Altabey, W. A. (2024). Integrating self-powered medical devices with advanced energy harvesting: A review. Energy Strategy Reviews, 52, 101328 https://doi.org/10.1016/j.esr.2024.101328
17. Varzi, A., Thanner, K., Scipioni, R., Di Lecce, D., Hassoun, J., Dörfler, S., Altheus, H., Kaskel, S., Prehal, C., & Freunberger, S., A. (2020). Current status and future perspectives of lithium metal batteries. J. Power. Sources., 480 (31).
18. Whittingham, M. S. (2004). Lithium batteries and cathode materials. Cheminform, 35(50). https://doi.org/10.1002/chin.200450266
19. Zhang, Y., Zhang, P., Hu, J., Zhang, C., Zhang, L., Wang, Y., & Zhang, W. (2024). Fault diagnosis Method of Lithium-Ion battery leakage based on electrochemical impedance spectroscopy. IEEE Transactions on Industry Applications, 60(1), 1879-1889.

# Assessing Fabric Dyeing Potential of Natural Dyes Derived from Fruit and Vegetable Peels

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**Abstract**

Natural dyes are important substances from natural resources like plants, minerals, and insects. These dyes are non-toxic and free of health hazards like skin allergies. The aim of the present study is to analyze the performance of plant dyes extracted from different plant parts (fruit peels and vegetable peel) in cotton and silk fabrics, to compare color retain ability in fabrics using different mordants and to analyze the fastness properties of plant dyes on silk and cotton fabrics. In this paper ten fruit peel extract is used as a natural source of dyeing cotton and silk fabric. This work aims to achieve twin objectives of effective recycling waste from food industry and extraction and application of natural dyes from fruit waste and also enhance the dye stuffs performance characteristics. Alum, vinegar and salt were used in premordanting. Visible bright colors were dyed in both the fabrics. All the ten plants dyes show excellent results in rub fastness test. Silk fabric shows good dye retaining capacity of natural dye when compared to cotton fabric.

***Keywords:*** Dye, Fruit peels, Mordants, Salt, Vinegar

## Introduction

Natural pigments are applied to various plant-based fabrics. Textile production necessitates an abundance of diverse substances: Beyond dyes, numerous auxiliary materials are required, such as leveling agents for achieving even dye absorption or finishing agents for enhancing resistance to wrinkles or water repellency. Colorants can be grouped based on their chemical composition. Synthetic dyes such as azo, anthraquinone, and metal-complex compounds are commonly used. Azo dyes are characterized by their stability, chemical versatility, high fixity, and other properties. Azo dyes resist light and moisture, making them the most commonly used type. Industrial size. The dangers from unprocessed waste disposal exist. Azo dyes found in the environment usually

come from their main products. Aromatic amines are formed by breaking down the central azo bonds. These substances were identified by IARC in 2010 as major cancer-causing agents posing severe risks to public health. Consumers prefer natural colorants for their soft texture, earthy tones, deodorizing qualities, and compatibility with natural surroundings. Color staying bright after washing is hard because dye can't be used much. The use of mordants is essential for the natural dye to interact with the fabric fibers, thereby improving their dyeing capabilities. The interaction between fiber, mordant, and dye significantly affects the durability of natural textiles. [6].

Natural dye alternatives have become increasingly important due to their sustainability. Natural dyes come from various natural sources without chemical additives. They can be made from plants like indigo and saffron, insects like cochineal beetles and lac scale insects, animals like certain mollusks, and minerals like ochre. Clay is also used for dyeing. [1]. Fruit peels, shells, seeds, and other agricultural waste contain pigments that can be used to create natural colors. Synthetic dyes used in textiles cause water pollution due to their non-biodegradable and carcinogenic nature, posing disposal difficulties. In today's world, more people care about nature and want eco-friendly choices, so there's been an interest in using plants' colors to dye clothes [14]. The current study aims to evaluate how plant dyes derived from various plant parts (such as fruit peels and vegetable peels) affect the quality of cotton and silk textiles. To see how well clothes keep their colors when treated differently with dyes. To examine how well plants colors stay on silk and cotton clothes.[12].

Due to their non-biodegradability and carcinogenic properties, synthetic dyes utilized in textile coloring contribute to water pollution and possess harmful health hazards challenges. In the present day, a global awareness of environmental issues increases and the demand for sustainable ways rises, the application of natural dyes for fabric coloring has become better choice [3]. The current study aims to evaluate how plant dyes derived from various plant parts (such as fruit peels and vegetable peels) affect the quality of cotton and silk textiles. To see how well clothes keep their colors when treated differently with dyes. To examine how well plants colors stay on silk and cotton clothes.

## Materials and Methods

**Collection of Plant Material:** The fruit peel of *Punica granatum L, Prunus domestica L, Garcinia mangostana L., Persea americana Mill., Citrus × sinensis (L.) Osbeck, Beta vulgaris L, Daucus carota L., Cucurbita pepo L. Selenicereus undatus* (Haw.) D. R. Hunt and *Vitis vinifera L*. were collected from in and around Nagercoil. The collected plant materials are used for extracting dyes. The cleaned samples (50 grams) were ground up and mixed with 100 milliliters of water. The mixture was heated in a hot water bath for 30 minutes to extract

quickly. At the end of thirty minutes, all colors were collected. The hot mixture was passed through a filter, resulting in a clear liquid that's utilized for coloring cotton and silk fabrics

**Dye Extraction:** The collected plant materials used for extraction of dye. The cleaned samples (50g) were crushed and then boiled with 100 ml of water for 30 mins in a hot water bath for quick extraction. At the end of 30 minutes, the total color was extracted. The hot solution was filtered and a clear solution was obtained which is used for dyeing cotton and silk fabric [10].

**Dyeing Materials:** Cotton (2 mm size), silk (2 mm size) cloths were used to test the dyeing ability of extracts.

**Preparation of Mordant**

**Alum:** 0.748g of alum and 0.187g of washing soda were mixed with 100ml of water and stored for further use.

**Vinegar:** Mix 50 ml of 5% acetic acid with 100 ml of water. From this amount, 25 ml is taken and combined with 100 ml of distilled water

**Salt:** 5 g of sodium chloride was mixed with 100ml of distilled water and was used as a mordant and stored for further use.

**Premordant Dyeing:** Sodium chloride, vinegar and alum were used as mordants. The extracts obtained were filtered and used for dyeing textile material. The fabric materials used for dyeing were first washed with water. Then the fabric materials were transferred to fixative (mordants) 100ml salt/ 125 ml vinegar and allowed to boil for one hour at 100° C. The fabric is simmed in the fixative for at least an hour. The fixed fabric is immersed in the dye which is already extracted. It is then boiled and simmed until the fabric takes up the dye at least for an hour. After an hour, the fabric is carefully pulled out from the simmering fixative and is wrung out completely. The fabric is then placed on the newspaper or tile to dry. The shade dried fabric was further evaluated for its light, rub and wash fastness. Wash fastness was tested by washing with soapy water [12].

**Evaluation of Colour Fastness:** The evaluation of colour fastness involves testing how well the dye stays on the fabric after being washed according to standards set by ISO: 105-A02-1995, which uses an ISO-3 wash fastness method. The wash fastness rating was determined by measuring the loss of shade depth according to ISO-105-A02 and the extent of staining based on ISO-105-AO3 standards using grey scale. Colour fastness to rubbing (dry and wet) was assessed manually by hand rubbing one sample ten times and grey scale as per ISO-105-$AO_3$ extent of staining. [2].

**Results and Discussion**

Dying was an old-fashioned way of doing things before writing came along. Fabric creation hinges on this crucial element. The introduction of synthetic dyes led to a significant reduction in the usage of natural dyes for textile dyeing by 1856. The world uses about 30 million tonnes of textiles annually, projected to increase by 3% every year. This large number of textiles requires about 700,000 tons of dyes, leading to the emission of an enormous volume of unused and unbound synthetic colors into the environment according to [13]. Synthetic dyes are replaced by natural colorants, which have been increasing annually at about 2%. Natural hues are more digestible than artificial ones. Natural dyeing of textiles resulted in unsatisfactory colors and low durability. Mordants are employed to eliminate such complications. Metal ions from mordants donate electrons to dye molecules forming soluble complexes which make them water-soluble [9].

In the present study, different parts used in extraction of dyes. Fruit and vegetable peel of plants used in extracting dyes (Plate 1: Table 4.1)

In the present study, the peel of Beta vulgaris L. produced Golden brown colour extract and of dying, it produced a range of pinkish brown shades on different textile material studied. It produces light pinkish brown colour in silk fabric and pale brown colour in cotton fabric. Among the mordant used the best color developed in all mordant such as salt, vinegar and alum.

***Table 4.1 Plants That Yielded Substantive Dyes***

| **Sl. No** | **Binomial name** | **Family** | **Dye yielding plant part** |
|---|---|---|---|
| 1 | *Beta vulgaris L.* | Amaranthaceae | Peel |
| 2 | *Citrus sinensis* (L.)Osbeck | Rutaceae | Peel |
| 3 | *Cucurbita pepo* L. | Cucurbitaceae | Peel |
| 4 | *Daucus carota* L. | Apiaceae | Peel |
| 5 | *Garcinia mangostana* L. | Clusiaceae | Peel |
| 6 | *Persea americana* Mill. | Lauraceae | Peel |
| 7 | *Prunus domestica* L. | Rosaceae | Peel |
| 8 | *Punica granatum* L. | Lythraceae | Peel |
| 9 | *Selenicereus undatus* (Haw.) D. R.Hunt | Cactaceae | Peel |
| 10 | *Vitis vinifera* L. | Vitaceae | Peel |

The peel of *Citrus sinensis* produced orange yellow color extract and of dying, it produced a range orange and yellow shades on different textile material studied.

It produces pale yellow colour in silk fabric and orange yellow colour in cotton fabric. Among the mordant used the best color developed in vinegar and alum.
The peel of *Cucurbita pepo* produced bright yellow color extract and of dying, it produced a range of light yellow to bright yellow shades on different textile material studied. It produces bright yellow colour in silk fabric and pale yellow in cotton fabrics but mostly there is no colour developed in cotton fabrics. Among the mordant used the best color developed in alum.
The peel of *Daucus carata* produced yellow orange color extract and of dying, it produced a range of light brown yellow shades on different textile material studied. It produces golden yellow colour both cotton and silk fabric, treated with alum mordant and pale-yellow colour is observed in both cotton and silk fabrics with salt and vinegar mordant. Great golden colour is observed in the fabrics with alam mordant. Among the mordant used the best color developed in alum mordant.
The peel of *Garcinia mangostana* L. produced creamy brown color extract and of dying, it produced a range of pale brown shades on different textile material studied. It produces beige colour in silk fabric and cotton with the fabric treated with vinegar mordant and pale brown colour in both silk and cotton fabrics treated with alum and salt mordant. Among the mordant used the best color developed in vinegar.
The peel of *Persea americana* produced dark violet colour extract is obtained and of dying, it produced a range of violet shades are observed on different textile material studied. It produced blackish violet colour in silk fabrics treated with vinegar and alum mordant and violet grey colour is observed in the cotton fabrics treated with vinegar and alum mordant and pale violet colours in fabrics treated with salt. Among the mordant used the best colous developed in vinegar and alum.
The peel of *Prunus domestica* produced violet colour extract is obtained and of dying, it produced a range of pink color shades on different textile material studied. It produces very pale colour in both cotton and silk fabrics with the alam mordant and whitish pink colour is observed in both cotton and silk fabrics with vinegar and salt mordant. Among the mordant used the best color developed in all mordants such as salt, vinegar and alum.
The peel of *Punica granatum* produced creamy brown color extract was obtained and of dying, it produced a range of golden yellow shades on different textile material studied. It produces golden yellow colour in silk fabric and pale golden yellow in cotton fabrics. Among the mordant used the best color developed in all mordants such as salt, vinegar and alum.
The peel of *Selenicereus undatus* produced bright red color extract and of dying, it produced a range of yellowish pink and pink red shades on different textile material studied. It produces yellowish pink colour in

silk fabric and pale yellowish pink in cotton fabrics. Among the mordant used the best color developed in vinegar and alum.

The peel of *vitis vinifera* produced various shades of purple brown colour dye. When the cotton fabric is treated with sodium chloride mordant, whitish pink colous is developed when treated with vinegar it developed a pale pink and treated with alum it developed whitish pink colour. In silk fabric it developed a baby pink colour in a sodium chloride mordant, a pink shade produced in a vinegar mordant and a baby pink colous is produced in alum mordant. The test results showed that the boiling of dye grapes for more than 40 minutes gives significant colour dye to the sample and its dye. Among the mordant used the best color developed in salt and alum.

The dyed samples showed moderate to good wash- fastness ratings ranging from 3-4 on gray scale for change in colour. The wet rub- fastness of the dyed samples was found to be between 4 and 5 and dry rub- fastness ratings were 5.

Alum treated dyes were tolerant to washing and showed good light and rub fastness. The Red colored dye was extracted from *Selenicereus undatus* peels. Different shades of brown colors obtained from the dye extracted from *Garcinia mangostana* and *Beta vulgaris*. Vinegar and alum treated fabrics showed good washing, rubbing and light fastness than the mordant salt. The Vitis vinifera fruits yield a light pink color dye. Various shades of violet were observed in both cotton and silk materials from the dyes of Persea americana and Prunus domestica. The peel of beta vulgaris yield a brown-colored dye. Various shades of yellow color were observed in cotton and Silk fabrics, but there is no color developed in cotton treated with salt and vinegar except alum treated silk cloth exhibited pale yellow shade. Good fastness observed in cotton treated with alum. The process is economically viable as the raw materials are available at low cost and so cost of production is also very low.

Based on the results an observation made that the cotton fabric material developed good colours with alum, vinegar and sodium chloride mordants when compared to silk fabric material.

The mordants give distinct color varieties after being added to the dye color, allowing the shade to go from the original plant to mordant to different shades of color: From mordants of the same dye plant you can prepare different shades of colours by adding different mordants: mordants: alum, and acid of the type of cream of tartar is added to the dye, whereas, when using vinegar, salt in the mordant to the fabric: this results in a better dye colour as compared to mordant addition directly to the dye and when using heat mordant sticks well to the material. When the cloth undergoes exposure to the heat of this mordant it also makes the fabric sticks to the mordant very well as well and then, the color of the mordant does not go with time [5].

Plant leaves, vegetables, meat, red wine, green tea, fruits, and insects are used to make natural dyes and pigments. These raw materials are then processed to create plant-based, animal-based, and mineral-based dyes, as well as pigments like carotenoids, anthocyanins, betaines, quercetin, chlorophyll, and phycocyanin, which are widely used in printing inks, textiles, food and beverage, cosmetics, and other applications. Apart from phenolic compounds (flavonoids), nitrogenous compounds (chlorophyll derivatives), tocopherols, carotenoids, and ascorbic acid are among the phytochemical substances that have antioxidant activity. These dyes are safe and environmentally friendly and contain medicinal and therapeutic properties. Growing plants for dye extraction encourages forestation, resulting in a great ecological balance. Synthetic dyes are made using various carcinogenic chemicals, and the effluents that are dumped into rivers or vented into the atmosphere pollute the environment.[8].

While natural dyes originate from botanical, zoological, and geological sources, the vibrant hues derived from plant and insect-based dyes, unlike single-pigment synthetic alternatives, are typically the result of a complex interplay of various phytochemicals. These often include tannins, anthocyanins, and flavonoids, meaning that the final color rarely attributable to a solitary chemical compound [15].

The ability of a dye's color to endure through various cleaning processes is known as wash fastness, an essential characteristic for both synthetic and natural coloring agents. This property indicates how well a dye resists fading, bleeding, or altering its shade after being subjected to repeated washing and laundering cycles. Several factors influence the wash fastness of natural dyes, including the specific type of dye, the mordants employed, and the textile material to which it is applied. It is crucial to recognize that natural dyes generally exhibit less resistance to washing-induced degradation compared to synthetic dyes. To enhance the color stability of natural dyes against washing, a range of techniques can be implemented. These include the strategic use of mordanting agents, the application of dye fixatives, and various post-treatment procedures. Additionally, selecting natural dyes inherently known for their superior wash fastness and utilizing appropriate dyeing methodologies can significantly contribute to achieving more durable results.The resilience to washing varies considerably among different natural dyes. The choice of mordant also plays a decisive role; for instance, the absence of mordants for color fixation typically leads to diminished wash fastness. Furthermore, the type of fabric on which the dye is applied significantly affects its performance. Natural fibers such as cotton, silk, and wool interact uniquely with dyes and respond differently to washing, which, in turn, impacts how effectively the dye adheres to the textile [7].

***Table 4.2 Plant synthesized color dyes using different mordants***

| **Sl. No** | **Binomial name** | **Family** | **Dye yielding plant part** | **Fabric material** | **Vinegar** | **Sodium chloride** | **Alum** |
|---|---|---|---|---|---|---|---|
| 1 | *Beta vulgaris* | *Amaranthaceae* | Peel | Cotton | Pinkish brown | Pinkish brown | Pinkish Brown |
| | | | | Silk | Pinkish brown | Pinkish Brown | Pinkish brown |
| 2 | *Citrus sinensis* | Rutaceae | Peel | Cotton | Pale yellow | Yellow | Pale yellow |
| | | | | Silk | Yellow | Yellow | Pale yellow |
| 3 | *Cucurbita pepo* | Cucurbitaceae | Peel | Cotton | Yellow | Pale yellow | pale yellow |
| | | | | Silk | Yellow | Pale yellow | Bright yellow |
| 4 | *Daucus carota* | Apiaceae | Peel | Cotton | Pale yellow | Pale yellow | Golden yellow |
| | | | | Silk | Pale yellow | Pale yellow | Golden yellow |
| 5 | *Garcinia mangostana* | Clusiaceae | Peel | Cotton | Beige | Pale brown | Pale brown |
| | | | | Silk | Beige | Pale brown | Pale brown |
| 6 | *Persea americana* | Lauraceae | Peel | Cotton | Violet grey | Pale violet | Violet grey |
| | | | | Silk | Blackish purple | Pale violet | Blackish purple |
| 7 | *Prunuus domestica* | Rosaceae | Peel | Cotton | whitish pink | Whitish pink | Pale pink |
| | | | | Silk | Whitish pink | Whitish pink | Pale pink |
| 8 | *Punica granatum* | Lythraceae | Peel | Cotton | Golden yellow | Golden yellow | Golden yellow |
| | | | | Silk | Golden yellow | Golden yellow | Golden yellow |
| 9 | *Selenicereus undatus* | Cactaceae | Peel | Cotton | Yellowish pink | Yellowish pink | Pale pink |
| | | | | Silk | Yellowish pink | Yellowish pink | Pale pink |
| 10 | *Vitis vinifera* | Vitaceae | Peel | Cotton | Pale pink | Pale pink | Pale pink |
| | | | | Silk | Baby pink | Baby pink | Baby pink |

***Table 4.3 Colour fastness of dyed fabrics using Nacl as mordant***

| Sl. No. | Binomial name | | Fastness | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Wash fastness | | | | Light fastness | | | | Rub fastness | | | |
| | | | 1 | 2 | 3 | 4 | 1 | 2 | 3 | 4 | 1 | 2 | 3 | 4 |
| 1 | *Beta vulgaris* | Cotton | | | ✓ | | | | | ✓ | | | | ✓ |
| | | Silk | | | ✓ | | | | | ✓ | | | | ✓ |
| 2 | *Citrus sinensis* | Cotton | | | ✓ | | | | ✓ | | | | | ✓ |
| | | Silk | | | ✓ | | | | ✓ | | | | | ✓ |
| 3 | *Cucurbita pepo* | Cotton | ✓ | | | | | | ✓ | | | | ✓ | |
| | | Silk | ✓ | | | | | | ✓ | | | | | ✓ |
| 4 | *Daucus carota* | Cotton | | | ✓ | | | | ✓ | | | | | ✓ |
| | | Silk | | | ✓ | | | | ✓ | | | | | ✓ |
| 5 | *Garcinia mangostana* | Cotton | | ✓ | | | | | ✓ | | | | | ✓ |
| | | Silk | | ✓ | | | | | ✓ | | | | | ✓ |
| 6 | *Persea americana* | Cotton | | | | ✓ | | | | ✓ | | | | ✓ |
| | | Silk | | | | ✓ | | | | ✓ | | | | ✓ |
| 7 | *Prunus domestica* | Cotton | | | ✓ | | | | | ✓ | | | | ✓ |
| | | Silk | | | ✓ | | | | | ✓ | | | | ✓ |
| 8 | *Punica granatum* | Cotton | | | ✓ | | | | | ✓ | | | | ✓ |
| | | Silk | | | ✓ | | | | | ✓ | | | | ✓ |
| 9 | *Selenicereus undatus* | Cotton | | ✓ | | | | | ✓ | | | | | ✓ |

| | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Silk | | ✓ | | | | | ✓ | | | | | |
| 10 | *Vitis vinifera* | Cotton | | | | ✓ | | | | ✓ | | | | ✓ |
| | | Silk | | | | ✓ | | | | ✓ | | | | ✓ |

1- Poor, 2-Weak, 3–Good, 4- Excellent

**Table 4.4 Colour fastness of dyed fabrics using vinegar as mordant**

| Sl. No | Binomial name | | Fastness | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Wash fastness | | | | Light fastness | | | | Rub fastness | | | |
| | | | 1 | 2 | 3 | 4 | 1 | 2 | 3 | 4 | 1 | 2 | 3 | 4 |
| 1 | *Beta vulgaris* | Cotton | | | ✓ | | | | ✓ | | | | | ✓ |
| | | Silk | | | ✓ | | | | ✓ | | | | | ✓ |
| 2 | *Citrus sinensis* | Cotton | | | ✓ | | | | ✓ | | | | | ✓ |
| | | Silk | | | ✓ | | | | ✓ | | | | | ✓ |
| 3 | *Cucurbita pepo* | Cotton | ✓ | | | | | | ✓ | | | | ✓ | |
| | | Silk | ✓ | | | | | | ✓ | | | | | ✓ |
| 4 | *Daucus carota* | Cotton | | ✓ | | | | | ✓ | | | | | ✓ |
| | | Silk | | ✓ | | | | | ✓ | | | | | ✓ |
| 5 | *Garcinia mangostana* | Cotton | | | ✓ | | | | ✓ | | | | | ✓ |
| | | Silk | | | ✓ | | | | ✓ | | | | | ✓ |
| 6 | *Persea americana* | Cotton | | | | ✓ | | | | ✓ | | | | ✓ |
| | | Silk | | | | ✓ | | | | ✓ | | | | ✓ |
| 7 | *Prunus domestica* | Cotton | | | ✓ | | | | | ✓ | | | | ✓ |
| | | Silk | | | ✓ | | | | | ✓ | | | | ✓ |
| 8 | *Punica granatum* | Cotton | | | ✓ | | | | ✓ | | | | | ✓ |
| | | Silk | | | ✓ | | | | ✓ | | | | | ✓ |
| 9 | *Selenicereus undatus* | Cotton | | ✓ | | | | | ✓ | | | | | ✓ |
| | | Silk | | ✓ | | | | | ✓ | | | | | ✓ |

| | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 10 | *Vitis vinifera* | Cotton | | | | ✓ | | | ✓ | | | | | ✓ |
| | | Silk | | | | ✓ | | | ✓ | | | | | ✓ |

1- Poor, 2-Weak, 3–Good, 4- Excellent

***Table 4.5 Colour fastness of dyed fabrics using alum as mordant.***

| **Sl. No** | **Binomial name** | | **Fastness** | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | **Wash fastness** | | | | **Light fastness** | | | | **Rub fastness** | | | |
| | | | **1** | **2** | **3** | **4** | **1** | **2** | **3** | **4** | **1** | **2** | **3** | **4** |
| 1 | *Beta vulgaris* | Cotton | | | ✓ | | | | | ✓ | | | | ✓ |
| | | Silk | | | ✓ | | | | | ✓ | | | | ✓ |
| 2 | *Citrus × sinensis* | Cotton | | | ✓ | | | | | ✓ | | | | ✓ |
| | | Silk | | | ✓ | | | | | ✓ | | | | ✓ |
| 3 | *Cucurbita pepo* | Cotton | | ✓ | | | | | | ✓ | | | | ✓ |
| | | Silk | | ✓ | | | | | ✓ | | | | | ✓ |
| 4 | *Daucus carota* | Cotton | | | ✓ | | | | ✓ | | | | | ✓ |
| | | Silk | | | | ✓ | | | ✓ | | | | | ✓ |
| 5 | *Garcinia mangostana* | Cotton | | | ✓ | | | | ✓ | | | | | ✓ |
| | | Silk | | | ✓ | | | | ✓ | | | | | ✓ |
| 6 | *Persea americana* | Cotton | | | | ✓ | | | | ✓ | | | | ✓ |
| | | Silk | | | | ✓ | | | | ✓ | | | | ✓ |
| 7 | *Prunus domestica* | Cotton | | | ✓ | | | | | ✓ | | | | ✓ |
| | | Silk | | | ✓ | | | | | ✓ | | | | ✓ |
| 8 | *Punica granatum* | Cotton | | ✓ | | | | | ✓ | | | | | ✓ |
| | | Silk | | ✓ | | | | | ✓ | | | | | ✓ |
| 9 | *Selenicereus undatus* | Cotton | | | ✓ | | | | | ✓ | | | | ✓ |
| | | Silk | | | ✓ | | | | | ✓ | | | | ✓ |
| 10 | *Vitis vinifera* | Cotton | | | | ✓ | | | | ✓ | | | | ✓ |
| | | Silk | | | | ✓ | | | | ✓ | | | | ✓ |

Poor, 2-Weak, 3–Good, 4- Excellent

**Fastness Results**

Wash, light and rub fastness tests were conducted to determine whether the identified dyes would withstand washing and drying when used to colour everyday clothes.

**Washing Fastness**

The results of washing, light and rub fastness of the dyed fabrics are shown in table 3. Variable color shades were formed on both silk and cotton fabrics after fastness tests. *Beta vulgaris* L. dyed in both cotton and silk fabrics recorded good wash fastness results (3) in vinegar, sodium chloride and alum mordants. Citrus × sinensis dyed fabrics of cotton and silk were recorded a good wash fastness result (3) in vinegar, sodium chloride and alum mordrants.

*Cucurbita pepo* dyed fabrics showed a poor (1) wash fastness in sodium chloride and vinegar mordants, it shows weak (2) in alum mordant. Daucus carota recorded a good (3) wash fastness in sodium chloride mordant and shows weak (2) in vinegar mordant and shows good (3) in cotton fabric with alum and excellent (4) in silk fabric with alum mordant. *Garcinia mangostana* L. exhibited a good (3) wash fastness on both cotton and silk fabrics in alum and sodium chloride mordants and shows weak (2) in vinegar mordant. *Persea americana* gave excellent (4) wash fastness in all the mordants such as alum, sodium chloride and vinegar. *Prunus domestica* recorded a good (3) wash fastness on both cotton and silk in the selected mordants. Punica granatum L. gave a weak (2) result in alum mordant and shows Weak (3) in sodium chloride and alum mordants. Selenicereus undatus exhibited a good (3) wash fastness result in alum and weak (2) result in sodium chloride and vinegar. *Vitis vinifera* recorded a excellent (4) wash fastness result in all the mordants

**Light Fastness**

*Beta vulgaris* L. good (3) light fastness result in vinegar mordant and shows excellent (4) in both alum and sodium chloride mordants. *Citrus sinensis* and *Curcubita pepo* recorded good (3) light fastness in both vinegar and sodium chloride mordant and shows excellent (4) in alum mordant. The three selected mordant *Daucus carota* and *Garcinia mangostana* registered good (3) light fastness in sodium chloride, alum and vinegar. The mordants venigar and alum mordanted samples dyed with Persea americana and prunus domestica showed excellent (4) light fastness on both cotton and silk fabrics. *Slenicereus undatus* showed good (3) light fastness results in vinegar and sodium chloride mordants. *Vitis vinifera* showed good (3) light fastness results in vinegar mordant and showed excellent (4) in alam and sodium chloride mordants. The salt, alum and vinegar treated dyes were tolerant to washing and showed good washing fastness. It also had a good light and rub fastness.

**Rub Fastness**

In the present study, dyed fabrics from all the plant materials *Beta vulgaris*, *Citrus sinensis, Cucurbita pepo, Daucus carota, Garcinia mangostana, Persea americana, Prunus domestica, Punica granatum, Selenicereus undatus, Vitis vinifera* recorded good (3) and excellent (4) results with all the three mordants in rub fastness test. All the mordants showed moderate washing, rubbing and light fastness except the silk treated with vinegar showed poor fastness. *Selenicereus undatus* and *Persea americana* dyes show no change in colour after wash fastness test, indicating their ability to withstand laundering. this suggests their viability as dyes for colouring everyday clothes.

**Fastness Test**

Most of the natural dyes have poor light stability as compared to that of the best synthetic dyes, and hence the colours in historical old textile are often different from their original colours. Fading of colour on the textile occurs on exposure to light. This degradation occurs when light breaks chemical bonds in dyes. Sunlight is made up of ultra violet light, visible light and infrared radiation. While short wave UV causes most of the physical property damage to fibres, it is generally the longer wave UV and visible light that causes dyed textile fade. Poor light fastness of some of the natural dyes can be attributed to propensity of the dye chromophore to the photochemical oxidation. The chromophore in some classes can be protected from photochemical oxidation by forming complex with transition metals, where by a six-member ring is formed. The photons sorbed by the chromophoric group dissipate their energy by resonating within the ring and hence dye is protected. The post mordanting process with metal increases the light fastness of natural dyed samples. However, the post mordanting with metal salts also results in change in hue of the dyed fabric, hence the post treatment should be selected rightly. To improve the light fastness properties of popular natural colourants namely turmeric, henna, madder and pomegranate rind using tannin containing natural mordants namely tamarind seed coat and gooseberry fruits and also metallic mordant (safer limit) combinations. The rate of fading has been greatly reduced due to the combined effect of natural tannins and metal mordant bonding. Additionally, the dyed fabrics holds good antimicrobial property and thus can be used as eco-friendly [11].

**Washing Fastness**

Some of the natural dyes undergo a little change in their hue on washing, this may be due the alkaline nature of the washing mixture mainly the pH. In general, natural dyes (on wool) have only moderate wash fastness as assessed by the ISO 2 test. However, logwood and indigo dyes exhibit better fastness when applied to different textiles. The nature of detergent solution suitable for conservation of

natural coloured art work has been examined. A liquor containing 1g/l of sodium polyphosphate is found to be best resulting marginal changes in hue with natural dyes applied on wool or silk. The small increase in cleaning efficiency attributable to the alkali must be balanced against possible colour change in the natural dyes, apart from possible damage to the protein fibre under alkaline conditions. In general, the fastness of a colour can vary with the type of dye, the particular shade used, the depth of shade and the dyeing process and mainly the nature of the washing mixture. Some natural dyes undergo marked changes in colour on washing, shown to be attributed to even small amounts of alkali in washing mixtures, high-lighting the necessity of knowing the pH of alkaline solutions used for cleaning of textiles dyed with natural dyes [4].

Derived from nature, plant-based dyes represent an inherently sustainable and non-polluting coloration method. However, achieving effective adhesion of these dyes to fabric fibers typically necessitates the use of auxiliary compounds known as mordants. The shift from synthetic to natural dyes plays a crucial role in ecological restoration. Despite their benefits, research and development into standardizing natural dye processes remain limited. Furthermore, a common concern revolves around their colorfastness properties, particularly resistance to washing and light exposure. These performance limitations can be significantly improved through judicious selection of natural mordants, optimized extraction methods, and the intelligent application of both technology and environmentally conscious processes. Such advancements are vital for the widespread commercialization of these botanical colorants for textile dyeing, offering substantial benefits to local rural artisans and plant growers. Consequently, the industrial-scale application of natural dyes in textile manufacturing is transitioning from aspiration to reality, establishing its place in the market for eco-friendly textiles.

**Summary and Conclusion**

In the present study of various ranges of dyes extracted from ten plant materials. *Beta vulgaris, Citrus sinensis, Cucurbita pepo, Daucus carota, Garcinia mangostana, Persea americana, Prunus domestica, Punica granatum, Selenicereus undatus, Vitis vinifer*a were the plants used for the extraction of dyes. Nacl, vinegar and alum are mordants used as fixatives of dyes. Plant parts like fruit and vegetables peels were used for extracting dyes. Fabrics like cotton and silk were used for dyeing. *Vitis vinifera* and *Persea americana* dyes show no change in colour after wash fastness test.

This investigation explores the effectiveness of fruit and vegetable skins, typically discarded, as environmentally sound alternatives for producing natural dyes in textile applications. Outer layers from ubiquitous produce such as beetroot, carrots, and pumpkins were subjected to water-based methods to

specifically isolate their inherent colorants. The resulting pigments were then applied to both cotton and silk fibers, using various natural mordants to thoroughly assess their take-up rate, their resistance to fading (colorfastness), and their overall aesthetic appeal. The outcomes definitively illustrate that these organic refuse materials (the fruit and vegetable peels) successfully impart vivid and durable color hues, particularly when the fabrics are pre-treated with fixatives like alum and common salt. This research underscores the dual benefits of environmental responsibility and financial viability associated with repurposing kitchen waste as a substitute for synthetic dyes. It champions an ecological methodology for fabric coloration and actively contributes to initiatives that transform waste into valuable resources.

***References***

1. Aravin, P.P: Natural dyeing of cellulose fbers using syzygium cumini fruit extracts and a bio-mordant: A step toward sustainable dyeing. Sustain Mater. Technol., 33:1-9(2022).
2. Bide, M: Colour measurement and fastness assessment. Colour Measurement: Principles, Advances and Industrial Applications. 196-217 (2010).
3. Bishal,A: Kazi Asraf Ali, Somnath Ghosh, Pijus Parua, Bratati Bandyopadhyay, Souvon Mondal, Mayukh Jana, Aveek Datta, Karan Kumar Das, Biplab Debnath. 2023. Natural dyes: Its Origin, Categories and Application on Textile Fabrics in brief, Euro Chem Bull., 12(8):9780-9802(2023).
4. Duff, D.G., Sinclair, R.S and Stirling, D.1977. studies in conservation.22:170-176.
5. Geetha B and Judia,V. H.S: Extraction of Natural Dyes from Plants. Int J Chem Pharma Sci, 1(8): 502-509 (2013).
6. IARC, International agency for research in cancer. 2010. Some Aromatic Amines, Organic Dyes, and Related Exposures. ISBN: 978-92-832-1299-7. (2010).
7. Indumathi, T.R and Matheshwaran, S. Extraction of Natural Dye from Fruit Peels Permeate in Cotton Embroidery Threads. Int J Emer Technol Inno Res., 11 (3)465-475(2024).
8. Lokman, H. and Kirtiraj, G. 2022. Natural Dyes from Plants for Smart Packaging Printing and Applications. J Advan Chemi Eng.12(9): 1-8 (2022).
9. Mongkholrattanasit, R., Krystufek, J., Wiener, J. and Vikova, M. Dyeing, fastness and UV protection properties of silk and wool fabrics dyed with eucalyptus leaf extract by the exhaustion process. Fibers & Textiles, 19(3): 94- 99. (2011).
10. Opoku-Asare, N.A., Korankye, O and Adu Baah, K: Assessing the Efficacy of Dyes Extracted from Some Local Plants for Colouring Cotton Fabrics. J

Sci Technol, 33(3): 51-59 (2013).
11. K.H. and Aniket, S B: Plant based natural dyes and mordnats: A Review. J. Nat. Prod. Plant Resour., 2 (6):649-664 (2012)
12. Redwan J: 2014. Dyeing of Silk Using Natural Dyes Extracted from Local Plants. Int. J.Sci. Eng. Res., 5(11): 809-818 (2014).
13. Samanta, S.K and Agarwal, P: Application of natural dyes on textiles. Ind J Fibre & Textile Res., 34: 384-399 (2009).
14. Sara K Natural Dyes: A Traditional Craft Experiencing New Attention, The Delta Kappa Gamma Bulletin, page no:14 (2008).
15. Siva, R: Status of Natural Dyes and Dye-Yielding Plants in India. Current Science, 92, 916-925. (2007).

# A Brief Study on Myxomycetes

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**Abstract**

Myxomycetes, commonly known as slime molds, are a unique group of protists that exhibit characteristics of both fungi and protozoa. They play a crucial role in ecosystems as decomposers, aiding in nutrient cycling. This review provides a comprehensive overview of Myxomycetes, focusing on their taxonomy, ecology, distribution, and research progress in India. Over the years, significant studies have contributed to the identification of diverse species, with approximately 450 species reported from India. Recent advancements in molecular techniques have improved species identification and phylogenetic studies, yet several challenges remain, including limited field surveys and underexplored habitats. This review highlights key research findings, identifies gaps in knowledge, and suggests future research directions, emphasizing the importance of Myxomycetes in ecological studies and conservation efforts.

***Keywords:*** Myxomycetes, Slime Molds, Protozoa, Taxonomy.

## Introduction

Myxomycetes, commonly known as slime molds, are a unique group of protists that exhibit characteristics of both fungi and protozoa. They belong to the class Myxogastria under the phylum Amoebozoa and are known for their complex life cycle, which alternates between an amoeboid plasmodial stage and a spore-producing fruiting body. Found primarily in decaying organic matter, Myxomycetes play a significant role in nutrient cycling and ecosystem functioning. Historically, Myxomycetes were classified with fungi due to their spore-producing structures, but modern molecular studies have placed them closer to amoebozoans. Their distribution spans diverse habitats, including forests, grasslands, and even extreme environments such as deserts and alpine regions. India, with its varied climatic zones and rich biodiversity, provides a suitable environment for Myxomycetes, yet comprehensive studies on their diversity and ecological roles remain limited.

Over the past few decades, research on Myxomycetes in India has significantly advanced, with numerous species being documented. However, challenges persist

in terms of underexplored habitats, seasonal variations affecting their occurrence, and the need for advanced
Molecular techniques for accurate identification. This review aims to consolidate existing research on Myxomycetes, focusing on their taxonomy, ecology, distribution, and significance, while identifying key knowledge gaps and future research directions

**Occurrence and Distribution:** Myxomycetes, commonly known as plasmodia slime molds, are widely distributed throughout the world and occur in a variety of terrestrial ecosystems. They are most commonly found in moist, shaded environments where organic matter is abundant. Myxomycetes occur on decaying plant materials such as leaf litter, rotting wood, bark, soil, and occasionally on living plants, stones, and animal dung.

**Global Distribution:** Myxomycetes have a cosmopolitan distribution and are reported from tropical, subtropical, temperate, and even Polar Regions. However, species richness is generally higher in tropical and subtropical regions due to favourable environmental conditions such as high humidity, moderate temperatures, and dense vegetation. Forest ecosystems, especially tropical rainforests and moist deciduous forests, support the greatest diversity of myxomycetes.

**Habitat Preference:** The occurrence of myxomycetes is closely related to moisture availability and substrate type. Decaying wood and leaf litter are the most preferred substrates, as they provide nutrients and microbial food sources. Bark of living and dead trees also supports many corticolous species. Some myxomycetes are adapted to unusual habitats, including stones, mosses, grasslands, and even arid regions, where they survive unfavourable conditions as spores or sclerotic.

**Seasonal Distribution:** Seasonal variation plays an important role in the distribution of myxomycetes. Fruiting bodies are most commonly observed during the rainy season and immediately after rainfall, when humidity levels are high. In temperate regions, myxomycetes are more abundant during spring and autumn, while in tropical regions, they are most frequently recorded during the monsoon season.

**Distribution in India:** In India, myxomycetes are widely distributed, with significant records from the Western Ghats, Eastern Himalayas, Central India, and north-eastern states. Coastal regions and forested areas with high rainfall support rich myxomycete diversity. However, many regions remain underexplored, and further surveys are necessary to document their true distribution.

**Life Cycle of Myxomycetes**

Myxomycetes (slime molds) exhibit a unique biphasic life cycle that alternates between a unicellular amoeboid stage and a multicellular spore-producing stage. Their life cycle consists of the following key stages.

**1. Spore Stage (Dormant Phase)**

The life cycle begins with spores, which are typically dispersed by wind, water, or animals Spores are resistant structures that remain dormant until favorable environmental conditions (moisture, temperature, and nutrients) trigger germination.

**2. Germination**

When conditions are suitable, the spore germinates by breaking open and releasing a single haploid protoplast. This protoplast develops into one of two forms

a) Myxamoeba (amoeboid form) – moves using pseudopodia.

b) Swarm Cell (flagellated form) – moves in water using flagella.

The ability to switch between these two forms allows Myxomycetes to adapt to different environmental conditions.

**3. Growth and Fusion (Plasmodium Formation)**

Under appropriate conditions, two compatible myxamoebae or swarm cells fuse (syngamy) to form a diploid zygote. The zygote undergoes repeated nuclear division without cell division, leading to the formation of a multinucleated plasmodium.

**4. Plasmodium Stage (Vegetative Phase)**

The plasmodium is the active, feeding stage of Myxomycetes. It moves through cytoplasmic streaming, engulfing bacteria, fungi, and organic matter via phagocytosis. Depending on environmental conditions, plasmodia can be:

I. Protoplasmodium – small and simple.
II. Aphanoplasmodium – transparent and delicate.
III. Phaneroplasmodium – large and well-developed.

**5. Sporulation (Reproductive Phase)**

When environmental conditions become unfavorable, the plasmodium differentiates into fruiting bodies (sporophores), leading to sporulation. The fruiting body develops a peridium (outer covering), capillitium (sterile filaments), and spores within a sporangium.Spores are released and dispersed to restart the cycle.

This alternation between a unicellular and multicellular phase makes Myxomycetes unique among protists. Their ability to switch between amoeboid and plasmodial forms allows them to adapt to various environmental conditions

**Study Methods: It includes following Steps**

**1. Collection:**

The collection of myxomycetes involves several methods depending on their life stages: spores, plasmodia, and fruiting bodies. The most common approach is collecting fruiting bodies (sporocarps), which are easier to identify and preserve.

**A. Direct Collection of Fruiting Bodies:** Myxomycetes are found on decaying wood, leaf litter, bark, soil, and even living plants. Look for them in moist, shaded environments, especially during the rainy season. Carefully collect entire fruiting bodies using forceps or a scalpel. Place them in small paper boxes, envelopes, or herbarium packets to avoid crushing. Label samples with date, location, substrate type, and environmental conditions

**B. Collecting Bark and Litter for Moist Chamber Cultures:** If fruiting bodies are not visible, collect pieces of decaying wood, bark, or leaf litter. Store samples in paper bags to prevent mold growth. Later, use moist chamber cultures in the lab to induce fruiting.

**2. Moist Chamber Culture Method**

This is a standard technique to encourage myxomycetes to fruit under controlled conditions.

**Steps**

I. Place collected bark, leaves, or soil samples in shallow plastic or glass Petri dishes
II. Add a layer of filter paper or tissue to absorb water.
III. Moisten with distilled water and drain excess after 24 hours.
IV. Keep at room temperature in indirect light.
V. Check regularly for myxomycete fruiting bodies, which may appear in 1–4 weeks.

**3. Preservation Methods**

Air-dry specimens in paper envelopes or small boxes. Store in a dry place or a desiccator with silica gel.

**A. Microscopic Slides:** Make permanent slides using Hoyer's medium or PVL (polyvinyl lacto phenol). Mount spores for identification.

**B. Herbarium Storage:** Keep dried specimens in airtight containers. Store at low humidity to prevent mold or insect damage.

## 4. Identification

Use stereomicroscopes and compound microscopes to examine spore size, ornamentation, and capillitium structure. Molecular techniques (e.g., DNA sequencing) are increasingly used for precise identification.

## Literature Review

Research on Myxomycetes in India has a rich history, with significant contributions from various scholars. A comprehensive checklist published in 2012 documented 373 species across 50 genera and 11 families, highlighting the country's diverse Myxomycetes flora. Pioneering work in the early 20th century by researchers like Drake laid the foundation for Myxomycetes studies in India. Later, scientists such as K.S. Thind in North India and V. Agnihothrudu in South India made substantial contributions, describing numerous species and enhancing the understanding of their distribution and ecology.

Ecological studies have examined the distribution patterns of Myxomycetes in relation to environmental factors. For instance, research indicates a higher occurrence of these organisms at altitudes above 500 meters, with temperature ranges between 15–20°C and varying rainfall levels influencing their growth and sporulation. For a detailed historical perspective, the chapter "History and Development of Myxomycetes Research in India" provides an in-depth overview of the progression of studies in this field. These resources collectively offer a thorough understanding of the research conducted on Myxomycetes in India, highlighting significant contributions and developments over the years.

Myxomycetes, commonly known as slime molds, are a group of fungus-like organisms that have been the subject of extensive research in India. The study of these organisms in the country can be categorized into three distinct periods:

**Period I (up to 1950):** Research began with A. Drake's collections between 1911 and 1927, leading to the first published records and monographs on Indian Myxomycetes. This foundational work laid the groundwork for future studies.

**Period II (1952–1980):** This era witnessed significant research activity across various regions of India. Notably, Thind and his students published a series of 29 research papers between 1955 and 1980, describing 170 species, including 17 new species, 2 new varieties, and 4 new forms. Thind also published the first comprehensive monograph on 'Myxomycetes of India', detailing 186 species, 3 varieties, and 6 forms up to 1973. Additionally, six doctoral theses focusing on Myxomycetes were produced during this period.

**Period III (1980 – 2000):** Research continued with the publication of another monograph in 1981, describing 293 species of Myxomycetes. During this time, four individuals received doctorates on different aspects of Myxomycetes, further

contributing to the field.

**Period III (2000 onwards):** Temperature, humidity, rainfall, and topography are the main factors that determine the distribution of Myxomycetes. An assessment of myxomycete species diversity in India is still restricted to few places (Ranade et al. 2012) and only few papers have been published recently (Hashmi et al. 2020). Ranade et al. (2012) provided the check list of myxomycetes from India. In this checklist about the 373 species, 17 varieties and 4 forms within 50 genera, 11 families and 6 orders of myxomycetes were reported. Ranadive et al. (2017) developed the first online database of Myxomycetes from India. This database contains 394 records from 11 families, 50 genera and 351 species, and can be accessed at www.fungifromindia.com.

Most of the studies reported from the India are based on herbarium collections of myxomycete fruiting bodies (sporocarps) collected over decades in the field while the studies based on moist chamber culture technique is scarce.

Tembhurne and Nanir (2013) during their floristic study of the myxomycetes of South-West of Maharashtra which include the Solapur, Satara, Sangli and Kolhapur discussed four species namely *Lepidodermopsis leonina, L. martini, Physarina echinospora* and *Comatrichia aequalis*. Hashmi et al. 2020 recently published their work on myxomycetes from Jammu and Kashmir. Chimankar (2022) studied the myxomycetes from Jalgaon district of Maharashtra. He explained *Lamproderma scintillans* and *Stemonitis flavoginata* being described for the first time from the Jalgaon district.

Tetiana et al. (2020) provided checklist which contains 143 species and infra-specific taxa of myxomycetes representing six orders, 12 families and 29 genera known from the Seychelles Islands. Carlos Lado and de Basanta (2008) evaluated richness of myxobiota in different countries and gaps in current information and unexplored areas.

Since 2000, research on myxomycetes, or plasmodial slime molds, has advanced significantly, particularly in the areas of molecular phylogenetics, biodiversity, and ecological roles.

**Molecular Phylogenetics and Classification**

Advancements in molecular techniques have reshaped the understanding of myxomycete taxonomy and evolutionary relationships. Studies utilizing small-subunit ribosomal DNA sequences and elongation factor EF-1A gene analyses have provided insights into their phylogenetic placement. For instance, research by Baldauf and Doolittle (1997) and Baldauf et al. (2000) indicated that myxomycetes belong to the phylum Amoebozoa, aligning them more closely with amoeboid organisms than with fungi.

**Biodiversity and Biogeography**

Field studies have expanded knowledge of myxomycete diversity across various ecosystems. Investigations in tropical forests, such as those in Costa Rica, revealed unique assemblages of species associated with specific microhabitats, differing from patterns observed in temperate regions. Research by Schnittler and Stephenson (2000) highlighted these distinct ecological patterns. In regions like Vietnam, systematic reviews have documented 173 myxomycete species, including six new species discovered since 2009, underscoring the importance of ongoing biodiversity assessments in understudied areas.

**Ecological Roles**

Myxomycetes play crucial roles in ecosystems, particularly in nutrient cycling and as indicators of environmental health. Their presence and diversity can reflect ecological conditions, making them valuable in studies of ecosystem dynamics.

**Recent Developments**

A 2023 review by Stephenson emphasized the historical context and ongoing field-based studies of myxomycetes, highlighting the progression from early discoveries to current research initiatives. Overall, post-2000 research has significantly enhanced the understanding of myxomycetes, integrating molecular data with ecological and biogeographical studies to provide a comprehensive view of their diversity and function in various ecosystems.

For a comprehensive review of Myxomycetes research in India, the chapter titled "History and Development of Myxomycetes Research in India" provides an in-depth historical account and is a valuable resource for understanding the progression of studies in this field. Additionally, the "Checklist of Myxomycetes from India" offers a detailed list of species documented up to its publication and can serve as a reference for researchers interested in the diversity of Myxomycetes in the country.

These resources collectively offer a thorough overview of the research conducted on Myxomycetes in India, highlighting significant contributions and developments over the years

**Diversity and Taxonomy of Myxomycetes in India**

India is rich in diversity of Myxomycetes, with studies documenting over 373 species distributed across 50 genera and 11 families. The highest diversity has been recorded in forested ecosystems, particularly in the Western Ghats, Eastern Himalayas, and the Northeastern states, which provide the optimal humid conditions for their growth. Many species remain classified based solely on morphological characteristics, highlighting the need for further integrative

taxonomic studies.

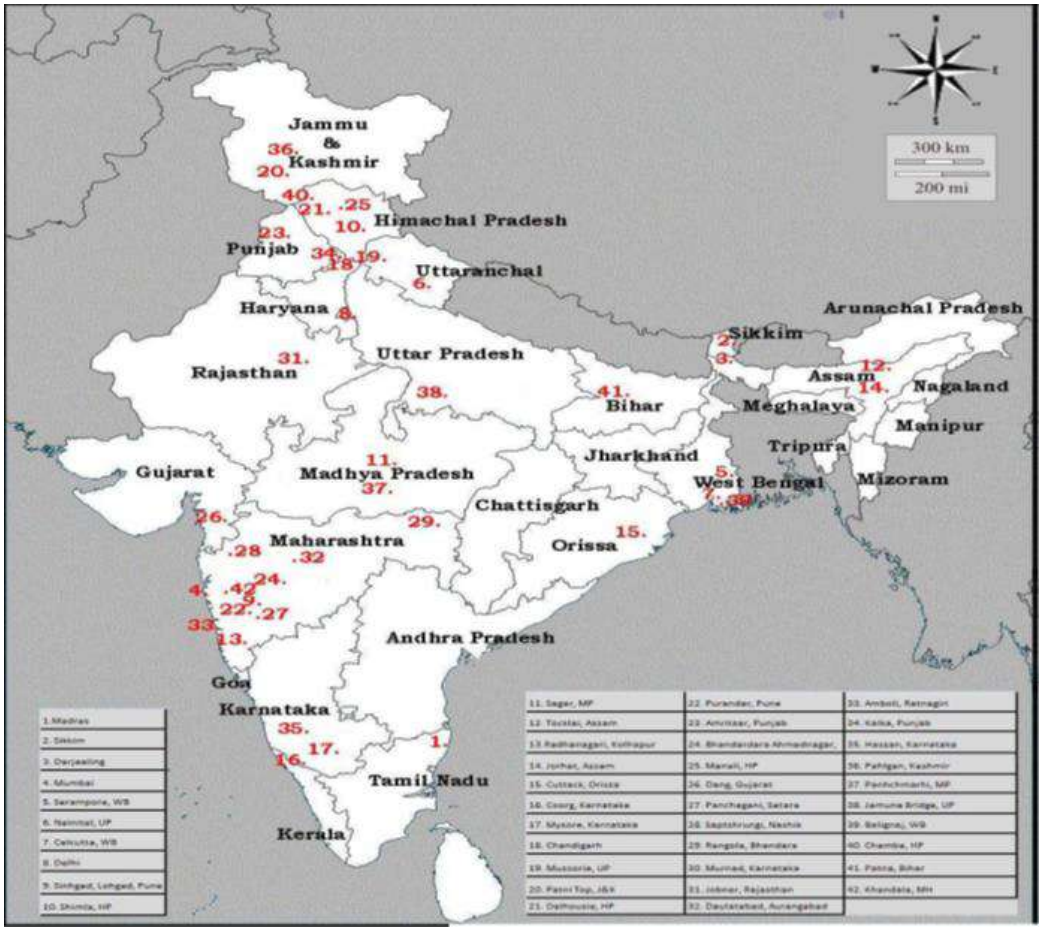


***Fig. Study Areas in India***

## *References*

1. Agnihothrudu V. 1968. Some slime moulds of Southern India – 10. Sydowia, 22:179-182.
2. Bhide V.P., Sathe A.V., Pande Alaka, Patwardhan P.G. and Rao V.G. 1987. Fungi of Maharashtra Supplement I. Maharashtra Association for the Cultivation of Science, Pune – 411004, 1-2.
3. Bilgrami K.S., Jamaluddin S. and Rizwi M.A. 1979. Fungi of India Part – I, List and References. Today & Tomorrow's Printers and Publishers, New Delhi – 110005, 1–467.
4. Bisby G.R. 1914. Some observations on the formation of capillitium and the development of Physarella mirabilis and Stemonitis fusca Roth. American Journal of Botany. 1: 274-288.

5. Bruhl P. and Gupta J.S. 1927. Indian Slime Fungi (Myxomycetes or Mycetozoa)- First contribution. Journal Department of Science, University of Calcutta. 8:101-122.
6. Butler E.J and Bisby G.R. 1931. The Fungi of India. Imperial Council of Agriculture Research, India Science Monograph, 1. Calcutta, 1-237.
7. Butler EJ, Bisby GR revised by RS Vasudeva 1960 – The Fungi of India, Indian Council of Agricultural Research, New Delhi, 1–552.
8. Carlos Rojas, Martin Schnittler & Steven L. Stephenson. 2010. A review of the Costa Rican myxomycetes (Amebozoa). Brenesia. 73-74: 39-57.
9. Chavan P.D. and Kulkarni U.V. 1974. Additions to the fungi from Maharashtra, India. Maharashtra Vidyan Mandir Patrika. 9:132–139.
10. G. B. Chibhade, V. B. Mulye, A. S. Mohite, S. S. Sawant, M. T. Sharangdhar, V. V. Bansode and J. M. Koli. 2020. Soil and Water quality parameters of freshwater fish farms of Raigad District of Maharashtra. J. Exp. Zool. India. 23(1): 883-889.
11. Wrigley de Basanta, D., Lado, C., Estrada-Torres, A. et al. 2013. Biodiversity studies of myxomycetes in Madagascar. Fungal Diversity. 59:55–83.
12. Ghosh G.R. and Datta B.G. 1962a. Myxomycetes from Orissa (India)- I. Mycopathologia. 16:165–176.
13. Ghosh G.R. and Datta B.G. 1962b. Myxomycetes from Orissa (India)- II. Mycopathologia. 16: 209–218.
14. Ghosh G.R. and Datta B.G. 1962c. Myxomycetes from Orissa. Proceeding 49th Indian Science Congress, Part III, 243.
15. Ghosh G.R. and Datta B.G. 1963. Myxomycetes from Orissa (India)- III. Mycopathologia. 19:271–282.
16. Hashmi, S.A.J., Sharma, R. and Sharma, Y.P., 2020. Some interesting myxomycetes of Jammu and Kashmir, India. Indian Journal of Forestry, 43(2):178-182.
17. Indira P.U. 1968a. Some slime molds from Southern India - VIII. Journal of Indian Botanical Society. 47:155–186.
18. Indira P.U. 1968b. Some slime molds from Southern India - IX. Distribution, habitat and variation. Journal of Indian Botanical Society. 47:330-340.
19. Indira P.U. 1975. Some slime molds of Southern India -XI. Kavaka. 3:41–54.
20. Jagtap A.P. and Singh N.P. 2002. Biodiversity of The Western Ghats of Maharashtra-Current Knowledge. Published by Bishen Singh Mahendra Pal Singh, Dehradun, Appendix XXV, 339–342.
21. Kar A. 1964. Myxomycetes of Calcutta Suburbs. Indian Phytopathology 17: 222–223.
22. Karlekar S.N. and Thakurdesai. 2017. Geomorphological field guide book on Konkan and Goa coasts (Edited by Amal Kar). Indian Institute of

Geomorphologists, Allahbad.
23. Kaur S. and Mukherji K.G. 1996. Arcyria rufosa: A new Indian Myxomycetes. Mycotaxon. 59:479 – 481.
24. Kowalski D.T. and Lakhanpal T.N. 1973. A new species of Didymium from India. Mycologia. 65:474–477.
25. Lakhanpal T.N. 1966. Taxonomic studies on the Mxyomycetes of Simla and Kulu Hills (Himachal Pradesh). M. Sc. Thesis, Punjab University, Chandigarh.
26. Lado C. & Wrigley de Basanta D. 2008. A Review of Neotropical Myxomycetes (1828-2008). Anales Jard. Bot. Madrid 65(2): 211-254.
27. Lakhanpal T.N. 1978. Taxonomic studies on the Indian Myxomycetes – XVIII. Two species of Myxomycetes and a reappraisal of the genus Lepidodermopsis. Norwegian Journal of Botany. 25:196.
28. Lakhanpal T.N. and Mukherji K.G. 1981. Taxonomy of the Indian Myxomycetes. J. Cramer. FL – 9490 VADUZ, 01–411.
29. Lister G. 1924. Mycetozoa from North India. Journal of Botany. 62:16–20.
30. Mishra R.L. and Ranade V.D. 1979. Mxyomycetes of Maharashtra - II. The genus Diderma. Maharashtra Vidnyan Mandir Patrika. 14:33–41.

# Marine Bacteria for Bioremediation of Polluted Marine Environments: A Blue Revolution Approach

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## Abstract

Escalating contamination of marine ecosystems by hydrocarbons, heavy metals, xenobiotics, and microplastics threatens ecosystem stability and public health, while conventional remediation approaches remain costly, inefficient, or ecologically disruptive. This paper explores the potential of marine bacteria in the bioremediation of polluted marine environments, aligning with the goals of the Blue Revolution. This approach moves beyond traditional reliance on naturally occurring bioremediation rates by employing targeted bacterial insights to enhance the metabolic capabilities of marine microorganisms. Bioremediation is a process used to clean up contaminated sites using biological systems. Microorganisms capable of degrading most constituents of crude oil and xenobiotics are present in the marine environment. Generally, consortia of microorganisms are necessary to degrade complex molecules in pollutants. Most bioremediation technologies involve naturally occurring microorganisms and the improvement of nutrient conditions and environmental factors to enhance biodegradation. The Blue Revolutionary Strategy promises sustainable use of marine resources for ecosystem benefit. Marine bacteria show altered metabolism as a strategy against metal-induced stress. Understanding these strategies to avoid toxic effects of heavy metals can help devise novel biotechnological applications for ocean clean-up. Using biological tools for remediation has advantages, as it does not involve harmful chemicals and shows greater flexibility to environmental fluctuations. This research will contribute significantly to achieving sustainable marine resource management goals, fostering a healthier and more productive marine environment.

***Keywords:*** Bioremediation, blue revolution, marine bacteria, sustainable marine resource, biodegradation.

## Introduction

Microorganisms are widely distributed in the biosphere because they can grow easily in a wide range of environmental conditions. The nutritional versatility of microorganisms can also be exploited for biodegradation of pollutants, a process termed bioremediation. It is based on the ability of certain microorganisms to convert, modify, and utilize toxic pollutants to obtain energy and produce biomass (Tang, Criddle, & Leckie, 2007). It is regarded as a more economical, environmentally friendly, and scalable technology than traditional physicochemical treatment techniques such as pump and treat, soil vapour extraction, air sparging, chemical oxidation, and thermal desorption. The method depends on promoting the growth of specific microorganisms that use pollutants as a food and energy source. This can be accomplished using techniques like biostimulation, which offers required nutrients and conditions for current microorganisms to flourish, and bioaugmentation, which involves introducing appropriate microbes to the environment. The process of bioremediation is based on promoting the growth of specific microorganisms that use pollutants such as oil, solvents, and pesticides as food and energy sources. These microorganisms break down pollutants into innocuous gasses like carbon dioxide and small volumes of water. Proper temperature, nutrients, and food must all be present for bioremediation to occur. Lack of these components could prolong the clean-up process. Environmental remediators such as molasses, vegetable oil, or air might be added to enhance unfavorable conditions for bioremediation (He & Su, 2015).

## Objective

The primary objective of this review is to evaluate the role and mechanisms of marine bacteria in the bioremediation of polluted marine environments, focusing on hydrocarbons, heavy metals, and microplastics. Additionally, this paper aims to discuss strategies such as bioaugmentation and biostimulation to enhance bioremediation efficiency, contributing to sustainable marine resource management under the Blue Revolution framework

## Data and Methodology

This paper is based on a comprehensive review of existing scientific literature pertaining to marine bioremediation. Databases such as Google Scholar, PubMed, and Science Direct were searched using keywords including “marine bacteria bioremediation,” “hydrocarbon degradation,” “heavy metal biosorption,” “microplastic biodegradation,” “bioaugmentation,” and “biostimulation.” Articles from peer-reviewed journals published between 2000 and 2023 were selected.

Data from these studies were synthesized to discuss bacterial species, mechanisms, and strategies for effective marine bioremediation.

**Types of Marine Bacteria and Target Pollutants in Bioremediation**:
The ocean is home to a plethora of microorganisms such as bacteria, fungi, microalgae, and cyanobacteria. They are important components of the marine ecosystem, performing several services like food production, decomposition of organic matter, nutrient cycling, and breaking down harmful chemicals (Pandolfo, Caracciolo, & Rolando, 2023). Carbon is the most important nutrient for microorganisms. Microbes from a variety of environments are used for bioremediation, such as Achromobacter, Alcaligenes, Xanthobacter, Arthrobacter, Pseudomonas, Bacillus, Mycobacterium, Corynebacterium, Flavobacterium, and Nitrosomonas (Sharma et al., 2021).

**Heavy Metals**
Heavy metals cannot be destroyed biologically (no degradation; changes occur in the nuclear structure of the element) but can be transformed from one oxidation state or organic complex to another. Bacteria are efficient in heavy metal bioremediation. Microorganisms have developed capabilities to protect themselves from heavy metal toxicity through various mechanisms such as adsorption, uptake, methylation, oxidation, and reduction. They uptake heavy metals actively (bioaccumulation) and/or passively (adsorption). Microbial methylation plays an important role because methylated compounds are frequently volatile. For example, mercury (Hg II) can be biomethylated by bacterial species such as Alcaligenes faecalis, Bacillus pumilus, Bacillus sp., Pseudomonas aeruginosa, and Brevibacterium iodinium to gaseous methyl mercury (Jaysankar, Ramaiah, & Vardanyan, 2008).

**Hydrocarbons**
Widespread hydrocarbon pollution of marine systems, particularly in coastal regions with significant anthropogenic pressure, has prompted studies to determine which bacterial taxa are most efficient in eliminating these pollutants (Dell'Anno et al., 2021). Petroleum and its derivatives are complex mixtures of hydrocarbons, and no bacterial species can degrade all components. Bacteria exhibit hydrocarbon specificity depending on the enzymes they produce to metabolize specific hydrocarbon components (Gao et al., 2021; Rajasekar et al., 2007). For instance, *Alcanivorax* sp. strain 24 can break apart alkanes by producing enzymes such as oxygenase and dehydrogenase, converting hydrocarbons into fatty acids, which are then metabolized into carbon dioxide and water (Xue, Yu, Bai, Wang, & Wu, 2015; Zadjelovic, Gibson, Dorador, & Christie-Oleza, 2020). Polycyclic aromatic hydrocarbons (PAHs) are another component degraded by bacteria such as *Cycloclasticus* sp. strain 78-ME using

oxygenase and hydrolase enzymes (Xue et al., 2015; Messina et al., 2016).

**Microplastics and Nanoplastics**

Micro/nanoplastic particles have primary and secondary sources. Primary microplastics come from industrial and domestic sources, including personal hygiene products and laundry fibers. Secondary microplastics result from the breakdown of macroscopic oceanic plastic debris (Zicarelli, Multisanti, Falco, & Faggio, 2022; Li, Tse, & Fok, 2016). Bacteria such as *Muricauda sp., Pelomonas sp., Sphingomonas sp., Acinetobacter sp., Staphylococcus epidermidis*, and *Thalassospira sp.* have shown degradation activity toward polyethylene (Debroas, Mone, & Ter Halle, 2017). Microplastics cause malnutrition, inflammation, chemical poisoning, growth thwarting, decreased fecundity, and death in marine life. Nanoplastics can cross biological barriers, leading to bioaccumulation in vital organs (Peng & Fu, 2020).

**Bacterial Mechanisms of Bioremediation**

**Enzymatic Degradation**

Marine bacteria play a significant role in the degradation of organic materials. Species such as Pseudomonas, Shewanella, and Marinobacter have shown promise in plastic degradation (Auta, Emenike, Jayanthi, & Fauziah, 2018). Enzymes produced by marine microorganisms are key to plastic biodegradation, targeting specific bonds in plastic polymers and facilitating breakdown into smaller, less harmful components. For example, Alcanivorax sp. produces enzymes that degrade polystyrene (Zhang et al., 2022). Enzymatic degradation typically occurs through hydrolysis, oxidation, or cleavage of chemical bonds. Esterases and lipases catalyze hydrolysis of ester bonds, while proteases cleave peptide bonds in protein-based plastics. Integrating plastic-degrading enzymes into recycling technologies offers advantages such as increased efficiency, reduced energy consumption, and ability to process mixed plastic waste (Zadjelovic et al., 2022).

**Biosorption**

Microorganisms use their cellular structure to absorb heavy metal ions, which are sorbed onto binding sites of the cell wall in a process known as biosorption (Malik, 2004). This is a passive uptake mechanism independent of the metabolic cycle. Biosorption is an effective detoxification method for removing heavy metals at low concentrations. The process depends on pH and involves modulation of the isoelectric point, impacting overall negative charge and changes in ligands' ionic state, such as carboxyl, phosphate, sulfhydryl, and amino groups (Sağ, Özer, & Kutsal, 1995).

**Bioaccumulation**

Bioaccumulation refers to ingestion and subsequent accumulation of toxic substances in organism tissues, while biomagnification transfers toxins across trophic levels. Some compounds can be degraded by digestive processes, while others persist. Investigations into heavy metal bioaccumulation are essential. Heavy metals like Cu and Zn are essential cofactors but become toxic at high concentrations. Assessing their concentration in surface waters and bioaccumulation capacity using bioindicators such as Mytilus galloprovincialis is important (Boudjema, Badis, & Moulai-Mostefa, 2022).

**Biotransformation**

Biotransformation involves structural modification of chemical compounds into more polar molecules (Asha & Vidyavathi, 2009). In microbial bioremediation, hazardous metals and organic molecules are transformed into less dangerous forms. Microbial transformations include oxidation, reduction, hydrolysis, methylation, and demethylation. Bacteria and fungi possess enzymatic systems that catalyze biotransformation of heavy metals, aiding in environmental detoxification (Pande et al., 2022; Kapahi & Sachdeva, 2019).

**Strategies and Techniques for Enhancing Bioremediation**

Bioremediation strategies can be divided into bioaugmentation (addition of hydrocarbon-degrading biomass) and biostimulation (addition of nutrients to stimulate indigenous degraders) (Tyagi, da Fonseca, & de Carvalho, 2011). Microorganisms are central to both processes (Abatenh, Gizaw, Tsegaye, & Wassie, 2017). Direct introduction of microbes may fail due to environmental stresses such as extreme temperature, pH, salinity, and pollutant toxicity (Paniagua-Michel, 2015). These issues may be overcome by modifying selected microorganisms to enhance bioaugmentation effectiveness.

**Bioaugmentation**

Bioaugmentation involves adding pollutant-degrading microorganisms (natural, exotic, or engineered) to enhance the biodegradative capacity of indigenous populations. Microbes are collected from the site, cultured, genetically modified, and reintroduced. For example, in sites contaminated with chlorinated ethenes, bioaugmentation ensures complete degradation to non-toxic ethylene and chloride (Niu et al., 2009). Genetically modified microorganisms can increase degradative efficiency for a wide range of pollutants (Malik & Ahmed, 2012). Engineered microbes degrade pollutants faster and compete effectively with indigenous species (Sayler & Ripp, 2000; Thapa, Kumar, & Ghimire, 2012).

**Biostimulation**

Biostimulation involves injecting specific nutrients at the site to stimulate

indigenous microorganisms. This includes supplying fertilizers, growth supplements, trace minerals, and optimizing environmental conditions like pH, temperature, and oxygen (Adams, Fufeyin, Okoro, & Ehinomen, 2015; Kumar, Bisht, Joshi, & Dhewa, 2011). Small amounts of pollutants can also act as stimulants by turning on operons for bioremediation enzymes. Nutrients such as nitrogen, phosphorus, and carbon are essential for microbial energy, biomass, and enzyme production (Madhavi & Mohini, 2012).

**Bioattenuation**

Bioattenuation or natural attenuation involves eradication of pollutants through biological processes (aerobic/anaerobic biodegradation, plant/animal uptake), physical phenomena (advection, dispersion, dilution, volatilization, sorption), and chemical reactions (ion exchange, complexation) (Mulligan & Yong, 2004). Natural attenuation mechanisms include microbial digestion, sorption to soil, dilution, and volatilization. If natural attenuation is insufficient, bioremediation can be enhanced via biostimulation or bioaugmentation (Li, Wong, & Tam, 2010).

**Results and Discussion**

Marine bacteria demonstrate significant potential for bioremediation of diverse pollutants through mechanisms such as enzymatic degradation, biosorption, bioaccumulation, and biotransformation. Species like Alcanivorax, Pseudomonas, and Cycloclasticus exhibit specialized metabolic pathways for hydrocarbon degradation, while others like Bacillus and Alcaligenes mediate heavy metal detoxification. Microplastic degradation by bacteria such as Muricauda and Alcanivorax highlights emerging bioremediation avenues. Strategies like bioaugmentation and biostimulation enhance remediation efficiency but face challenges such as environmental stress and competition with indigenous species. Genetically modified microorganisms offer promising solutions but require careful ecological risk assessment. The Blue Revolution framework aligns with these biological approaches, promoting sustainable marine resource management. However, successful large-scale implementation requires integrated policies, monitoring, and community engagement.

**Conclusion**

Marine bacteria offer a sustainable, cost-effective solution for remediating polluted marine environments. Their diverse metabolic capabilities enable degradation of hydrocarbons, heavy metals, and emerging contaminants like microplastics. Strategies such as bioaugmentation and biostimulation can optimize bioremediation processes, supporting the goals of the Blue Revolution. Future research should focus on genetic engineering, field-scale applications, and

ecological impact assessments to harness the full potential of marine bacteria for ecosystem restoration and sustainable marine resource management.

***References***


1. Abatenh, E., Gizaw, B., Tsegaye, Z., & Wassie, M. (2017). Application of microorganisms in bioremediation-review. Journal of Environmental Microbiology, 1, 1-8.
2. Adams, G. O., Fufeyin, P. T., Okoro, S. E., & Ehinomen, I. (2015). Bioremediation, biostimulation and bioaugmentation: A review. International Journal of Environmental Bioremediation & Biodegradation, 3(1), 28-39.
3. Asha, S., & Vidyavathi, M. (2009). Cunninghamella—a microbial model for drug metabolism studies—a review. Biotechnology Advances, 27(1), 16-29.
4. Auta, H. S., Emenike, C. U., Jayanthi, B., & Fauziah, S. H. (2018). Growth kinetics and biodeterioration of polypropylene microplastics by Bacillus sp. and Rhodococcus sp. isolated from mangrove sediment. Marine Pollution Bulletin, 127, 15-21.
5. Boudjema, K., Badis, A., & Moulai-Mostefa, N. (2022). Study of heavy metal bioaccumulation in Mytilus galloprovincialis (Lamark 1819) from heavy metal mixture using the CCF design. Environmental Technology & Innovation, 25, 102202.
6. Debroas, D., Mone, A., & Ter Halle, A. (2017). Plastics in the North Atlantic garbage patch: A boat-microbe for hitchhikers and plastic degraders. Science of the Total Environment, 599-600, 1222-1232.
7. Dell'Anno, F., Rastelli, E., Tangherlini, M., Corinaldesi, C., Brunet, C., Balzano, S., ... & Dell'Anno, A. (2021). Highly contaminated marine sediments can host rare bacterial taxa potentially useful for bioremediation. Frontiers in Microbiology, 12, 584850.
8. Gao, J., Ming, J., Xu, M., Fu, X., Duan, L. F., Xu, C. C., ... & Xiao, X. F. (2021). Isolation and characterization of a high-efficiency marine diesel oil-degrading bacterium. Petroleum Science, 18 (2), 641–653.
9. He, Y. T., & Su, C. (2015). Use of additives in bioremediation of contaminated groundwater and soil. Advances in Bioremediation of Wastewater and Polluted Soil. https://doi.org/10.5772/60915
10. Jaysankar, D., Ramaiah, N., & Vardanyan, L. (2008). Detoxification of toxic heavy metals by marine bacteria highly resistant to mercury. Marine Biotechnology, 10(4), 471-477.
11. Kapahi, M., & Sachdeva, S. (2019). Bioremediation options for heavy metal pollution. Journal of Health and Pollution, 9(24), 191203.
12. Kumar, A., Bisht, B. S., Joshi, V. D., & Dhewa, T. (2011). Review on bioremediation of polluted environment: A management tool. International Journal of Environmental Sciences, 1(6), 1079-1093.

13. Li, C. H., Wong, Y. S., & Tam, N. F. (2010). Anaerobic biodegradation of polycyclic aromatic hydrocarbons with amendment of iron (III) in mangrove sediment slurry. Bioresource Technology, 101(21), 8083–8092.
14. Li, W. C., Tse, H. F., & Fok, L. (2016). Plastic waste in the marine environment: A review of sources, occurrence and effects. Science of the Total Environment, 566, 333–349.
15. Madhavi, G. N., & Mohini, D. D. (2012). Review paper on parameters affecting bioremediation. International Journal of Life Science and Pharma Research, 2(1), 77-80.
16. Malik, A. (2004). Metal bioremediation through growing cells. Environment International, 30(2), 261–278.
17. Malik, Z. A., & Ahmed, S. (2012). Degradation of petroleum hydrocarbons by oil field isolated bacterial consortium. African Journal of Biotechnology, 11(3), 650–658.
18. Messina, E., Denaro, R., Crisafi, F., Smedile, F., Cappello, S., Genovese, M., ... & Yakimov, M. M. (2016). Genome sequence of obligate marine polycyclic aromatic hydrocarbons-degrading bacterium Cycloclasticus sp. 78-ME, isolated from petroleum deposits of the sunken tanker Amoco Milford Haven, Mediterranean Sea. Marine Genomics, 25, 11–13.
19. Mulligan, C. N., & Yong, R. N. (2004). Natural attenuation of contaminated soils. Environment International, 30(5), 587–601.
20. Niu, G. L., Zhang, J. J., Zhao, S., Liu, H., Boon, N., et al. (2009). Bioaugmentation of a 4-chloronitrobenzene contaminated soil with Pseudomonas putida ZWL73. Environmental Pollution, 157(3), 763–771.
21. Pande, V., Pandey, S. C., Sati, D., Bhatt, P., & Samant, M. (2022). Microbial interventions in bioremediation of heavy metal contaminants in agroecosystem. Frontiers in Microbiology, 13, 824084.
22. Pandolfo, E., Barra Caracciolo, A., & Rolando, L. (2023). Recent advances in bacterial degradation of hydrocarbons. Water, 15(2), 375.
23. Paniagua-Michel, J. (2015). Bioremediation of petroleum contaminants in marine environments: An overview. Journal of Bioremediation & Biodegradation, 6(5), 273.
24. Peng, L., & Fu, D. (2020). Micro- and nano-plastics in marine environment: Source, distribution and threats—A review. Science of the Total Environment, 698, 134254.
25. Rajasekar, A., Babu, T. G., Pandian, S. T. K., Maruthamuthu, S., Palaniswamy, N., & Rajendran, A. (2007). Role of Serratia marcescens ACE2 on diesel degradation and its influence on corrosion. Journal of Industrial Microbiology and Biotechnology, 34(9), 589–598.

26. Sağ, Y., Özer, D., & Kutsal, T. (1995). A comparative study of the biosorption of lead (II) ions to Z. ramigera and R. arrhizus. Process Biochemistry, 30(2), 169–174.
27. Sayler, G. S., & Ripp, S. (2000). Field applications of genetically engineered microorganisms for bioremediation processes. Current Opinion in Biotechnology, 11(3), 286–289.
28. Sharma, P., Pandey, A. K., Kim, S. H., Singh, S. P., Chaturvedi, P., & Varjani, S. (2021). Critical review on microbial community during in-situ bioremediation of heavy metals from industrial wastewater. Environmental Technology & Innovation, 24, 101826.
29. Tang, C. Y., Criddle, C. S., & Leckie, J. O. (2007). Effect of flux (transmembrane pressure) and membrane properties on fouling and rejection of reverse osmosis and nanofiltration membranes treating perfluorooctane sulfonate containing wastewater. Environmental Science & Technology, 41(6), 2008-2014.
30. Thapa, B., Kumar, K. C., & Ghimire, A. (2012). A review on bioremediation of petroleum hydrocarbon contaminants in soil. Kathmandu University Journal of Science, Engineering and Technology, 8(1), 164-170.
31. Tyagi, M., da Fonseca, M. M. R., & de Carvalho, C. C. C. R. (2011). Bioaugmentation and biostimulation strategies to improve the effectiveness of bioremediation processes. Biodegradation, 22(2), 231–241.
32. U.S. Environmental Protection Agency. (n.d.). Bioremediation: Overview. Retrieved from https://clu-in.org/techfocus/default.focus/sec/Bioremediation/cat/Overview/
33. Xue, J., Yu, Y., Bai, Y., Wang, L., & Wu, Y. (2015). Marine oil-degrading microorganisms and biodegradation process of petroleum hydrocarbon in marine environments: A review. Current Microbiology, 71(2), 220–228.
34. Zadjelovic, V., Gibson, M. I., Dorador, C., & Christie-Oleza, J. A. (2020). Genome of Alcanivorax sp. 24: A hydrocarbon degrading bacterium isolated from marine plastic debris. Marine Genomics, 49, 100686.
35. Zadjelovic, V., Erni-Cassola, G., Obrador-Viel, T., Lester, D., Eley, Y., Gibson, M. I., & Christie-Oleza, J. A. (2022). A mechanistic understanding of polyethylene biodegradation by the marine bacterium Alcanivorax. Journal of Hazardous Materials, 436, 129278.
36. Zhang, K., Hu, J., Yang, S., Xu, W., Wang, Z., Zhuang, P., & Luo, Z. (2022). Biodegradation of polyester polyurethane by the marine fungus Cladosporium
37. Zicarelli, G., Multisanti, C. R., Falco, F., & Faggio, C. (2022). Evaluation of toxicity of Personal Care Products (PCPs) in freshwaters: Zebrafish as a model. Environmental Toxicology and Pharmacology, 94, 103923.

# The Mosquito Repellents Are Silent Environmental Chemical Toxicant to the Human Health

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## Abstract

Mosquitoes are major carriers of various diseases and pests, causing millions of deaths worldwide each year due to mosquito-borne illnesses. The use of insect repellents significantly reduces the risk of mosquito bites. To protect against mosquitoes and related diseases, several types of repellents—such as liquid vaporizers, coils, and mats—have been widely used. However, the current reliance on synthetic pesticides to control insects and other arthropods poses serious health risks to humans and has adverse effects on the environment. Given that complications from mosquito bites can be life-threatening, it is essential to explore safer and more sustainable control methods. Consequently, there has been growing interest in alternative strategies, particularly plant-based mosquito repellents. This review will highlight the potential side effects of excessive and uncontrolled use of conventional insect repellents and discuss alternative, eco-friendly solutions for mosquito control.

***Keywords:*** Humans, mosquito repellents, Diseases, potential toxicity, health hazards, strategies.

## Introduction

Globally, illnesses transmitted by insects pose a significant public health issue, especially in tropical and subtropical areas. Various diseases, including yellow fever, dengue hemorrhagic fever, malaria, different forms of encephalitis, West Nile virus, and filariasis, are transmitted by mosquitoes [1]. A report from the World Health Organization (WHO) indicated that malaria resulted in 247 million cases and 619,000 fatalities in 2021, with little advancement in malaria control efforts [2]. Diseases carried by mosquitoes threaten more than 40% of the global population and are increasingly recognized as a serious health challenge worldwide [3]. Dengue has become significantly more prevalent across the globe, with numerous documented epidemic regions [4]. Additionally, several major outbreaks affecting 89 countries and territories have recently emerged due to Zika, a newly identified mosquito-borne disease associated with neurological issues [5]. Furthermore, there are currently no effective treatments or vaccines available to the public for diseases such as dengue and Zika, and traditional insecticide-based strategies remain the primary approach for vector control [6].

A recent study indicates that mosquito species are likely to continue their global spread in the coming decades, placing approximately 50% of the world's population at risk of contracting a virus from mosquito-borne diseases by the year 2050 [7]. To mitigate the mosquito population, especially in residential zones, individuals utilize chemical insecticides in various forms, including creams, coils, vaporizers, and mats. In recent years, there has been a significant increase in insecticide resistance among mosquito populations [8, 9]. The extensive use of insecticides for mosquito control has led to environmental pollution and adverse effects on various organisms [10]. In India, a significant portion of the population lives in regions where malaria poses a threat. Similarly, dengue fever, which is endemic in around 112 countries globally and predominantly impacts tropical and subtropical urban areas, is increasing. Approximately 3.5 billion individuals are at risk of contracting the virus, particularly in Indian metropolitan regions. Other vector-borne diseases, such as Japanese encephalitis, lymphatic filariasis, and chikungunya, are also significant concerns in certain areas of India [11-17].

India's climate creates an ideal environment for mosquito reproduction. It is impossible to completely eliminate mosquitoes from the country. In this context, insect repellents prove to be beneficial. The insect repellent market in India is estimated to exceed Rs 4 billion. Families with young children frequently utilize personal insect repellents. These repellents are available in various forms, such as oils, patches, sprays, and creams. Although the immediate effects of using mosquito repellents may not be obvious, prolonged exposure to smoke and odors

can pose serious health risks to individuals. Diseases transmitted by mosquitoes not only inflict physical pain and fatalities but also lead to significant economic repercussions, reduced productivity, and a decline in quality of life [18]. Mosquitoes are responsible for spreading diseases that affect humans, as well as several illnesses and parasites that are particularly harmful to dogs and horses [19]. Outbreaks of these diseases can instill fear, resulting in job loss and decreased productivity due to illness, disability, and death [20-21].

Repellents cannot entirely prevent mosquito bites; however, they can reduce an individual's attractiveness to mosquitoes [22]. The effects of these repellents may last for 6 to 8 hours, while liquid mosquito incense can endure for up to 60 days [23]. Many individuals apply repellents daily without fully comprehending the ingredients or the necessary safety measures [24]. A single mosquito coil releases fine particulate matter comparable to that of 75 to 137 cigarettes [25]. The combustion of mosquito repellents can emit harmful pollutants, including particulate matter, carbon monoxide (CO), volatile organic compounds (VOCs), sulfur dioxide (SO2), and nitrogen dioxide (NO2) [26-29]. The persistent or prolonged use of various repellents (for at least 8 to 10 hours daily) can lead to acute respiratory infections such as colds, asthma attacks, and pneumonia [30]. Numerous studies have been conducted on diseases transmitted by mosquitoes and the associated health risks of using repellents [31-35]. Mosquitoes act as vectors for a variety of human diseases caused by protozoa, helminthes, and viruses [36-37]. Mosquito-borne diseases represent a significant threat to over 80% of the global population, making them a leading cause of the burden of human vector-borne diseases.

Transmission primarily occurs through mosquito bites, which are crucial in the dissemination of these diseases [38]. Collectively, vector-borne diseases represent over 17% of all infectious diseases worldwide and result in more than 700,000 deaths each year. Among these, malaria and dengue are responsible for nearly 450,000 fatalities [39]. In 2017, the World Health Assembly approved the "Global Vector Control Response" (GVCR) for the years 2017–2030. This initiative highlights the urgent need to enhance vector control as a fundamental strategy for disease prevention and outbreak management, providing strategic guidance to countries. Research on mosquito control is currently concentrating on understanding the resistance of mosquitoes to synthetic insecticides and developing new methods to address these resistance challenges. There is a growing interest in natural compounds as potentially more effective and less toxic alternatives to synthetic insecticides. Bioinsecticides derived from botanical or plant-based sources have emerged as a promising alternative due to their minimal toxicity to human health and the environment. This review presents the latest information on the synthetic insecticides utilized in mosquito control, their impact on the prevalence of insecticide resistance in mosquitoes, significant

plant-based insecticides, their mechanisms of action, and ongoing research regarding their compatibility with mosquitoes.

**Chemical Mosquito Repellents**

This text also offers a comprehensive insight into how biochemical compounds can be advantageous compared to synthetic ones, as well as strategies to address insecticide resistance challenges in combating the spread of mosquito-borne diseases. Chemical Mosquito Repellents development partners are urged to reorganize vector control strategies, enhance infrastructure, improve technical capabilities, strengthen monitoring and surveillance systems, and engage the community. The objective of the GVCR is to facilitate the achievement of both national and international targets concerning specific diseases, alongside the Sustainable Development Goals and Universal Health Coverage, by implementing a holistic approach to vector control. Currently, India is striving to eliminate these diseases by the year 2030 [40].

**Common Types of Chemical Mosquito Repellents**

**Topical Repellents**

- These are applied directly to the skin or clothing to prevent mosquito bites.
- Active Ingredients in Mosquito Repellents DEET (N, N-diethyl-meta-toluamide)
- The main active ingredient found in most topical insect repellents. It was originally developed by the US Department of Agriculture in 1946 as a protective measure for military use and received public approval in 1957 [41].

**DEET (N, N-diethyl-meta-toluamide) and Allethrin (d-trans-ALLETHRIN)**

Sulfuric acid Pyrethroid insecticide that is commonly utilized as a coil mosquito repellent and is recognized as the first commercially synthesized product, introduced in 1949.

Allethrin is extensively utilized in various products, such as coils, mats, aerosol sprays, and liquid lotions [42].

**Allethrin (d-trans-ALLETHRIN)**

Picaridin (KBR 3023) Icaridin, often known as picaridin, serves as a topical insect repellent that is effective on both skin and clothing. This synthetic compound was developed in the 1980s. It is marketed under trade names like Saltidin and Bayrepel. Its design mimics piperine, a naturally occurring substance found in the plant family that produces black pepper. When applied to human skin or clothing, picaridin successfully repels ticks, fleas, chiggers, mosquitoes, and biting flies. These products may come in the form of wipes, aerosols, liquids, or pump sprays. Picaridin (KBR 3023)

**Ethyl 3-[acetyl (butyl) amino] propanoate (IR3535)**

Ethyl 3-[acetyl (butyl) amino] propionate (IR3535), an insect repellent developed by Merck and Company in 1975, exhibits less irritation and toxicity in mammals compared to DEET when applied topically or ingested [43].

**Ethyl 3- [acetyl (butyl) amino] propanoate (IR3535)**

Diethyl phthalate (DEP) Diethyl phthalate, a colorless liquid produced through the reaction of sulfuric acid, phthalic anhydride, and ethanol, is used in various industrial applications. It is not only a component of insecticide sprays and mosquito repellents but is also found in skincare products and cosmetics [44]. Diethyl phthalate (DEP) Due to the global resistance issues associated with pyrethroids and DDT, these alternatives have been employed as insecticides in IRS. However, they exhibit shorter residual effectiveness, higher toxicity to mammals, and increased costs, which restrict their long-term use.

**Diethyl Phthalate**

Diethyl phthalate, a color less liquid is produced in the industry by the reaction of ethanol with phthalic anhydride along with sulfuric acid. Not only it is used in insecticide sprays and mosquito repellents, diethyl phthalate is also used as an ingredient in cosmetics and skin care product. The compound can bind to cosmetics owing to its solvent nature. Some of the medical devices such as intestinal tubing has the use of diethyl phthalate.

**Permethrin**

Worldwide insect repellent can be characterized into 2 basic chemical classes: synthetic chemicals and plant-derived oils. Permethrin belongs to the plant-derived insect repellent. Permethrin has been demonstrated to provide maximum protection against malarial mosquitoes, ticks, and flies. Permethrin is manufactured from crushed dried flowers of Chrysanthemum. An effective response against insects requires a direct contact of permethrin as it blocks

**Picaridin**

Picaridin is a repellent for mosquitoes and other insects which is similar in effectiveness to DEET, but more pleasant to use and much less likely to cause skin irritation. This piperidine derivative is available worldwide to be used as insect repellent. Picaridin can be used on human skin or clothing to repel mosquitoes, biting flies, ticks, fleas, and chiggers. Picaridin contains two stereo centers: one where the hydroxyethyl chain attaches to the ring, and one where the sec-butyl attaches to

**Spatial or Indoor Repellents**

These repellents release chemicals into the surrounding environment to repel or kill mosquitoes.

- **Mosquito Coils:** Contain pyrethroids such as allethrin; burning coils release smoke containing toxic particulate matter and harmful gases.
- **Liquid Vaporizers:** Use synthetic pyrethroids (e.g., prallethrin, transfluthrin) that vaporize when heated, providing prolonged indoor protection.
- **Aerosol Sprays:** Contain pyrethroids and propellants that can be harmful when inhaled in enclosed spaces.
- **Mats and Electric Repellents:** Release insecticidal vapors when heated electrically, similar to liquid vaporizers.

**Adverse Effects of Mosquito Repellents**

The most frequently reported adverse effects of excessive DEET exposure include skin reactions, neurological issues, and cardiovascular disorders [45]. Human self-poisoning through the ingestion or excessive application on the skin has been noted to lead to neurological effects, including encephalopathy and seizures [46]. Skin and Eye Irritation, redness, rashes, itching, or burning sensation when applied directly. Accidental eye contact may cause conjunctival irritation. Research involving human nasal mucosal cells [47], Hodgkin lymphoma, and soft tissue sarcomas has indicated that DEET may possess carcinogenic properties. The most toxic structural variant of allethrin utilized for parasite control in animal systems is the allethrin d-trans isomer [48]. This compound can exhibit neurotoxic effects as it modifies the membrane's permeability to Na+ and K+ ions, thereby stimulating neurons [49]. Overexposure to allethrin is the primary cause of skin and respiratory allergies [50]. Although it requires time, individuals who frequently handle chemicals or sprayers are at risk of this condition. While ingestion is exceedingly rare and typically unintentional, it can lead to potentially life-threatening symptoms such as vomiting, nausea, sore throat, abdominal pain, oral ulcers, increased secretions and/or dysphagia, coma, and convulsions [51].

**Mosquito Repellent Effect on Pregnant Women**

Pregnant women, small children, and infants are especially susceptible to these effects. Pregnant women, small children, and infants are especially susceptible to it. Eye irritation caused by excessive use of IR3535 [52]. Researchers have found that the vapor phase of mosquito repellents contains carbonyl compounds, including formaldehyde and acetaldehyde, which have a strong irritating effect on the upper respiratory tract [53]. Pyrethroids induce oxidative stress and decrease antioxidant levels [54]. The smoke and vapors emitted from mosquito repellents contain volatile organic compounds and free radicals, which are significant contributors to DNA and tissue damage. They may trigger apoptosis, necrosis, inflammation, and carcinogenesis. These repellents are mainly used at night, particularly in Asian countries, where the vapors released or burned during

the night can have harmful effects on the body as a whole [55]. When a mosquito coil is burned overnight, it releases heavy metals, aldehyde, and carbon particles. Inhalation of these substances leads to cellular damage in the lung tissues and destruction of the mucous membrane. As insecticides, pyrethrins and allethrins are found in most mosquito repellents.

Given that pyrethrum-based insecticides exhibit rapid metabolism and minimal accumulation, they are considered relatively low-risk toxins for mammals. Nevertheless, prolonged exposure to these highly toxic repellents can result in significant adverse health effects due to their high concentration of toxic chemicals. Furthermore, the act of burning or spraying these repellents generates a substantial amount of toxic particulate matter, which contaminates indoor air and ultimately contributes to air pollution [55]. Pesticides serve as crucial instruments in managing insect pests that impact both agriculture and public health; however, their indiscriminate application can lead to negative repercussions.

## Conclusions

Mosquito repellents, though widely used for protection against vector-borne diseases, act as silent environmental chemical toxicants with potential risks to human health. Prolonged and indiscriminate use of chemical repellents—such as coils, vaporizers, sprays, and lotions—can lead to respiratory irritation, skin allergies, neurological effects, hormonal disruption, and increased vulnerability in children, pregnant women, and the elderly. These products release toxic compounds into indoor and outdoor environments, contributing to air pollution and cumulative chemical exposure. While mosquito control remains essential for public health, excessive dependence on synthetic repellents highlights the need for safer alternatives, regulated usage, increased public awareness, and promotion of eco-friendly and biological mosquito control methods. Balancing disease prevention with environmental and human health protection is crucial for sustainable living. Furthermore, public health initiatives should educate the public about the negative effects associated with chemical mosquito repellents, while promoting a reduction in the use of these chemical-based products and an increase in the adoption of natural alternatives.

## *References*

1. Rozendaal JA.World Health Organization: Vector Control: Methods for Use by Individuals and Communities.Vol. 13. Geneva, Switzerland: World Health Organization; c1997.
2. Franklinos LHV, Jones KE, Redding DW, Abubakar I. The effect of global change on mosquito-borne disease. Lancet Infect Dis. 2019; 19(9):302–312. DOI: 10.1016/s1473-3099(19)30161-6

3. World Health Organization () World malaria report; c2023a. Available at: https://www.who.int/publications/i/item/9789240064898 (Accessed January 10, 2023).
4. World Health Organization. Dengue and severe dengue. Available at: https://www.who.int/news-room/factsheets/detail/dengue-and-severe-dengue (Accessed January 10, 2023.
5. World Health Organization. Zika virus. Available at: https://www.who.int/news-room/fact-sheets/detail/zikavirus (Accessed January 10, 2023); c2023b.
6. Namias A, Jobe NB, Paaijmans KP, Huijben S. The need for practical insecticide-resistance guidelines to effectively inform mosquito-borne disease control programs. Elife; c2021.DOI: 10.7554/eLife.65655.
7. Kraemer MUG, Reiner RC,Brady OJ, Messina JP, Gilbert M, Pigott DM,et al.Past and future spread of the arbovirus vectors Aedesaegypti and Aedesalbopictus. Nat. Microbiol. 2019; 4:854-863.
8. World Health Organization. Global report on insecticide resistance in malaria vectors: 2010–2016; c2018. Available at: https://apps.who.int/iris/handle/10665/272533 (Accessed January 10, 2023).
9. Peng ZY, He MZ, Zhou LY, Wu XY, Wang LM, et al.Mosquito repellents: Efficacy tests of commercial skin-applied products in China. Molecules, 2022, 27(17). DOI: 10.3390/molecules27175534
10. Deng S, Huang Q, Wei H, Zhou L, Yao L, Li D, et al. Beauveria bassiana infection reduces the vectorial capacity of Aedesalbopictus for the zika virus. J. Pest Sci.2019;92(2):7 781-789. DOI: 10.1007/s10340-019- 01081-0.
11. Boratne AV, Datta SS,Zile Singh Z, PurtyAJ,Jayanthi V, Senthilvel V.Attitude and practices regarding mosquito borne diseases and socio demographic determinants for use of personal protection methods among adults in coastal Pondicherry, Indian Journal of Medicine. 2010; 1(2):91-96.
12. Guzman MG, Kouri G. Dengue: An update, The Lancet Infectious Diseases. 2002;2(1):33-42. 13. Halstead SB. Dengue, the Lancet, 2007; 370(9599):1644–1652.
13. Cheng V, Lee HR, Chen CS.Morphological changes in the respiratory system of mice after inhalation of mosquito coil smoke, Toxicology Letters, 1992;62(2-3):163–177.
14. Brar R, Suri V, Suri V, Singh MP, Biswal M, Sikka P. Fever during pregnancy: Etiology and fetomaternal outcomes. Journal of Obstetrics and Gynecology of India. 2022; 72:102–108.
15. 16.Rathod GK, Jain M, Sharma KK, Das S, Basak A, Jain R. New structural classes of anti-malarial. European Journal of Medicinal Chemistry. 2022; 242:114653.

16. Valecha N. Keeping the momentum: suggestions for treatment policy updates in the final push to eliminate malaria in India. Malaria Journal. 2023; 22:128.
17. Bhavsar AT, Shepard DS, Suaya JA, Mafowosofo M, Hurley CL. A private hospital-based study assessing knowledge, attitudes, practices and costs associated with dengue illness in Surat, India. World Health Organization encephalopathy associated with use of DEET insect repellents: A case analysis of its toxicity in children. Human & Experimental Toxicology. 2001; 20:8–14. DOI: 10.1191/096032701676731093.
18. Omits TG, Murphy JV, Fell LA, Page B. Adverse events associated with the use of insect repellents containing N, N-diethyl-m-toluamide (DEET). Regulatory Toxicology and Pharmacology. 2010; 56:93–9. DOI: 10.1016/j.yrtph.2009.09.004.
19. Wiles D, Yee J, Castillo U, Russell J, Spiller H, Casavant M, et al. Lethal case of DEET toxicity due to intentional ingestion. Journal of Analytical Toxicology. 2014; 38:696–8. DOI: 10.1093/jat/bku082.
20. Tavares EM, Judge BS, Jones JS. Bug off! Severe toxicity following inhalational exposure to N, N-diethylmeta-toluamide (DEET). American Journal of Emergency Medicine. 2019; 37:1395. e3–1395.e4. DOI: 10.1016/j.ajem.2019.07.013.
21. U.S. Environmental Protection Agency (U.S. EPA). Reregistration Eligibility Decision (RED): DEET. U.S. EPA 738-R-98-010. Washington (DC): U.S. EPA; September 1998,118.
22. Sudakin DL, Trevathan WR. DEET: A review and update of safety and risk in the general population. Journal of Toxicology and Clinical Toxicology. 2003; 41:831–839.
23. Abou-Donia MB, Wilmarth KR, Jensen KF, Oehme FW, Kurt TL. Neurotoxicity resulting from co-exposure to pyridostigmine bromide, deet, and permethrin: implications of Gulf War chemical exposures. Journal of Toxicology and Environmental Health. 1996; 48:35–56. DOI: 10.1080/009841096161456.
24. Parihar VK, Hattiangady B, Shuai B, Shetty AK. Mood and memory deficits in a model of Gulf War illness are linked with reduced neurogenesis, partial neuron loss, and mild inflammation in the hippocampus. Neuro psycho pharmacology. 2013; 38:2348–2362. DOI: 10.1038/npp.2013.158.
25. Tisch M, Schmezer P, Faulde M, Groh A, and Maier H. Genotoxicity studies on permethrin, DEET and diazinon in primary human nasal mucosal cells. European Archives of Oto-Rhino-Laryngology. 2002; 259:150–153.
26. Pahwa P, et al. Hodgkin lymphoma, multiple myeloma, soft tissue sarcomas, insect repellents, and phenoxyherbicides. Journal of Occupational and

Environmental Medicine. 2006; 48:264–274. DOI: 10.1097/01.jom.0000183539.20100.06.
27. Pérez-Fernández V, García MÁ, Marina ML. Characteristics and enantiomeric analysis of chiral pyrethroids. Journal of Chromatography. A. 2010; 1217:968–989. DOI: 10.1016/j.chroma.2009.10.069.
28. Arif A, Quds R, Mahmood R. Bioallethrin enhances generation of ROS, damages DNA, impairs the redox system and causes mitochondrial dysfunction in human lymphocytes. Scientific Reports. 2021; 11:8300. DOI: 10.1038/s41598-021-87799-3.
29. Ravula AR, Yenugu S. Effect of oral administration of a mixture of pyrethroids at doses relevant to human exposure on the general and male reproductive physiology in the rat. Ecotoxicol. Environmental Safety. 2021; 208:111714. DOI: 10.1016/j.ecoenv.2020.111714.
30. Bhatt P, Zhou X, Huang Y, Zhang W, Chen S. Characterization of the role of esterases in the biodegradation of organophosphate, carbamate, and pyrethroid pesticides. Journal of Hazardous Materials. 2021; 411:125026. DOI: 10.1016/j.jhazmat.2020.125026.
31. Murugesan A, Nguyen P, Ramesh T, Yli-Harja O, Kandhavelu M, Saravanan KM. Molecular modeling and dynamics studies of the synthetic small molecule agonists with GPR17 and P2Y1 receptor. Journal of Biomolecular Structure and Dynamics; c2021. p. 1-9. DOI: 10.1080/07391102.2021.1977707.
32. Saravanan A, Kumar PS, Karishma S, Vo DVN, Jeevanantham S, Yaashikaa PR, George CS. A review on biosynthesis of metal nanoparticles and its environmental applications. Chemosphere. 2021;264(2):128580. DOI: 10.1016/j.chemosphere.2020.128580.
33. Karri RR, Ravindran G, Dehghani MH. Chapter 1 – Wastewater - Sources, Toxicity, and Their Consequences to Human Health. In; Karri, R.R., Ravindran, G., Dehghani, M.H. B.T.-S.C.T. in S.W. and W.M., Eds.; Elsevier; c2021. p. 3-33.
34. Pitzer EM, Williams MT, Vorhees CV. Effects of pyrethroids on brain development and behavior: Deltamethrin. Neurotoxicol. Teratol. 2021; 87:106983. DOI: 10.1016/j.ntt.2021.106983.
35. O'Malley M, Ngo MA, Maibach HI. Pesticide-Related Dermatoses BT - Kanerva's Occupational Dermatology. In; John, S.M., Johansen, J.D., Rustemeyer, T., Elsner, P., Maibach, H.I., Eds.; Springer International Publishing: Cham; c2020. p. 1029-1057
36. 37.Zhang H, Li J, Saravanan KM, Wu H, Wang Z, Wu D, et al. An Integrated Deep Learning and Molecular Dynamics Simulation-Based Screening Pipeline Identifies Inhibitors of a New Cancer Drug Target TIPE2. Front.

Pharmacol. 2021; 12:1-13. DOI: 10.3389/fphar.2021.772296.
37. Puccetti G. IR3535 (ethyl butylacetylaminoproprionate) In: Debboun M, Frances SP, Strickman D, editors. Insect repellent. CRC Press; Boca Raton; c2007. p. 353–360.
38. Koo LC, Ho JH. Mosquito coil smoke and respiratory health among Hong Kong Chinese: results of three epidemiological studies. Indoor Built. Environ. 1994; 3:304-310.
39. Chang JY, Lin JM. Aliphatic aldehydes and allethrin in mosquito coil smoke. Chemosphere. 1998; 36:617-624.
40. Demerdash FM El. Oxidative stress and hepatotoxicity induced by synthetic pyrethroids-organophosphate insecticides mixture in rat. Journal of Environmental Science and Health Part C: Environmental Carcinogenesis & Ecotoxicology Reviews. Rev. 2011; 29:145-158.
41. Mossa AT, Refaie AA, et al. Amelioration of Prallethrininduced oxidative stress and hepatotoxicity in rat by the administration of Origanummajorana essential oil. BioMed Research International. 2013:1-11.
42. Cheng H, Yang W. Large-scale spraying of bednets to control mosquito vectors and malaria in Sichuan, China. Bull. World Health Organ. 1995; 73(3):321–328.
43. Liu WK, Wong MH. Toxic effects of mosquito coil (a mosquito repellent) smoke on rats. Toxicol. Lett. 1987; 39:223–239.
44. John NA, John J. Prolonged use of mosquito coil, mats, and liquidators: a review of its health implications. Int. J Clin. Experimental Physiology. 2015; 2:209–213.
45. Valbuena D, Cely-Santos M, Diana Obreg´on. Agrochemical pesticide production, trade, and hazard: Narrowing the information gap in Colombia. Journal of Environmental Management. 2021; 286:112141. DOI: 10.1016/j.jenvman.2021.112141.
46. Govindarajan M, Rajeswary M, Senthilmurugan S, Vijayan P, Alharbi NS, Kadaikunnan S. Larvicidal activity of the essential oil from AmomumsubulatumRoxb. (Zingiberaceae) against Anopheles subpictus, Aedesalbopictus and Culextritaeniorhynchus (Diptera: Culicidae), and nontarget impact on four mosquito natural enemies. Physiological and Molecular Plant Pathology. 2018; 101:219-224. DOI: 10.1016/j.pmpp.2017.01.003.
47. Govindarajan M, Rajeswary M, Hoti SL, &Benelli G. Larvicidal potential of carvacrol and terpinen-4-ol from the essential oil of Origanumvulgare(Lamiaceae) against Anopheles stephensi, Anopheles subpictus, Culexquinquefasciatus and Culextritaeniorhynchus (Diptera:

Culicidae). Research in Veterinary Science. 2016; 104:77-82. DOI: 10.1016/j.rvsc.2015.11.011.
48. Madhavi M, Shravani G, Divya Sri K, DayanikaJadav, Navya P, Nivarna Reddy M, et al. Evaluating the Efficacy of Thyme, Camphor, and Eucalyptus Essential Oils as Larvicides Against Aedesaegypti Mosquitoes, International Journal of Innovative Science and Research Technology. 2023a; 8(5):766-768.
49. Madhavi M, Mahesh L. Murrayapaniculata leaf extracts as effective larvicides against the larvae of Aedesvittatus. (Insecta; Diptera; Culicidae), International Journal of Entomology Research. 2023b;8(5):21-26.
50. Madhavi M, Srikanth M, Malleshwari B Kavitha K, Mahesh L. Biological control of the Aedesaegypti larvae by the aqueous extracts of Argemonemexicana, International Journal of Multidisciplinary Research and Development. 2023c;10(5):23-25.
51. Sandeep Paarakh PM. Jasminumgrandiflorum Linn (Chameli): ethnobotany, phytochemistry and pharmacology: A review. Pharmacology Online. 2009; 2:586–595.
52. Jilani G, Saxena CR, Rueda BP. Repellent and growth inhibiting effects of turmeric oil, sweetflag oil, neem oil, and margosan-O on red flour beetle (Coleoptera: Tenebrionidae). Journal of Economic Entomology. 1988; 81:1226-1230

# Environmental Biotechnology and Waste Management

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## Abstract

Many environmental problems caused by human activities need to be addressed using methods that are both cost-effective and environmentally sustainable. This paper proposes a comprehensive framework for managing and controlling solid waste in the south-east region of Nigeria. The key activities include collecting and transporting waste to designated sites, separating recyclable materials, and converting biodegradable waste into organic fertiliser and biogas.

These processes require the active involvement of local communities, industries, and Small- and Medium-Scale Enterprises (SMEs) that can apply environmentally integrated processing and production technologies. Biotechnology is used to transform organic waste into enriched compost and biogas, while SMEs can use waste materials as raw inputs to produce other goods and services. This approach represents the use of value-adding environmental technologies.

Overall, the framework promotes the use of modern and forward-looking technologies to effectively manage existing waste problems and prevent future accumulation. Strong collaboration between government bodies, the public, and research institutions is essential for successful implementation.

## Introduction

Environmental biotechnology can be described as the use and control of microbial communities to deliver beneficial services to humans. According to Rittmann (2006a), these services mainly fall into two broad categories.

First, microorganisms are able to remove or neutralize harmful pollutants present in water, soil, sediments, and sludge. By doing so, these contaminated resources can be cleaned and reused for beneficial purposes.

Second, microbial communities can transform the energy stored in different forms of biomass—often spread out and sometimes dangerous—into useful and usable energy forms such as methane, hydrogen, and electricity.

Both of these functions depend on oxidation–reduction (redox) reactions driven by microorganisms. While redox reactions are fundamental to all living systems, microorganisms are exceptionally efficient at carrying them out in ways that supply energy for their own growth and simultaneously generate valuable products for human use. This creates a mutually beneficial or "win–win" situation.

Environmental biotechnology is a specialized branch within the broader field of biotechnology. What makes it distinct is that its scientific foundation lies mainly in microbial ecology, which focuses on understanding how microbial populations interact with each other and with their environment.

Microbial ecology focuses on understanding a microbial community by examining three main aspects. First, it identifies the different kinds of microorganisms present, which defines the community's phylogenetic composition. Second, it determines the metabolic activities these microorganisms perform, which represent the community's functional capabilities. Third, it studies how microorganisms interact with one another and with their surrounding environment. In most situations, these metabolic activities are the actual services that benefit human society.

Over the last two decades, major progress in genome-based techniques has made it possible to study microbial communities in much greater detail. These advances have led to the identification of previously unknown microorganisms, newly discovered metabolic pathways, and innovative biotechnological applications. As a result, a new discipline known as molecular microbial ecology has emerged.

To ensure that microbial communities consistently deliver the desired benefits, they must be actively managed. This management often involves designing engineered systems that combine microbial communities with advanced materials and physical or chemical processes. Fortunately, developments in materials science and engineering are keeping pace with progress in molecular microbial ecology. Therefore, as our knowledge of microbial community structure and function grows, it can be effectively integrated into increasingly advanced engineered systems that guide microbial activity toward meeting societal needs.

Proper management of medical waste is a critical issue in the healthcare sector because inadequate handling can pose serious risks to both the environment and public health. Medical waste is generally categorized as infectious, hazardous, or non-hazardous, and each category requires specific treatment and disposal methods. According to the World Health Organization (WHO), healthcare activities during 2020–2021 generated approximately

87,000 tonnes of personal protective equipment (PPE), 0.73 million litres of chemical waste, and an additional 0.15 million tonnes of medical waste associated with the administration of more than 8 billion vaccine doses. Data on solid medical waste generation from the world's top 50 countries further illustrate the scale of the problem.

WHO estimates that around 85% of healthcare waste consists of general, non-hazardous materials similar to household waste, while the remaining 15% is classified as hazardous, including infectious, chemical, and radioactive waste. If these wastes are not managed properly, they can negatively affect both environmental quality and human health. For instance, uncontrolled burning or improper incineration of healthcare waste can release harmful pollutants such as dioxins, furans, and particulate matter, which are known to be carcinogenic and capable of causing respiratory illnesses.

Improper handling and disposal of sharps waste—such as needles, syringes, scalpels, and blades—can result in injuries and the spread of infections among healthcare workers and waste handlers. Similarly, pharmaceutical waste, including expired or contaminated medicines and vaccines, may pollute soil and water bodies, thereby harming aquatic organisms and agricultural productivity. In addition, radioactive medical waste originating from diagnostic and therapeutic procedures poses long-term radiation exposure risks to humans and animals.

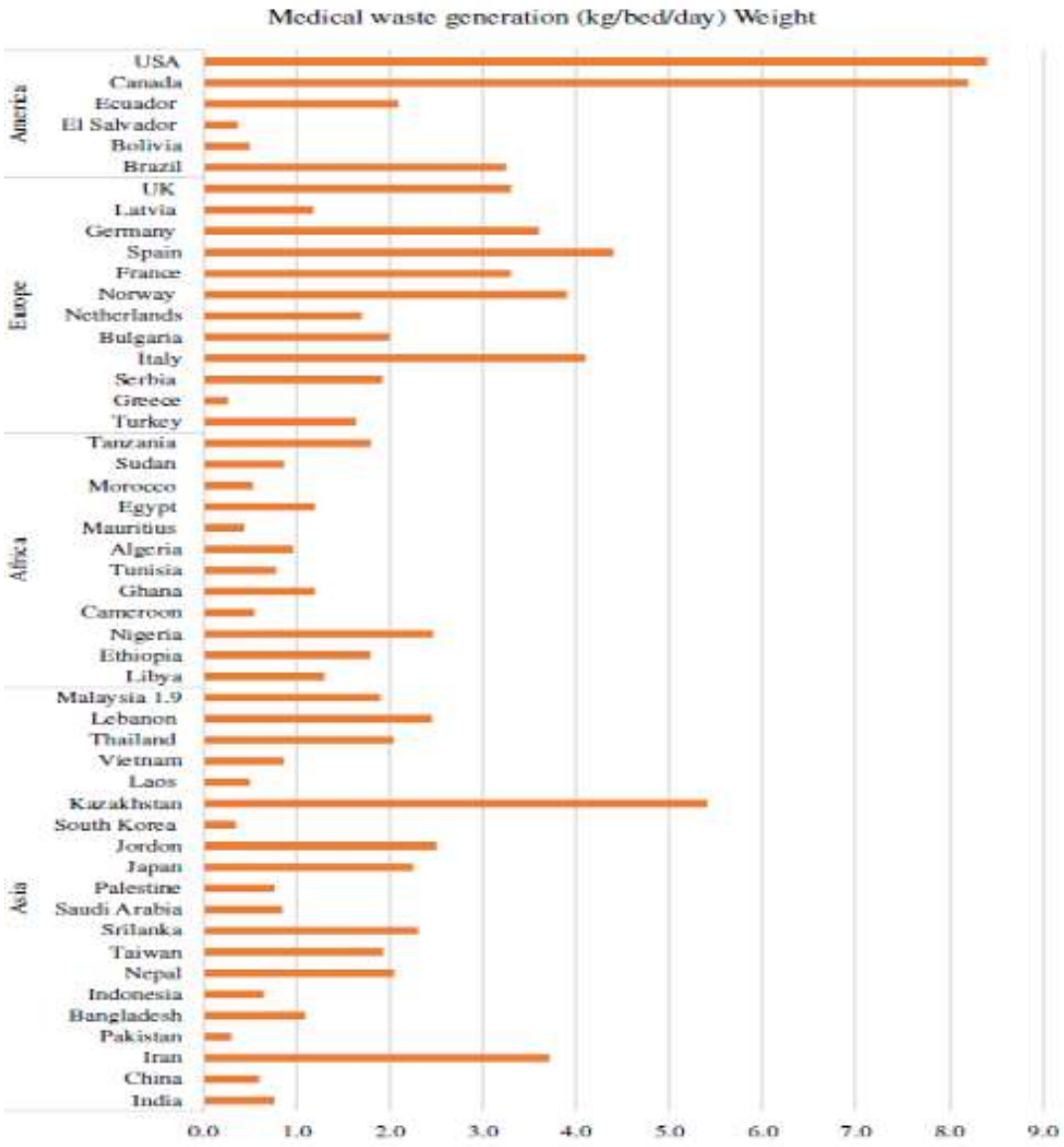
The quantity and composition of healthcare waste vary widely depending on the type of healthcare facility, the level of economic development, and the medical practices employed. Consequently, a single universal approach to healthcare waste management is not effective. Instead, integrated and context-specific waste management strategies that combine multiple treatment and disposal methods are required to address local needs and conditions. An average medical waste generation in kg per bed in one day of the top 50 countries.

**Environmental Impact of Medical Waste**

Despite the continuous publication of research on medical waste treatment and disposal, effective management of medical waste remains a major global challenge that demands ongoing research and innovation. The volume and variety of medical waste have increased significantly due to advancements in healthcare technologies and the occurrence of epidemic and pandemic outbreaks in recent years. Examples include the COVID-19 pandemic caused by SARS-CoV-2 in 2019, the SARS outbreak in 2003, and the H1N1 influenza pandemic in 2009–2010.

As a result, medical waste management guidelines and disposal practices must be frequently revised and improved. Failure to do so can lead to serious environmental pollution and health hazards affecting humans, animals, and ecosystems through contamination of air, water, and soil.

Environmental biotechnology plays a crucial role in addressing these challenges by using biological processes to control pollution, restore environmental quality, and support sustainable development. It has demonstrated significant potential in reducing the harmful environmental impacts associated with medical waste.

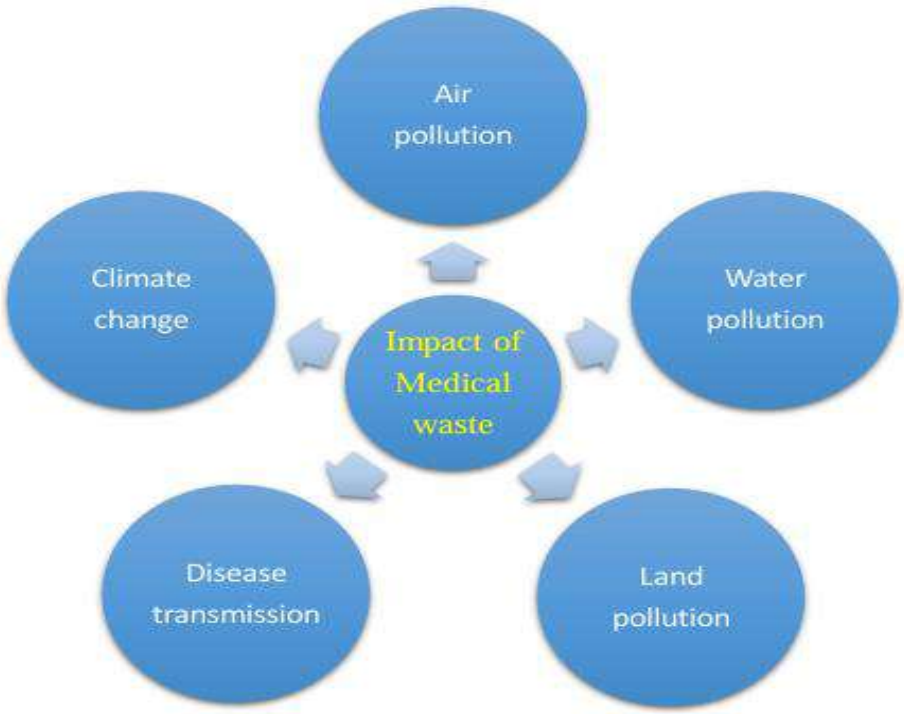



An average medical waste generation in kg per bed in one day of the top 50 countries.

Accordingly, this review presents an updated and comprehensive overview of medical waste management practices and highlights the contribution of environmental biotechnology in minimizing the environmental consequences of

medical waste. The review discusses all stages of waste management, including waste generation, segregation, collection, transportation, treatment, and final disposal, emphasizing the importance of biotechnology at each step.

In addition, the review evaluates various environmental biotechnological approaches used in medical waste management, such as composting, anaerobic digestion, microbial fuel cells, and phytoremediation. The advantages, limitations, and practical applications of these technologies are examined. Social and economic aspects of implementing environmental biotechnology for medical waste management are also considered. Overall, the review focuses on the role of environmental biotechnology in mitigating the environmental impact of medical waste while assessing existing management strategies and future solutions.



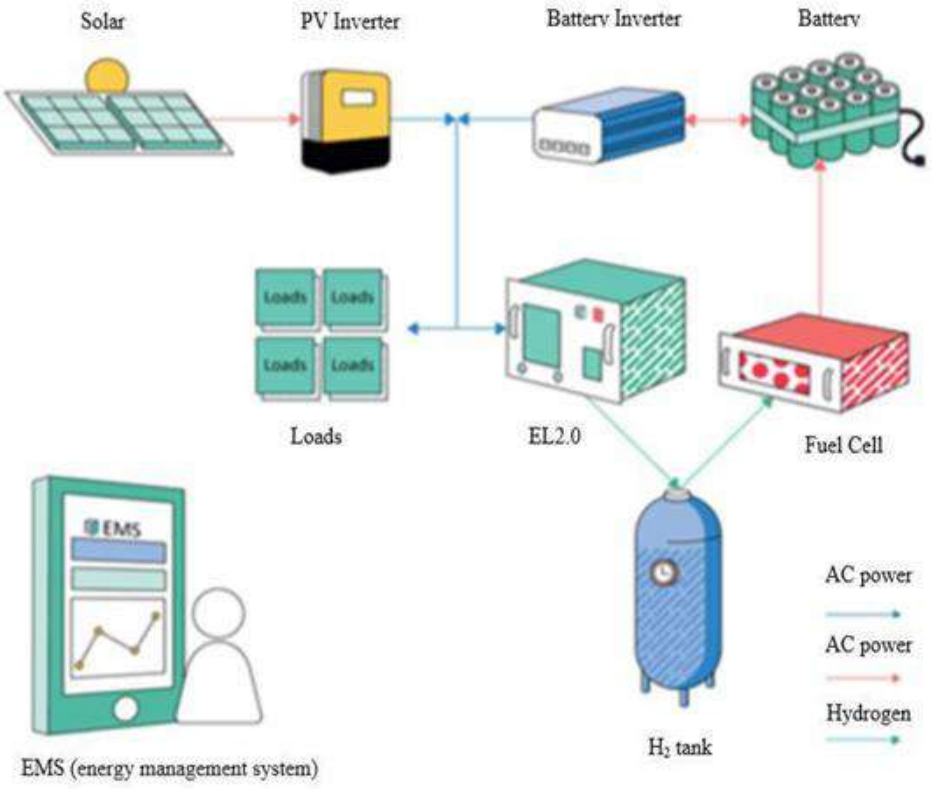

Medical waste generated by healthcare facilities such as hospitals, clinics, and laboratories can cause serious environmental harm if it is not managed correctly. Improper handling and disposal of medical waste can negatively affect the environment in several ways, including air, water, and land pollution, the spread of diseases, and contribution to climate change.

Burning medical waste releases toxic substances such as dioxins, furans, and mercury into the air, leading to air pollution and posing health risks to nearby populations. When medical waste is disposed of improperly, harmful substances and pathogens can seep into water bodies, contaminating drinking water sources

and threatening both human and aquatic life. Medical waste dumped in landfills can pollute soil, degrade land quality, and create long term environmental and health hazards.

Additionally, medical waste may contain infectious microorganisms such as bacteria, viruses, and parasites, which can spread diseases to humans and animals. Improper disposal methods, including open burning and uncontrolled decomposition, also release greenhouse gases that contribute to global warming and climate change.

Waste-to-energy (WTE) processes, medical waste is transformed into a source of renewable energy. Healthcare activities generate various types of medical waste, including used needles, expired pharmaceuticals, and biological materials. If these wastes are not managed properly, they can pose serious threats to public health and the environment. WTE technology provides an effective solution by converting medical waste into useful energy through different methods. One commonly used method is incineration, where waste is burned at very high temperatures to produce heat and steam that can be used for electricity generation or heating. Another method is gasification, in which medical waste is heated without oxygen, resulting in the formation of combustible gas that can be used to generate power (Manegdeg et al., 2022).



The waste-to-energy (WTE) conversion of medical waste offers several benefits. It significantly reduces the volume of waste sent to landfills, thereby decreasing the risk of environmental pollution and ecological damage. In addition, WTE helps meet increasing energy demands by generating electricity from a renewable source. This process also contributes to the reduction of greenhouse gas emissions by preventing the release of methane and other harmful gases that are normally produced when medical waste decomposes in landfills.

However, WTE conversion of medical waste also faces certain challenges. One major concern is the possible emission of toxic substances during incineration or gasification, which may pose serious health risks to nearby communities. Another limitation is the high cost associated with the installation, operation, and maintenance of WTE facilities and the supporting infrastructure (Chen et al., 2022)

**Sustainable Municipal Solid Waste (MSW) Management**

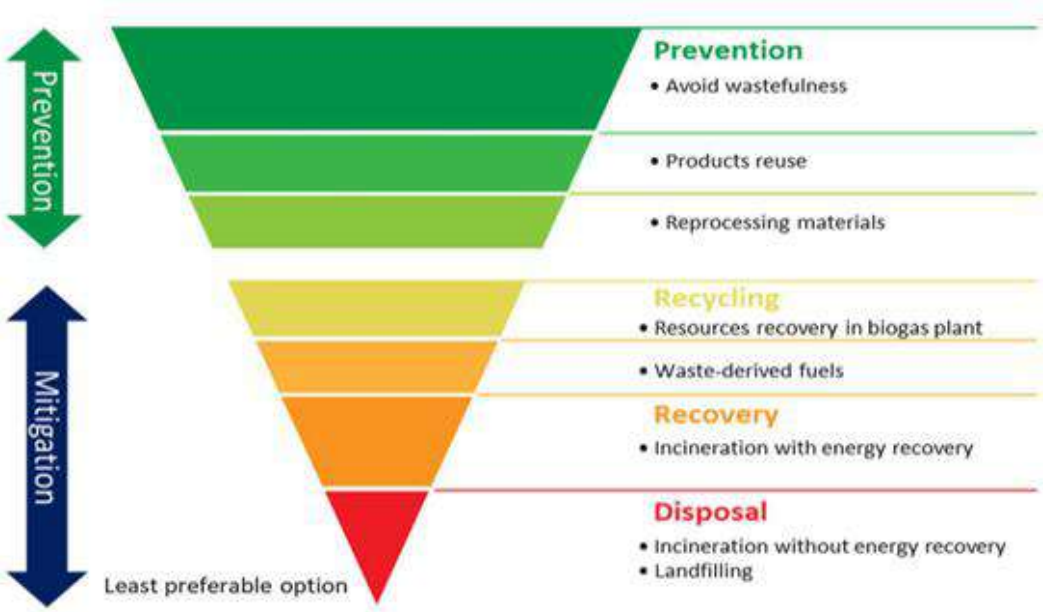
Municipal solid waste management has existed as a public service since the mid-18th century and includes all policies and practices involved in handling solid, liquid, and gaseous waste—from the point of generation to final disposal. In its early stages, the primary focus of MSW management was improving sanitation. However, over recent decades, the approach has shifted significantly due to the growing volume of waste. The emphasis has moved from rapid collection and disposal, mainly through landfilling, to more sustainable practices such as recycling and even urban mining.

Although waste management strategies differ depending on a country's level of development, the fundamental objectives have remained the same: conserving natural resources and protecting the environment. Sustainability has become the central principle of modern MSW management, aiming to reduce environmental impacts and safeguard resources for future generations.

When waste management systems are planned or evaluated, the waste management hierarchy is commonly used as a guiding framework, ranking waste treatment methods based on their sustainability. Landfilling, considered the least desirable option, is still widely practiced worldwide. Sanitary landfills involve controlled waste disposal with systems for leachate treatment and biogas recovery. However, landfilling recyclable materials discourages public participation in recycling and promotes wasteful behavior, which contradicts the concept of a circular or closed-loop system.

Moreover, landfills can cause soil contamination, unpleasant odors, and groundwater pollution due to the release of harmful leachate. In densely populated urban areas, landfill operations have become increasingly expensive and will continue to impose higher costs over time. As a result, global waste

management policies increasingly emphasize waste reduction and municipal recycling to conserve limited landfill space. This push for recycling has also gained strong political attention in many cities.




**Conclusion**

The government can collaborate with the private sector, including small and medium enterprises (SMEs), as well as local communities, to jointly implement production- and process-integrated environmental technologies for the sustainable treatment of municipal and industrial solid waste. This cooperative approach is likely to encourage the creation of numerous SMEs that utilize such waste-processing technologies.

These improvements can be achieved by adopting a pollution control framework based on the Best Available Technology Not Entailing Excessive Costs (BATNEEC), which emphasizes waste reduction at the source along with recycling and reuse of waste materials. In addition, alternative solutions guided by the Best Practicable Environmental Option (BPEO) can be implemented, such as waste separation and composting facilities, manual sorting, anaerobic digestion of biodegradable waste, and composting within landfills.

Microbial ecology forms the scientific foundation of environmental biotechnology and has progressed significantly over the past two decades due to the development of advanced genomics-based tools that enable detailed analysis of microbial community structure and function. These genomic techniques provide valuable insights into which components of microbial communities must be controlled or optimized to ensure they deliver specific environmental services.

By applying this knowledge, management objectives can be achieved through the use of modern materials along with physical and chemical processes to create conditions that favor the growth and activity of desired microorganisms. Technologies such as the membrane biofilm reactor (MBfR), microbial fuel cell (MFC), and microbial electrolysis cell (MEC) are strong examples of innovative systems that have emerged directly from this targeted microbial management approach.

***References***

1. Onwurah IN, Ogugua VN, Otitoju OF. Integrated environmental biotechnology-oriented framework for solid waste management and control in Nigeria. International journal of environment and waste management. 2006 Jan 1;1(1):94-104.
2. Rittmann BE. Environmental biotechnology in water and wastewater treatment. Journal of Environmental Engineering. 2010 Apr;136(4):348-53.
3. Chandra V, Arpita K, Yadav P, Raghuvanshi V, Yadav A, Prajapati S. Environmental biotechnology for medical waste management: A review of current practices and future directions.
4. Al-Barakah FN, Radwan SM, Abdel-Aziz RA. Using biotechnology in recycling agricultural waste for sustainable agriculture and environmental protection. Int. J. Curr. Microbiol. App. Sci. 2013;2(12):446-59.
5. Dille D, Archana K. Biotechnology for solid waste management–a critical.
6. Tsui TH, Wong JW. A critical review: emerging bioeconomy and waste-to-energy technologies for sustainable municipal solid waste management. Waste Disposal & Sustainable Energy. 2019 Nov;1(3):151-67.
7. Narancic T, O'Connor KE. Microbial biotechnology addressing the plastic waste disaster. Microbial biotechnology. 2017 Jul 17;10(5):1232.
8. Narancic T, O'Connor KE. Microbial biotechnology addressing the plastic waste disaster. Microbial biotechnology. 2017 Jul 17;10(5):1232.
9. Scragg AH. Environmental biotechnology. 2nd ed. Oxford: Oxford University Press; 2005.
10. Atlas RM, Bartha R. Microbial ecology: fundamentals and applications. 4th ed. Menlo Park (CA): Benjamin/Cummings; 1998.
11. Khan FI, Husain T, Hejazi R. An overview and analysis of site remediation technologies. J Environ Manage. 2004;71(2):95–122.
12. United Nations Environment Programme (UNEP). Solid waste management. Nairobi: UNEP; 2005.

# Genetic Disorders, Screening and Therapeutics

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**Abstract**

Recent advances of biological drugs have broadened the scope of therapeutic targets for a variety of human diseases. This holds true for dozens of RNA-based therapeutics currently under clinical investigation for diseases ranging from genetic disorders to HIV infection to various cancers. These emerging drugs, which include therapeutic ribozymes, aptamers, and small interfering RNAs (siRNAs), demonstrate the unprecedented versatility of RNA. However, RNA is inherently unstable, potentially immunogenic, and typically requires a delivery vehicle for efficient transport to the targeted cells. These issues have hindered the clinical progress of some RNA-based drugs and have contributed to mixed results in clinical testing. Nevertheless, promising results from recent clinical trials suggest that these barriers may be overcome with improved synthetic delivery carriers and chemical modifications of the RNA therapeutics.

***Keywords:*** Genetic disorders, siRNAs, RNA-based drugs, autosomal chromosome

## Introduction

Genetic disorders are health problems that happen because of some type of abnormality in a person's genetic material. There are several types of genetic disorders. In some cases, a genetic change in a single gene can cause someone to have a disease or condition. In other cases, the gene does not have a genetic change, but a person has more or fewer copies of the gene than most people, and this causes a disease or condition. Some diseases or conditions occur when a person does not have the same number of chromosomes as most people or has part of a chromosome that is missing, extra, or not in the right place.

Most genetic disorders happen due to the combination of many genetic changes acting together with a person's behaviors and environment. These are sometimes called complex conditions.

## Types of Disorders

### Single Gene Disorder

DNA contains the instructions for making your body work. DNA is made up of two strands that wind around each other. Each DNA strand includes chemicals called nitrogen bases—T (thymine), A (adenine), C (cytosine), and G (guanine)—that make up the DNA code. Genes are specific sections of DNA that have instructions for making proteins. Proteins make up most of the parts of your body and make your body work the right way (CDC., 2005).

Some diseases and conditions happen when a person has a genetic change (sometimes called a mutation) in one of their genes. These types of diseases are called single gene disorders.

For example, part of a gene that usually has the sequence TAC is changed to the sequence TTC.

single gene disorders that affect a gene on one of the 22 autosomal chromosome pairs are called autosomal disorders. Disorders that affect the sex chromosome are called X-linked disorders. Disorders are further described according to whether the affected genetic change is dominant or recessive.

### Autosomal Disorder

Autosomal dominant diseases or conditions, a person only needs a genetic change in one copy of the gene to have the disease. If one parent has an autosomal dominant disease or condition, each child has a 50% (1 in 2) chance of inheriting the genetic change that causes the condition.

Examples of autosomal dominant conditions include hereditary breast and ovarian cancer caused by genetic changes (mutations) to the BRCA1 and BRCA2 genes; Lynch syndrome; and familial hypercholesterolemia (Theoder., 2012).

### Autosomal Recessive

With autosomal recessive diseases or conditions, a person needs a genetic change in both copies of the gene to have the disease or condition. While a person with a genetic change in only one copy of the gene will not have the disease or condition, they can still pass the genetic change down to their children. These parents are sometimes called "carriers" of the disease because they "carry" the genetic change that causes the disease or condition but do not have the disease themselves.

A parent who is a carrier of a disease has a 50% (1 in 2) chance of passing the gene with the genetic change on to each of their children. If both parents are carriers of the disease, each child has a 25% (1 in 4) chance of inheriting two genes with the genetic change and thus of having the disease. Carrier screening looks for autosomal recessive genetic changes in parents to see if they could have a child with the disease or condition.

**Examples of Autosomal Recessive Disorders Are Sickle Cell Disease and Cystic Fibrosis.**

**X-linked**

Females have two X chromosomes, and males have one X chromosome and one Y chromosome. Each daughter gets an X from her mother and an X from her father. Each son gets an X from his mother and a Y from his father (Theoder., 2011).

Some diseases or conditions happen when a gene on the X chromosome have a genetic change. Because males only have one copy of all the genes on the X chromosome, they are much more likely to be affected by X-linked genetic disorders than females. A female with a genetic change on only one of her two X chromosomes may not have the disease or condition at all. However, in some cases, females with the genetic change on one of their X chromosomes can have the disease or condition, but it is often a milder form of the disease than usually occurs in males.

Examples of X-linked conditions include fragile X syndrome, Duchenne muscular dystrophy, and hereditary hemophilia.

**Chromosomal Abnormalities**

**Different Number of Chromosomes**

People usually have 23 pairs of chromosomes. But sometimes a person is born with a different number. Having an extra chromosome is called trisomy. Missing a chromosome is called monosomy.

For example, People with Down syndrome have an extra copy of chromosome 21. This extra copy changes the body's and brain's normal development and causes intellectual and physical problems for the person. Some disorders are caused by having a different number of sex chromosomes. For example, People with Turner syndrome usually have only one sex chromosome, an X. Women with Turner syndrome can have problems with growth and heart defects.

**Changes in Chromosomes**

Sometimes chromosomes are incomplete or shaped differently than usual. Missing a small part of a chromosome is called a deletion. A translocation is when part of one chromosome has moved to another chromosome. An inversion is when part of a chromosome has been flipped over.

For example, People with Williams syndrome are missing a small part of chromosome 7. This deletion can result in intellectual disability and a distinctive facial appearance and personality.

**Complex Conditions**

Complex disorders are caused by genetic changes in many different genes working together with environmental factors. Environmental factors include

exposures and behaviors such as air pollution, smoking, alcohol use, the amount of exercise a person gets, or the foods they eat. Having a family health history of a complex condition can make you more likely to have that condition yourself. However, genetic testing would not be recommended because there is not a single genetic change causing the condition that could be found by genetic testing.

Most chronic diseases, such as most cases of heart disease, cancer, diabetes, osteoporosis, and asthma, are complex disorders. So are most cases of developmental disabilities, such as autism spectrum disorder and attention deficit / hyperactivity disorder (ADHD), and mental health conditions, such as depression and schizophrenia.

**Symptoms and Causes of Genetic Disorder**

Genetic disorder occurs when a mutation takes place with a wrong amount of genetic material jeans are made up of DNA which contains instruction for cell functioning and the characteristics that make you unique the half amount of your jeans from each biological parent and may in Heritage mutation from one parent or both sometimes Jean also changes due to some of the issues within the DNA with can arise with the risk of having a genetic disorder some may call symptoms at work while others develop over a period of time(Motulsky., 2011).

**There Are Many Types of Genetic Disorder**

1. Chromosomal disorder - Down syndrome, fragile X syndrome, Klinefilter syndrome, triple x syndrome, turner syndrome, trisomy 18, trisomy 13
2. Alzheimer's disease
3. Arthritis
4. Autism spectrum disorder
5. Cancer
6. Coronary artery disease
7. Diabetes
8. Migraine headache

**Monogenic Disorder:** Cystic fibrosis, Sickle cell disease, Tay Sachs disease

**Genetic Disorders May Also Cause Rare Diseases**

- Rare genetic disorder includes
- Mitochondrial diseases Usher syndrome
- AA amyloidosis

**Symptoms And Causes of Genetic Disorder**

Most of the DNA in the gene instruct the body to make up a protein this protein start complexes early interactions that helps us to stay healthy when a mutation occurs it affects the gene protein making instructions and operate protein may be

missing or the ones have do not function properly environmental factors also governed this process that could lead to genetic mutation includes- Chemical exposure radiation exposure smoking, UV exposure from the Sun

**Management and Treatment**

Most genetic disorder do not have a cure some have treatments that makes slow disease progression or legend their impact on life the type of treatment that is right for depends upon the type and severity of the disease with others may not have treatment but provide medical survey lenses to try to catch complications only.

- Medications to manage symptoms or chemotherapy to slow abnormal cell growth.
- Nutrition counselling or dietary supplements to help to get the body needs full field.
- Physical occupational or speech therapy to maximize ability.
- Blood transfusion to restore levels of healthy blood cells.
- Surgery to repair abnormal structures or treat complications.
- Specialise treatment such as radiation therapy for cancer.
- Organ transplant which is a procedure to replace a non-functioning organ with one from a healthy donor.

**Outlook or Prognosis**

Outlook for people with genetic disorder some condition including certain rare and congenital diseases have a dream prognosis. Children born with anencephali typically survive only a few days other conditions like and isolated cleft lip do not affect life span but need regular specialised care to stay comfortable.

**Prevention**

Often little can be done to prevent a genetic disorder. Genetic counselling and testing can help to learn more about the risk it can also know by lightly hood of passing some disorder on to children's. New born screening this test uses a sample of new born babies' blood and is performed on the on all babies born. Detecting genetic disorder early in life can help the child receive family care if needed.

**Screening**

Genetic disorder screening uses DNA tests to find genetic risks (prenatal, carrier, newborn, predictive), while therapeutics aim to treat these disorders, primarily through gene therapy (adding healthy genes) or supportive care (like enzyme replacement), with emerging options like CRISPR showing promise but facing hurdles like delivery and long-term safety. Screening informs family planning and early intervention, while gene therapies, though limited, are advancing,

offering hope for previously untreatable conditions.

**Genetic Screening**

Purpose: Identify genetic changes in DNA, chromosomes, or proteins to assess disease risk or diagnose conditions.

**Types**

1. **Carrier Screening:** Checks if parents carry genes for disorders (e.g., cystic fibrosis, sickle cell).
2. **Prenatal Diagnosis:** Tests fetuses via amniocentesis/CVS for conditions.
3. **Newborn Screening:** Checks infants for treatable disorders like PKU.
4. **Diagnostic Testing:** Confirms suspected disorders in symptomatic individuals.
5. **Predictive/Presymptomatic:** Identifies risk for adult-onset conditions (e.g., Huntington's) before symptoms.

PCR and sequencing are key genetic screening methods: PCR (Polymerase Chain Reaction) amplifies specific DNA segments, making them easier to detect, while DNA sequencing determines the exact order of nucleotides (A, T, C, G) to find disease-causing mutations, often using PCR products as the starting material for methods like Sanger or Next-Generation Sequencing (NGS). PCR is used for early detection of known mutations and for situations with limited DNA, while sequencing provides a comprehensive view, analyzing single genes or entire genomes for variants linked to genetic disorders.

**Polymerase Chain Reaction (PCR)**

**Function:** Copies a specific DNA sequence millions of times, a process called amplification, to generate enough material for analysis.

**Application in Screening**

1. **Early Detection:** Detects known mutations for disorders like cystic fibrosis or Huntington's disease.
2. **Limited Samples:** Essential for prenatal or single-cell testing where DNA is scarce (e.g., in embryos).
3. **Real-time PCR (qPCR):** Monitors amplification in real-time, allowing for quantification of viral load or gene expression.
4. **Mutation-Specific PCR:** Uses primers to specifically amplify mutated sequences, like ARMS-PCR.

**DNA Sequencing**

**Function**

Determines the precise order of the four nucleotide bases (A, T, C, G) in a DNA strand.

**Application in Screening**

1. **Sanger Sequencing:** The "gold standard" for looking at one or a few genes at a time, often used after PCR amplification.
2. **Next-Generation Sequencing (NGS):** Can sequence millions of DNA fragments at once, enabling whole-genome or whole-exome sequencing to find new or rare mutations.

**Diagnosis:** Identifies specific mutations, insertions, deletions, or rearrangements linked to thousands of genetic disorders.

**Genetic Therapeutics**

**Goal:** Correct faulty genes, restore function, or manage symptoms.

**Approaches**

**Genetic Screening Methods**

Screening is categorized by the timing and purpose of the evaluation:

- **Prenatal Screening:** Identifies fetal risks for chromosomal abnormalities (e.g., Down Syndrome) or single-gene disorders (e.g., Cystic Fibrosis) using Non-Invasive Prenatal Testing (NIPT) or invasive methods like amniocentesis (Baird., 1988).
- **Newborn Screening (NBS):** Mandated population-wide testing for treatable conditions like phenylketonuria (PKU) and spinal muscular atrophy (SMA).
- **Carrier Screening:** Determines if prospective parents carry recessive mutations (e.g., Tay-Sachs) that could be passed to offspring.
- **Adult Risk & Pharmacogenetic Screening:** Assesses susceptibility to late.

| Screening Type | Main Applications | Advantages | Limitations |
|---|---|---|---|
| Prenatal Genetic Screening | Identify fetal genetic diseases or chromosomal abnormalities | Early detection of health problems | Possibility of false positives/negatives, psychological and ethical issues |
| Newborn Genetic Screening | Detect and treat newborn genetic diseases | Timely diagnosis, prevent adverse outcomes | Difficult to interpret results, ethical issues |
| Adult Disease Risk Screening | Assess adult disease susceptibility | Formulate preventive measures | Complex results, psychological impact |
| Cancer Genetic Screening | Early detection of hereditary | Improve prognosis | Uncertain results, psychological burden |

| | | | |
|---|---|---|---|
| | tumors | | |
| Pharmacogenetic Screening | Personalized medication | Improve efficacy, reduce adverse reactions | Challenges in genotype interpretation |

**Gene Therapy**

1. **Gene Addition:** Adds a functional gene to cells (e.g., using viral vectors).
2. **Gene Editing (e.g., CRISPR):** Precisely edits DNA (still largely experimental for many diseases).
3. **Enzyme Replacement Therapy (ERT):** Supplements missing enzymes (e.g., for Pompe, Fabry disease).
4. **Small Molecules/Drugs:** Target specific pathways.

**Examples**

- **Casgevy:** First FDA-approved CRISPR therapy for sickle cell disease/beta-thalassemia.
- **Myozyme:** For Pompe disease (ERT).

**Therapeutic Strategies**

While many genetic disorders are managed through symptom control or diet, advanced therapeutics focus on the genetic cause:

**Gene Therapy**

1. **Gene Addition/Augmentation:** Inserts a functional copy of a gene into cells to produce a missing protein. Examples include Zolgensma for SMA and Luxturna for retinal dystrophy.
2. **Gene Silencing:** Uses antisense oligonucleotides (ASOs) or RNA interference (RNAi) to "turn off" harmful genes.
3. **Gene Editing:** Employs tools like CRISPR/Cas9 to precisely correct a mutation at its endogenous location.
4. **Enzyme Replacement Therapy (ERT):** Provides a manufactured version of a missing enzyme, commonly used for lysosomal storage disorders like Gaucher disease.
5. **Small Molecule Regulators:** Drugs that bypass specific genetic defects without altering DNA, such as "triple therapy" for Cystic Fibrosis (Anahid., 2025).

**Key Approved Gene Therapies (Selected)**

| Condition | Therapy Name | Strategy |
|---|---|---|
| **Spinal Muscular Atrophy (SMA)** | Zolgensma | In vivo Gene Addition |
| **Hemophilia B** | Hemgenix | In vivo Gene Addition |
| **Retinal Dystrophy (RPE65)** | Luxturna | In vivo Gene Addition |
| **β-Thalassemia** | Zynteglo | Ex vivo Stem Cell Therapy |
| **Duchenne Muscular Dystrophy** | Elevidys | In vivo Gene Addition |

**Ethical and Practical Considerations**

- **Informed Consent:** Essential due to the potential for uncertain results (Variants of Uncertain Significance) and psychological impact on families.
- **Access & Cost:** Many gene therapies cost over $1 million per dose, creating significant barriers to equitable delivery.
- **Regulation:** Authorities like the FDA and EMA are adapting trial designs to better evaluate treatments for rare diseases with small patient populations.

***References***

1. Anahid A. Assadourian., Julian A. Martinez-Agosto, Precision diagnostic and therapeutic interventions in rare genetic neurodevelopmental disorders., Pediatric research., 2025,
2. Baird PA, Anderson TW, Newcombe HB, Lowry RB. 1988. Genetic disorders in children and young adults: a population study. Am. J. Hum.
3. CDC, Genomics and health., U.S. Centres for disesses control and prevention., National library of medicine, A review, Screening of single gene genetic disorder., vol 60, issue 6., 2005.
4. Motulsky AG. 2010. History of human genetics. In Vogel and Motulsky's Human Genetics: Problems and Approaches, ed. Speicher MR, Motulsky AG, Antonarakis SE, pp. 13–29. Heidelberg, Ger.: Springer. 4th ed.
5. Rinaldi, B. et al. Smith-Magenis syndrome—clinical review, biological background and related disorders. Genes 13, 335 (2022).
6. Shendure J, Ji H. Next-generation DNA sequencing. Nat Biotechnol. 2008;26(10):1135–1145.
7. Soler A, Morales C, Mademont-Soler I, Margarit E, Borrell A, et al. 2017. Overview of chromosome abnormalities in first trimester miscarriages: a series of 1,011 consecutive chorionic villi sample karyotypes. Cytogenet. Genome Res 152:81–89.
8. Strachan T, Read A. Human Molecular Genetics. 5th ed. New York: Garland Science. 2018.

9. Theodore L Roth., Alexander Marson., Genetic Disease and Therapy, 2021 Jan 24;16:145–166.
10. Vogelstein B, Kinzler KW. Digital PCR. Proc Natl Acad Sci U S A. 1999;96(16):9236–9241.

# RNA Biology and Therapeutics

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**Abstract**

RNA is a versatile molecule with unique properties that allows it to fulfil many different tasks within a living cell. Besides its important role as a messenger between the DNA and protein worlds, RNA can adopt many different secondary and tertiary structures which allow also non-coding RNAs to engage in various cellular processes by serving as scaffolds to assemble protein complexes or acting as sponges that sequester RNAs or proteins These multifunctional properties of RNA as well as its ubiquitous distribution within cells and organisms have attracted much attention recently, especially in the field of medicine. Several initiatives have been launched to explore and leverage the therapeutic potential of RNA. One of the most impressive examples, both in terms of speed and efficacy, was the development of a mRNA vaccine for use in combating the coronavirus disease 2019 (COVID-19) (Chen.,2008). However, unique challenges remain during the drug discovery and manufacturing process, which need to be overcome in order to implement RNA therapeutics and leverage their full potential.

***Keywords:*** DNA, RNA, Protein Synthesis, ASOs, Aptamers.

## Introduction

Ribonucleic acid (RNA) is a molecule that is present in the majority of living organisms and viruses. It is made up of nucleotides, which are ribose sugars attached to nitrogenous bases and phosphate groups. The nitrogenous bases include adenine, guanine, uracil, and cytosine. RNA mostly exists in the single-stranded form, but there are special RNA viruses that are double-stranded. The RNA molecule can have a variety of lengths and structures. An RNA virus uses RNA instead of DNA as its genetic material and can cause many human diseases. Transcription is the process of RNA formation from DNA, and translation is the process of protein synthesis from RNA. The means of RNA synthesis and the way that it functions differs between eukaryotes and prokaryotes. Specific RNA

molecules also regulate gene expression and have the potential to serve as therapeutic agents in human diseases (Raina., 2014).
RNA biology studies the diverse roles of ribonucleic acid (RNA) in cells, while RNA therapeutics leverage these molecules (like mRNA, siRNA, ASOs) to treat diseases, offering new avenues beyond traditional drugs by directly targeting genetic pathways, exemplified by the success of COVID-19 vaccines and expanding into cancer, genetic disorders, and neurological conditions, though delivery and stability remain key challenges.

**Types of RNA**
Three main types of RNA are involved in protein synthesis. They are messenger RNA (mRNA), transfer RNA (tRNA), and ribosomal RNA (rRNA).

**mRNA**
mRNA is transcribed from DNA and contains the genetic blueprint to make proteins. Prokaryotic mRNA does not need to be processed and can proceed to synthesize proteins immediately. In eukaryotes, a freshly transcribed RNA transcript is considered a pre-mRNA and needs to undergo maturation to form mRNA. A pre-mRNA contains non-coding and coding regions known as introns and exons, respectively. During pre-mRNA processing, the introns are spliced, and the exons are joined together. A 5' cap known as 7-methylguanosine is added to the 5' end of the RNA transcript, and the 3' end is polyadenylated. Polyadenylation refers to the process where a poly(A) tail, which is a sequence of adenine nucleotides, is added to the transcript. The 5' cap protects the mRNA from degradation, and the 3' poly(A) tail contributes to the stability of mRNA and aids it in transport (Crick., 1958). Researchers are also studying mRNA as an anti-cancer treatment due to its ability to modify cells.

**tRNA**
tRNAs are RNA molecules that translate mRNA into proteins. They have a cloverleaf structure that consists of a 3' acceptor site, 5' terminal phosphate, D arm, T arm, and anticodon arm. The primary function of a tRNA is to carry amino acids on its 3' acceptor site to a ribosome complex with the help of aminoacyl-tRNA synthetase. Aminoacyl-tRNA synthetases are enzymes that load the appropriate amino acid onto a free tRNA to synthesize proteins. Once an amino acid is bound to tRNA, the tRNA is considered an aminoacyl-tRNA. The type of amino acid on a tRNA is dependent on the mRNA codon, which is a sequence of three nucleotides that codes for an amino acid. The anticodon arm of the tRNA is the site of the anticodon, which is complementary to an mRNA codon and dictates which amino acid to carry. tRNAs also regulate apoptosis by acting as a cytochrome c scavenger (Tony., 2024)

**RNA**

rRNA forms ribosomes, which are essential in protein synthesis. A ribosome contains a large and small ribosomal subunit. In prokaryotes, a small 30S and large 50S ribosomal subunit make up a 70S ribosome. In eukaryotes, the 40S and 60S subunit form an 80S ribosome. The ribosomes contain an exit (E), peptidyl (P), and acceptor (A) site to bind aminoacyl-tRNAs and link amino acids together to create polypeptides (David Wang., 2023)

**The Role of RNA in Biology**

Beyond its well-known role as a messenger (mRNA) carrying genetic information from DNA to the protein-making machinery (ribosomes), RNA performs a vast array of structural, regulatory, and catalytic functions. These include:

- **Protein Synthesis:** Messenger RNA (mRNA) provides the blueprint, while ribosomal RNA (rRNA) forms the core of ribosomes and transfer RNA (tRNA) brings the correct amino acids, collectively orchestrating protein production.
- **Gene Regulation:** Non-coding RNAs (ncRNAs), such as microRNAs (miRNAs), small interfering RNAs (siRNAs), and long non-coding RNAs (lncRNAs), modulate gene expression by influencing mRNA stability, translation, or chromatin structure.
- **RNA Processing and Editing:** Small nuclear RNAs (snRNAs) are essential components of the spliceosome, which removes non-coding introns from pre-mRNA. Other processes include chemical modifications (epitranscriptomics) and editing (A-to-I substitutions) that add further layers of control and diversity (Cohen., 2010).
- **Catalysis:** Some RNA molecules, called ribozymes, possess enzymatic activity, such as in RNA splicing and protein synthesis, highlighting the ancient "RNA world" hypothesis.

**RNA Therapeutics: A New Class of Drugs**

The in-depth understanding of RNA biology has enabled the development of various RNA-based therapeutic strategies. These therapies can be broadly classified into several categories:

- **Messenger RNA (mRNA) Therapeutics:** These involve introducing synthetic mRNA into cells, usually encapsulated in lipid nanoparticles (LNPs), to produce a desired protein.
    - **Application:** The most prominent examples are the highly effective COVID-19 vaccines (Pfizer-BioNTech and Moderna), which encode the SARS-CoV-2 spike protein to elicit an immune response. mRNA is also

being explored for protein replacement therapies for genetic diseases and cancer immunotherapies (Charles., 2022)

- **Antisense Oligonucleotides (ASOs):** These are short, single-stranded synthetic nucleic acids designed to bind to target RNA sequences and modulate gene expression. They can trigger the degradation of target mRNA (via RNase H) or alter RNA splicing patterns.
  - **Application:** Approved drugs include Nusinersen (Spinraza), which treats spinal muscular atrophy by promoting correct splicing of the SMN2 gene, and Mipomersen for severe hypercholesterolemia.
- **Small Interfering RNA (siRNA):** These double-stranded RNA molecules harness the cell's natural RNA interference (RNAi) pathway to specifically degrade target mRNA, thereby silencing gene expression.
  - **Application:** FDA-approved drugs include Patisiran for hereditary transthyretin amyloidosis, which was the first siRNA therapeutic to be approved.
- **Aptamers:** These are single-stranded RNA (or DNA) molecules selected to bind with high affinity and specificity to a variety of molecular targets, such as proteins, peptides, or even whole cells, functioning similarly to antibodies. There are at least 6 RNA-based aptamers or decoys that have been clinically tested including a VEGF-specific modified RNA aptamer (Macugen by Pfizer/Eyetech) that is now an FDA approved drug for the treatment of AMD (Keefe et al., 2010; Sanghvi, 2011; Thiel and Giangrande, 2009). In addition to their antibody-like abilities to inhibit or activate the functions of protein targets, aptamers also offer novel functions as therapeutic and diagnostic agents. By utilizing Watson-Crick pairing of nucleic acids, RNA aptamers can be engineered to undergo conformational changes in the presence and/or absence of other effector RNAs both in vitro and in vivo.
  - **Application:** Pegaptanib (Macugen) is an FDA-approved aptamer that targets VEGF to treat age-related macular degeneration.

**Catalytic RNAs: Ribozymes**

Ribozymes are catalytic RNAs that function as enzymes and do not require proteins for catalysis. Most known natural ribozymes are self-processing RNAs that catalyze RNA cleavage and ligation reactions. However, the substrate recognition domain of ribozymes can be artificially engineered to stimulate site-specific cleavage in cis (the same nucleic acid strand) or trans (a noncovalently linked nucleic acid) (Scherer and Rossi, 2003). Moreover, ribozymes are amenable to in vitro selection and directed evolution to generate improved properties and new functions for therapeutic and diagnostic reagents. Ribozymes can be engineered to be allosterically activated by effector molecules, which has led to the development of artificial “riboswitches” as biosensors and synthetic

biological tools (Liang et al., 2011; Wieland et al., 2010). There are numerous types of ribozymes in biology, but the most common ribozyme therapeutics are derived from either "hammerhead" or "hairpin/paperclip" motifs.
Several ribozymes against HIV have been clinically tested using a gene therapy-based approach in CD4+ T cells or CD34+ hematopoietic stem cells (HSCs), which differentiate into various hematopoietic lineages including CD4+ T cells (Amado et al., 2004; Macpherson et al., 2005; Michienzi et al., 2003; Wong-Staal et al., 1998).

**CRISPR-based Therapies:** This genome-editing technology uses a guide RNA (gRNA) to direct the Cas protein to a specific DNA sequence, allowing for precise genetic modifications to correct disease-causing mutations.

**Challenges and Future Directions**

Despite significant progress, challenges remain, primarily concerning efficient and targeted delivery of RNA molecules to specific tissues within the body and managing potential immune responses. The use of advanced nanocarriers, such as lipid nanoparticles (LNPs) and engineered exosomes, along with chemical modifications to enhance stability, is helping to overcome these hurdles. (Cho., 2009). The field is expanding rapidly, with research ongoing into circular RNAs (circRNAs) for more stable protein expression, small activating RNAs (saRNAs) to upregulate gene expression, and the integration of artificial intelligence (AI) to accelerate drug discovery and personalize treatments.

**Types of RNA Based Therapeutics**

Messenger RNA (mRNA) plays a central and essential role in protein production by acting as the carrier of the genetic code from the DNA to the cellular machinery that synthesizes proteins. The overall process involves two main stages: transcription and translation, which form the core of the central dogma of molecular biology (Charles., 2024)

**Modern Applications**

mRNA technology is used in vaccines (like COVID-19 vaccines) to teach our cells to make viral proteins, triggering an immune response.
It's also explored for cancer therapy and producing therapeutic proteins. mRNA (messenger RNA) has emerged as a revolutionary platform for vaccines and therapeutics. Harnessing the power of mRNA technology scientists have developed RNA vaccines and therapeutics with remarkable potential (Damase., 2024).
mRNA (messenger RNA) interacts closely with the immune system, playing a significant role in immune responses. mRNA vaccines stimulate the immune system by delivering synthetic mRNA encoding specific antigens. This triggers

the production of antibodies, which recognize and neutralize the targeted pathogen. The immune system also activates T cells, which further aid in immune defense.

Vaccines based on mRNA have gained prominence, particularly in the context of combating infectious diseases such as COVID-19 caused by the SARS-CoV-2 coronavirus. mRNA vaccines work on the basis of synthetic mRNA molecules encoding specific antigens to stimulate an immune response (e.g. to the spike protein). (Tony., 2024). These vaccines, like those developed by Moderna and Pfizer-BioNTech during the COVID-19 pandemic, offer advantages in terms of speed, scalability, side effects and adaptability

In the field of therapeutics, mRNA-based approaches hold immense promise. By introducing synthetic mRNA into cells, researchers can direct the production of therapeutic proteins, enabling treatment for various diseases. Furthermore, mRNA therapeutics have the potential to modulate immune responses. By introducing specific mRNA molecules, the production of immune-related proteins can be regulated, influencing immune system function and inflammation. This novel approach offers potential advantages such as personalized medicine, targeted therapies, and rapid development (Bernstein., 2001).

mRNA vaccines and therapeutics represent a paradigm shift in medical interventions. They leverage the cell's natural machinery to produce desired proteins, providing new avenues for preventive and therapeutic applications (Sunjoo., 2023).

**Advances in mRNA technology**

Recent years have witnessed remarkable advancements in mRNA (messenger RNA) technology, revolutionizing the fields of medicine and biotechnology.

**mRNA Vaccine Development**: mRNA vaccines, such as those developed by Moderna and Pfizer-BioNTech, have emerged as a game-changer in vaccine development. Their rapid development and effectiveness against infectious diseases, including COVID-19, demonstrate the potential of mRNA technology (Van., 2013).

**Personalized Medicine:** mRNA technology offers the promise of personalized medicine. By tailoring mRNA sequences to individual patients, it becomes possible to develop therapies that specifically target their unique genetic setup.

**mRNA Stability and Delivery:** Scientists have made significant progress in enhancing the stability of mRNA molecules, enabling their efficient delivery to target cells. Techniques like controlled freezing of mRNA have paved the way

for the development of mRNA-based therapies with improved effectiveness (Berk., 2012).

**Sustainability:** mRNA, or associated areas such as oligonucleotide manufacturing, may affect sustainability aspects. The integration of single-use technologies in mRNA manufacturing has facilitated streamlined and efficient production processes. Single-use bioreactors, mixers, and disposable systems can contribute to improved scalability, flexibility, cost-effectiveness and sustainability.

**ASO:** The ASOs are short single-stranded (ss) oligonucleotides (12–24 nucleotides) complementary to the specific RNA through Watson–Crick base-pairing. They can alter RNA and reduce, restore, or modify protein expression. ASOs are divided into occupancy-mediated degradation and occupancy-only models based on different post-hybridization mechanisms.

**Antisense Oligonucleotides (Asos) Can Modulate the Target Gene Expression Through Two Mechanisms**.

1. In the occupancy-mediated degradation way, ASOs trigger the target mRNA cleavage by RNase H1 or ribozymes. The Occupancy-only mechanisms do not directly degrade target RNA.
2. Instead, it regulates the gene expression in several ways:
3. Alter RNA splicing using splice-switching ASOs to induce exon skipping or exon inclusion; lead to nonsense-mediated mRNA decay (NMD); inhibit or activate translation; block the microRNAs binding to target mRNA.

**RNA Interference (RNAi)**

Long double-stranded RNA (dsRNA) and precursor microRNA (pre-miRNA) are processed by Dicer into short interfering RNA (siRNA). The antisense strand (indicated as a blue strand) of siRNA is loaded into the RNA-induced silencing complex (RISC) for RNA targeting, degradation or translation repression. (S Jeong., 2023)

**CRISPR/Cas-Based RNA Editing System**

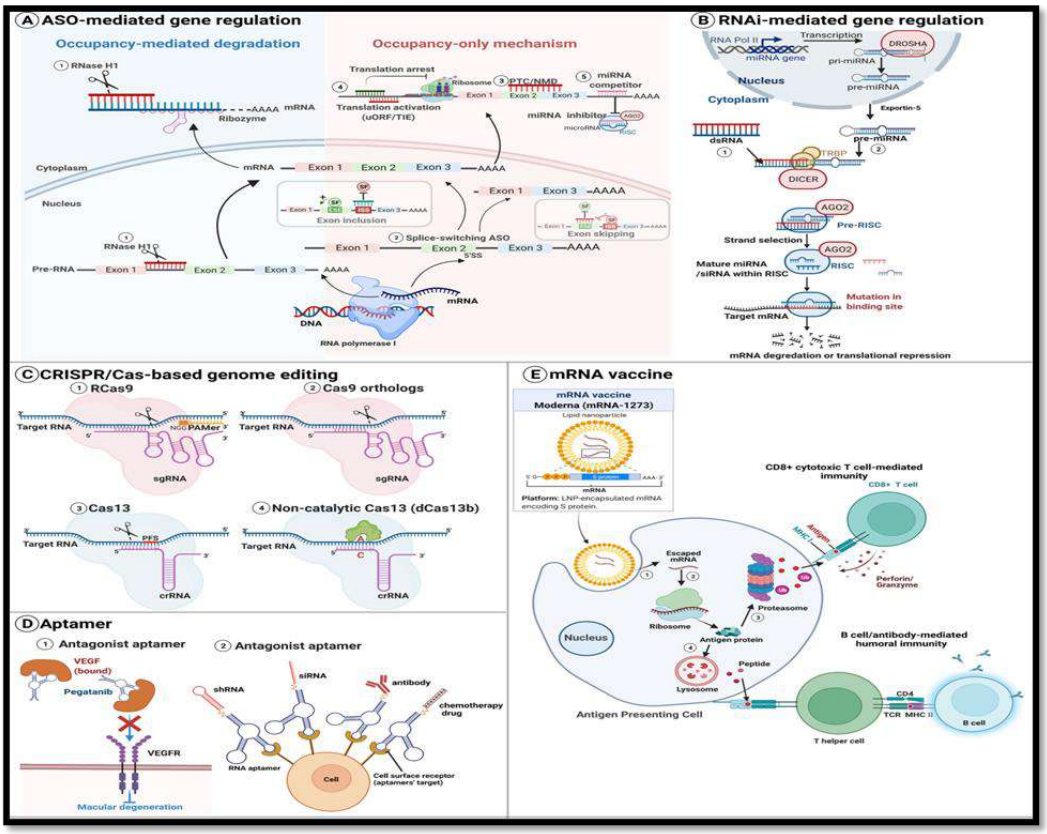
Includes two Cas nuclease categories, Cas9 and Cas13. A guide RNA (gRNA) binds to Cas9 to cleave ssRNA with or without the help of a protospacer-adjacent motif (PAM). A single CRISPR RNA (crRNA) guides Cas13 to target specific RNA having a protospacer flanking sequence (PFS). In addition to knockdown target RNA, a catalytically deactivated Cas13b (dCas9b) facilitates the A-to-I editing with ADAR.

**RNA Aptamers**

Function as agonizts or delivery agents.

1. As an antagonist aptamer, Pegaptanib interacts explicitly with vascular endothelial growth factor (VEGF) to inhibit the interaction of VEGF with its receptors, thus treating macular degeneration.
2. Cell type-specific RNA aptamers deliver agents (miRNA, siRNA, shRNA, antibody and chemotherapy drugs) by linking to or conjugating (Michael Eder., 2022)

***Fig. 1: Types of RNA-based therapeutics and modes of action.***




## Challenges and Future Directions

Despite significant progress, challenges remain, primarily concerning efficient and targeted delivery of RNA molecules to specific tissues within the body and managing potential immune responses. The use of advanced nanocarriers, such as lipid nanoparticles (LNPs) and engineered exosomes, along with chemical modifications to enhance stability, is helping to overcome these hurdles. The field is expanding rapidly, with research ongoing into circular RNAs (circRNAs) for more stable protein expression, small activating RNAs (saRNAs) to upregulate gene expression, and the integration of artificial intelligence (AI) to accelerate drug discovery and personalize treatments.

## Conclusion

Recent advances of biological drugs have broadened the scope of therapeutic targets for a variety of human diseases. This holds true for dozens of RNA-based therapeutics currently under clinical investigation for diseases ranging from genetic disorders to HIV infection to various cancers. These emerging drugs, which include therapeutic ribozymes, aptamers, and small interfering RNAs (siRNAs), demonstrate the unprecedented versatility of RNA. However, RNA is inherently unstable, potentially immunogenic, and typically requires a delivery vehicle for efficient transport to the targeted cells. These issues have hindered the clinical progress of some RNA-based drugs and have contributed to mixed results in clinical testing. Nevertheless, promising results from recent clinical trials suggest that these barriers may be overcome with improved synthetic delivery carriers and chemical modifications of the RNA therapeutics (John., 2012).

## *References*


1. A Lint S, Heirman C, Thielemans K, Breckpot K. mRNA: From a chemical blueprint for protein production to an off-the-shelf therapeutic. Hum Vaccin Immunother. 2013 Feb;9(2):265-74.
2. Annu. Rev. Pharmacol. Toxicol. 2010; 50:237-257, Burnett, J.C. · Rossi, J.J., Stem cells, ribozymes and HIV., Gene Ther. 2009; 16:1178-1179
3. Berk, P.D., An editor's look-back, Hepatology. 2006; 43: S13-S30
4. Bernstein, E. · Caudy, A.A. · Hammond, S.M., Role for a bidentate ribonuclease in the initiation step of RNA interference, Nature. 2001; 409:363-366
5. Biotechnol. J. 2011; 6:1130-1146 Castanotto, D. · Rossi, J.J.The promises and pitfalls of RNA-interference-based therapeutics, Nature. 2009; 457:426-433
6. Bouchard, P.R. · Hutabarat, R.M. · Thompson, K.M., Discovery and development of therapeutic aptamers
7. Brenner S., Jacob F., Meselson M. An unstable intermediate carrying information from genes to ribosomes for protein synthesis. Nature. 1961; 190:576–581. doi: 10.1038/190576a0.
8. Burnett, J.C. · Rossi, J.J. · Tiemann, K.,Current progress of siRNA/shRNA therapeutics in clinical trials
9. Castanotto, D. · Sakurai, K. · Lingeman, R., Combinatorial delivery of small interfering RNAs reduces RNAi efficacy by selective incorporation into RISC, Nucleic Acids Res. 2007; 35:5154-5164
10. Charles H. Jones, John R. Androsavich, Nina So, Matthew P. Jenkins, Derek MacCormack et.al, Breaking the mold with RNA—a “RNAissance” of life science, Genomic medicines, 2, 2024.
11. Chen, C.H. · Dellamaggiore, K.R. · Ouellette, C.P., Aptamer-based endocytosis of a lysosomal enzyme Proc. Natl. Acad. Sci. USA. 2008;

105:15908-15913
12. Cho, W.G. · Albuquerque, R.J. · Kleinman, M.E., Small interfering RNA-induced TLR3 activation inhibits blood and lymphatic vessel growth Proc. Natl. Acad. Sci. USA. 2009; 106:7137-7142
13. Cohen, M.G. · Purdy, D.A. · Rossi, J.S.,First clinical application of an actively reversible direct factor IXa inhibitor as an anticoagulation strategy in patients undergoing percutaneous coronary intervention, Circulation. 2010; 122:614-622
14. Crick F.H. On protein synthesis. Symp. Soc. Exp. Biol. 1958; 12:138–163.
15. Damase T.R., Sukhovershin R., Boada C., Taraballi F., Pettigrew R.I., Cooke J.P. The limitless future of RNA therapeutics. Front. Bioeng. Biotechnol. 2021; 9:628137.
16. Dannull, J. · Lesher, D.T. · Holzknecht, R. Immunoproteasome down-modulation enhances the ability of dendritic cells to stimulate antitumor immunity. Blood. 2007; 110:4341-4350
17. David Wang; Aisha Farhana. Biochemistry, RNA structure, National library of Medicine., 2023.
18. John C. Burnett · John J. Rossi jrosss., 2012., RNA-Based Therapeutics: Current Progress and Future Prospects., Cell chemical biology., Volume 19, Issue 1P60-71.
19. Raina M, Ibba M. tRNAs as regulators of biological processes. Front Genet. 2014; 5:171. [PMC free article] [PubMed]
20. Sunjoo Jeong, RNA in biology and therapeutics., National Library of Medicines., 2023 46(1):1–2.
21. Tony Guchner., RNA therapeutics, RNA biology, Taylor and Francis., vol 21, IssUE 1. 2024.
22. Van Lint S, Heirman C, Thielemans K, Breckpot K. mRNA: From a chemical blueprint for protein production to an off-the-shelf therapeutic. Hum Vaccin Immunother. 2013 Feb;9(2):265-74. [PMC free article] [PubMed]
23. Yum K, Wang ET, Kalsotra A. Myotonic dystrophy: disease repeat range, penetrance, age of onset, and relationship between repeat size and phenotypes. Curr Opin Genet Dev. 2017 Jun; 44:30-37. [PMC free article] [PubMed]

# A Review On 3D Printing Technology in Biopharmaceutics and Its Techniques

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## Abstract

In the biopharmaceutical sector, 3D printing, also referred to as additive manufacturing, has emerged as a revolutionary technology that allows the creation of complex systems for delivering medications and customized drugs.

This chapter presents an in-depth investigation of the development, applications, and techniques of 3D printing in the biopharmaceutical and pharmaceutical industries. Fused Deposition Modeling (FDM), Inkjet printing, Stereolithography (SLA), Selective Laser Sintering (SLS), and Pressure-Assisted Microsyringe (PAM) are among the printing techniques covered, with an emphasis on their importance in the production of pharmaceuticals. While discussing pertinent regulatory issues, the chapter also explores the variety of materials utilized, including hydrogels, polymers, biodegradable scaffolds, and active pharmaceutical ingredients (APIs).

Personalized dose forms, controlled release systems, implantable variations, and bioprinting for tissue engineering are among the significant uses under investigation. Real-world case studies, like the FDA-approved 3D printing drug Spriam®, demonstrate the usefulness and promise of this technology. The chapter also provides a critical analysis of the regulatory landscape and quality control issues pertaining to 3D printed goods. It also draws attention to the consequences of upcoming developments in personalized biomedicine, including the incorporation of artificial intelligence, smart materials, and 4D printing. The unique significance of 3D printing in enhancing drug delivery techniques and impacting the future of healthcare is generally highlighted in this chapter.

## Introduction

In the field of biopharmaceutics, 3D printing, also known as additive manufacturing, is a cutting-edge technology that makes it easier to produce precise and customizable medication dosage forms and biomedical devices. A useful platform for the development of personalized medicine, this layer-by-layer manufacturing technique offers unmatched control over drug mix, release patterns, and geometric arrangements.(1) 3D printing is being researched in the field of biopharmaceutics to produce medication delivery devices, implants, and tablets tailored to individual patients. It supports complex formulations, such as medication combinations and controlled release techniques. In terms of resolution, material compatibility, and scalability, widely used technologies including stereolithography, inkjet printing, and fused deposition modeling (FDM) each provide unique benefits.

More effective and customized treatments are being made possible by this innovation, particularly for individuals with special medical requirements, including those in the pediatric or elderly populations. 3D printing has enormous potential to transform pharmaceutical manufacturing and individualized treatment as material science advances and regulatory frameworks change. It is possible to immediately 3D print dentures, splints, surgical guides, and both temporary and permanent restorations. The first 3D printed medication, Spritam® (levetiracetam), was approved by the Food and Drug Administration (FDA) in

2015. The dosage form of Spritam® is orodispersible tablets (ODT). Personalized medicine, controlled medication release, complex dose forms, and tissue engineering are the main uses. (2)

**Historical Overview of 3D Printing**

The concept of 3D printing or additive manufacturing, began in the early in the early 1980s with Charles Hull's invention of stereolithography (SLA) in 1984. This innovation enabled the creation aided design (CAD) models. (3) Initially used in industrial prototyping, the technology gradually attracted attention for its potential in medical and pharmaceutical applications. In 1990s, various methods such as fused deposition modelling (FDM), selective laser sintering (SLS), and inkjet printing were developed, laying the foundation for pharmaceutical use. For drug delivery and tissue engineering, researchers started experimenting with printing biomaterials, cells, and medications into precise forms and architectures. 3D printing made its debut in the pharmaceutical industry in the early 2000s, enabling the creation of personalized dosage forms with exact control over drug release, geometry, and dosage strength. This signaled the emergence of customized medicine, which customizes care to meet the needs of each patient. (4)

When the U.S. FDA approved Spritam® (levetiracetam), the first 3D-printed medication made by Aprecia Pharmaceuticals using ZipDose® technology, in 2015, it was a significant milestone. (3) The very porous tablets quickly dissolved, proving the viability of 3D printing in large-scale pharmaceutical production and stimulating more investigation in this field. Applications were further extended to include orodispersible films, transdermal patches, controlled release implants, and multilayer tablets. Additionally, the technology allowed hospitals and pharmacies to produce patient-specific medications on demand. employing bioinks made of living cells and biomaterials.

The researchers began employing 3D printing to create living tissues, scaffolds, and organ models. This development significantly decreased the need for animal testing and increased the precision of preclinical research by creating new opportunities for drug testing, illness modeling, and regenerative medicine.Biopharmaceutics has advanced more quickly in recent years thanks to the combination of artificial intelligence (AI), machine learning, and computer-aided drug design with 3D printing. Targeted drug delivery systems and customized therapeutic devices can now be developed because to the ability of modern 3D printers to create extremely complex structures with nanoscale precision. One of the most revolutionary developments in biopharmaceutics today is 3D printing, which connects pharmaceutical sciences, biomedical engineering, and individualized healthcare. It started out as a prototyping tool and has developed into a complex platform that has the potential to completely

change how medications are created, produced, and delivered to patients. Rapid manufacture of medical goods was aided by COVID-19 3D printing. (5)

**Importance of 3D Printing in the Pharmaceutical and Biopharmaceutical Sectors:**

3D printing's significance for the pharmaceutical and biopharmaceutical industries in pharmaceutics and biopharmaceutics, 3D printing, also known as additive manufacturing, has become a transformative technology with many benefits and new opportunities in drug development, manufacturing, and personalized treatment. Several important perspectives can be used to understand the significance of 3D printing in different sectors:

**1. Personalized Treatment and Customization**

3D printing makes it possible to create drug dosage forms that are perfectly tailored to each patient's needs, including dose, shape, size, and release profiles. In situations where traditional dosage forms are ineffective or inconvenient, this is particularly crucial for children, the elderly, and patients who need polypharmacy. Patient outcomes and adherence are enhanced by personalization. (6)

**2. Complex and Multidrug Formulations**

It enables the creation of complex geometries and multicompartmental dosage forms, including pills, which combine several drugs with various release rates in a single tablet. This simplification of medication schedules maintains efficient drug delivery while streamlining therapy. (7)

**3. Quick Prototyping and Flexible Manufacturing**

3D printing speeds up drug development timeframes and allows for on-demand manufacturing by facilitating quick prototyping and small batch production. This adaptability can improve responsiveness to market demands or clinical trial requirements while lowering costs and waste. (8)

**4. Controlled and Targeted Drug Release**

By adjusting internal structure porosity and infill density, the layered manufacturing technique enables precise control of drug release kinetics. Innovations in targeted, sustained, and regulated medication delivery systems are encouraged by this capacity. (9)

**5. Innovative Drug Delivery Systems**

3D printing facilitates the development of cutting-edge drug delivery devices, such as microneedles, bioresorbable implants, and scaffold-based tissue engineering products, extending the therapeutic applications beyond conventional dosage forms. (10)

### 6. Integration with Bioprinting and Regenerative Medicine

In biopharmaceutics, 3D bioprinting integrates biomaterials and living cells to create organ-on-a-chip models and tissue constructs, enabling drug screening and regenerative treatments. This combination promotes advancements in personalized treatment and improves precision medicine. (11)

### 7. Overcoming Conventional Manufacture's Limitations

Conventional pharmaceutical manufacture uses a lot of tools and batch processes, which are frequently rigid and expensive to modify. By enabling decentralized, patient-centric production and reducing inventory concerns, 3D printing solves these problems. (12)

### 8. Regulatory and Quality Benefits

The FDA approved the first 3D printed medication (Spritam®), demonstrating how new regulatory frameworks recognize the promise of 3D printing. In 3D printing, quality by design techniques improve reproducibility and traceability. (13)

## Technology Used 3D Printing

### 1. SLA Technology (Stereo Lithographic)

SLA technology is based on the principle of photo polymerization and uses laser scanning to harden liquid resin to manufacture 3D-printed objects layer by layer [14]. Stereo lithographic builds plastic parts or objects one layer at a time by tracing a laser beam on the surface of a vat of liquid photopolymer, inside of which is a movable stage to support the part being built. The photopolymer layers. The self-adhesive property of the material causes each succeeding layer to bond to the previous one and thus form a complete, three-dimensional objects out of many layers. Object which has overhangs or undercuts must be supported during the fabrication process by support structures. These are either manually or automatically designed with a computer program specifically developed for rapid prototyping. Once complete, the part is elevated above the vat and drained. Excess polymer is swabbed or rinsed away from the surfaces. In many cases, a final cure is given by placing the part in a UV oven. After the final cure, supports are cut off the part and surfaces are polished, sanded or otherwise finished. [15] quickly solidifies wherever the laser beam strikes the surface of the liquid. The platform is lowered by a distance equal to layer thickness (Typically 0.003-0.002 in), and a subsequent layer is formed on top of the previously completed

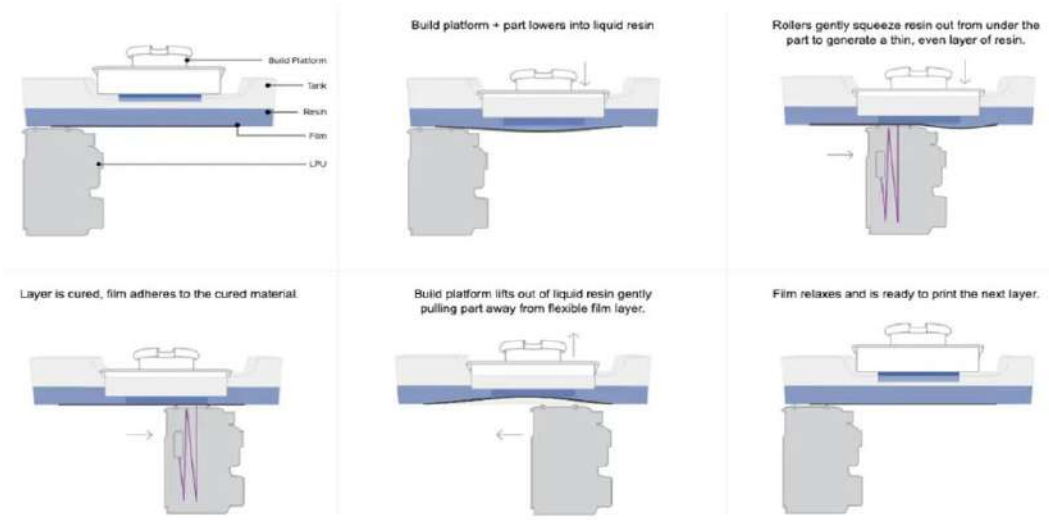


***Figure 1: SLA Printing builds layers***

**2. Inject Printing (Drop- On- Demand)**

Three-dimensional inkjet printing (IJP) is a material jetting technique in additive manufacturing (AM) [16]. AM includes different approaches to create components layer-by-layer. Apart from jetting processes, where liquid photopolymers or powder particles are solidified/bonded through an UV light source or a bonding agent, laser-based or extrusion-based methods are also commonly found in 3D printing. The former melts the powdered material layer-wise by a laser beam, while the latter heats up a filament-formed polymer, which is then extruded through a nozzle and deposited in a predefined path onto a building platform [17]. In the IJP process, a liquid photopolymer is jetted onto a substrate and immediately cured by an ultraviolet (UV) light source. Then, the platform with the substrate moves down and a new layer of drops is applied, cured, and connected to the already built part. In order to create overhang or a new layer after a hollow form, a support material is necessary. Hence, each printer contains at least two different print heads; each is equipped with a varying number, e.g., 16 to 1024, of linearly arranged nozzles [18].

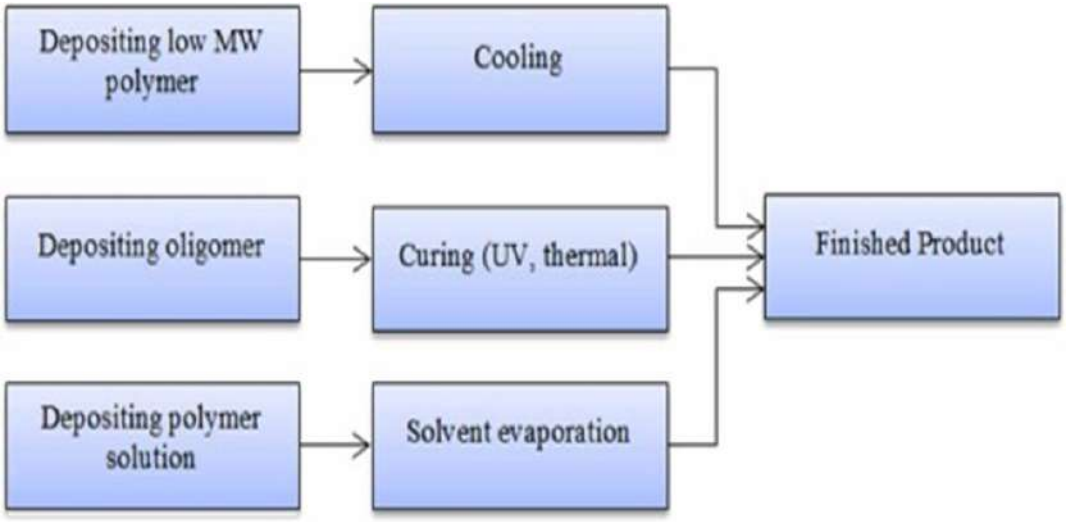


***Figure: 2 Methods of solidify polymer droplets in 3D printing***

**1. Fused Deposition Modelling (FDM)**

FDM is based on the melting and extrusion of a polymer filament. The filament is fed into and melted in a heated metal cylinder ending in a nozzle. As fresh filament is supplied continuously into this component, the molten polymer is pushed out of the nozzle, forming a thread roughly the size of the nozzle diameter [19]. Fused deposition modelling is basically based on the hot melt extrusion process, where the material is deposited layer by layer, according to the 3D model data, until a whole object is formed. The FDM process typically feeds a filament feedstock, with a diameter of 1.75 mm or 3.00 mm, into a printer via a circulating drive gear mechanism. A stepper motor is connected to one of the drive gears to provide the energy for moving the filament through the system. One or both of the drive gears may have a grooved or toothed surface to create sufficient friction for the drive gear to grab the filament and feed it to the liquefier without any slippage. The filament is then melted at the heated liquefier, while the solid portion at the back will act as a piston to push the melt material through the print nozzle [20]. PLA (Polylactic Acid) is amongst the most commonly available and the easiest material used in FDM. Since it is biodegradable, rigid and strong, it is used for concept modelling. The disadvantage of this material is that it is brittle and less resistant to heat and chemicals. ABS (Acrylonitrile Butadiene Styrene) Manufactured from the monomer Acrylonitrile, 1, 3- Butadiene and Styrene, ABS is a frequently used material which is known for its toughness and durability. This material is mainly used for functional prototyping but requires a heated bed for printing and ventilation. PETG (Polyethylene Terephthalate Glycol) PETG is mainly used for snap-fit components and is compatible with lower printing temperatures. It is

chemical resistant and has high transparency [21].

**2. SLS (Selective Laser Sintering)**

Selective Laser Sintering (SLS), an additive manufacturing process, uses a strong laser to fuse small powdered material particles into a solid, three-dimensional product. To begin the process, a thin layer of powder material—typically a polymer like nylon (polyamide)—is evenly deposited throughout the build platform. A computer-controlled laser that scans the surface in line with the digital 3D model then selectively sinters (melted and fused together) the powder particles in the required regions. When a layer is complete, the platform lowers slightly and is covered with a new layer of powder.Until the complete thing is constructed, this process is repeated layer by layer [22]. One of the main benefits of SLS is that it doesn't need any support structures because the printed object is naturally supported by the surrounding unsintered powder. This makes it possible to create elaborate interior structures and complex geometries. Because of its strength, endurance, and superior mechanical qualities, SLS is frequently utilized for functional prototypes, end-use parts, and components in industries like aerospace, automotive, and healthcare. Nylon (PA 11, PA 12), glass-filled nylon, and alumide (a mixture of aluminum and nylon powder) are common materials used in SLS. [23]

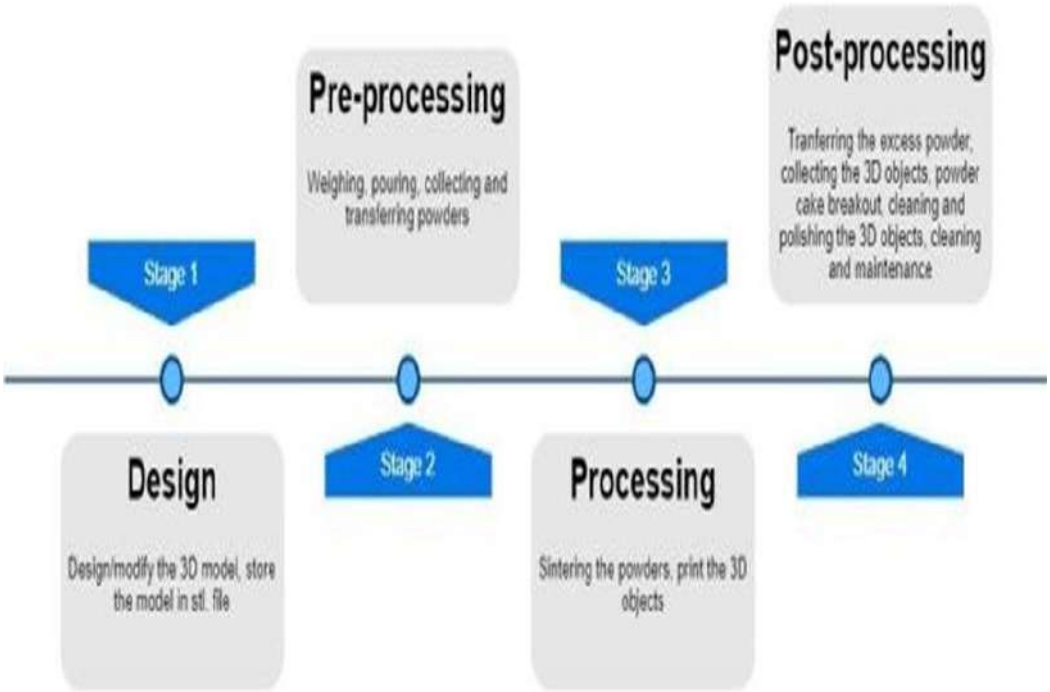


***Figure: 3 Typical Steps of SLS Process***

### 1. SSE (Semi – Solid Extrusion)

SSE is an additive manufacturing technology that deposits semisolid material layer by layer, where the extrusion head moves and extrudes the semisolid material in a set trajectory, stacking layers on top of each other until the product is printed [24]. The technology is based on FDM, with the difference that the print material used in this technology is in a semisolid form at room temperature, so the temperature should be controlled when heating to avoid too much material softening due to high temperatures and not being able to retain its shape during deposition. When printing, the print material is contained in a special syringe, and its extrusion can be driven by pneumatic pressure, mechanical energy, or an electromagnetic system [25]. Various materials can be used in SSE technology depending on the application. Commonly used materials include paper, plastic films (like PVC or ABS), and metal foils such as aluminium or titanium. Paper-based sheet lamination is inexpensive and used mainly for concept models, while metal-based methods are employed for producing functional parts that need greater strength and durability. [26]

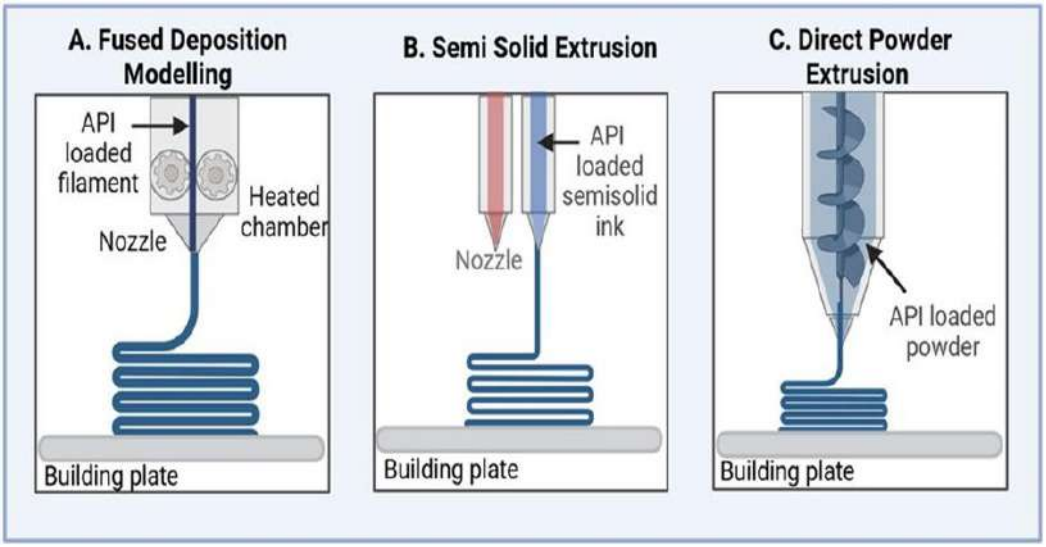


***Figure: 4 Extrusion based 3D printing***

### Materials Used In 3D Printing Technology

### Hydrogels

Hydrogels are usually categorized as either static or dynamic, depending on their capability to respond upon external stimulus. Static hydrogels are usually cross-linked via inflexible covalent bonds and can maintain stable physical and chemical properties in different conditions. In contrast, dynamic hydrogels have been designed to respond to a diversity of external stimuli such as pressure, strain, temperature, light, pH, ions, and magnetic field, giving rise to appealing

properties such as self-healing, self-deformation, or programmable actuating performance without catastrophic structural collapse [27]. Hydrogels formed from LMWGs can be prepared under different conditions to give different mechanical properties. Using hydrogels as cell carriers has also been shown to improve cell viability during bio-printing [28].

**Polymers**

3D printing technologies are widely used for the production of polymer components from prototypes to functional structures with difficult geometries. By using fused deposition modelling (FDM), it can form a 3D printed through the deposition of successive layers of extruded thermoplastic filament, such as polylactic acid (PLA), acrylonitrile butadiene styrene (ABS), polypropylene (PP) or polyethylene (PE). Lately, thermoplastics filaments with higher melting temperatures such as PEEK and PMMA can already be used as materials for 3D printing technology. 3D printing polymer materials in liquid state or with low melting point are widely used in 3D printing industry due to their low cost, low weight and processing flexibility. Mostly, the materials of polymers played important role in biomaterials and medical device products often as inert materials, by contributing to the efficient functioning of the devices as well as providing mechanical support in many orthopaedic implants [29]. PLA is considering a sustainable polymer. PLA fabricated by renewable materials like corn starch. PLA can be considering the easiest polymers to print, because it has ability to shrink slightly after finishing the process of 3D printing. This is considering as a type of a non-Newtonian pseudo-plastic liquid.[30].

**Resins**

The materials used in photocuring 3D printing is photosensitive resin. The photocuring mechanism would be chosen depending on the wavelength of the lamp and printing technology. Generally, the photosensitive resin which used in the SLA technique is based on the mechanism of cationic photopolymerization or hybrid photopolymerization. There are three reasons for choosing such mechanism. Firstly, the wavelength of laser beam of SLA is 355 nm. At this wavelength, both radical and cationic photopolymerization could be proceeded; Secondly, volume shrinkage is the fatal weakness to photopolymerization, it could induce the strong internal stress which caused the deformation of material, eventually, the material would be broken. Otherwise, volume shrinkage results in the decline of the precision of printing model. Thus, volume shrinkage is disadvantage to the photocuring 3D printing and scientists try to overcome it. It is well known that cationic photopolymerization has low or no volume shrinkage. Normally, the free radical photosensitive resin is used for DLP 3D printing. The reasons why do not use cationic photopolymerization are: Firstly, cationic

photoinitiator could hardly work under 405 nm irradiation, some cationic photoinitiator could work under 405 nm, however, the price is too high to constrain its application. Secondly, t the light intensity of DLP 3D printing is not high enough to photolysis the cationic photoinitiators, which can't induce the photopolymerization [31].

**Bioink**

Alginate is a type of anionic polysaccharide mostly present in brown algae. It is composed of a linear chain with two possible present blocks: G-blocks ((1-4) α-guluronate units) and M-blocks (β-D-mannuronate units) with alternate segments of M-G blocks. The existence of these blocks and their ratio on the main chain varies from each source and influence the hydrogel's resulting properties. Alginate hydrogels have shape-memory capability, biocompatibility, ability of degradation, nun-immunological effects, improved porosity and mechanical strength. The rheological properties of alginate allow a shearthinning behavior, reducing viscosity under shear stress. This characteristic is favourable for extrusion printing and cell survivability during the process. Hyaluronic acid It is one of the major extracellular matrixes (ECM) components in a variety of tissues such as central nervous system, cartilage, synovial and vitreous fluids and connective, epithelial and cardiovascular tissues. It is involved in several biological functions, such as regulation of cell adhesion, cell motility, cell differentiation and proliferation, and providing mechanical properties to tissues. HA is also responsible for providing the viscoelasticity of some fluids. Collagen has been utilized alone as bioink in extrusion-based bio printing. Droplet-based bioprinting also has an advantageous use in collagen; however, it needs to be deposited before the crosslinking onset. Due to its fibrous structure, the collagen use in inkjet bioprinting is highly limited, with micro-valve bioprinting being preferred [32].

**Excipients**

Carboxymethyl cellulose sodium (CMC) is the sodium salt of the carboxymethyl ether of cellulose, formed by the reaction of cellulose with monochloroacetic acid. It has been used to develop 3D-printed biocompatible structures for drug-delivery and tissue-engineering applications; however, this requires the development of new biocompatible (hydrogel) inks. CMC is a promising candidate for the preparation of hydrogels (inks) since it is a natural, biocompatible, and biodegradable polymer and has good solubility in water with multiple carboxyl groups. Among all cellulose ethers, CMC, in particular, has recently been reported as a useful structural component of bioinks for wound healing due to its matrix-forming capability, cell compatibility, and crosslinking feasibility. HPMC grades are unsuitable for thermal extrusion processes alone,

certain grades and combinations with other thermoplastic polymers and/or plasticizers help to facilitate the extrusion process to obtain filaments with adequate mechanical properties for 3D printing. Hydroxypropyl cellulose (HPC) is cellulose ether manufactured by reacting alkali cellulose with propylene oxide at an elevated temperature and pressure. The 3D-printed tablets showed acceptable ranges for tablet-breaking force, tablet friability, weight variation, and drug content. Abdella et al. fabricated estradiol-containing films using a formulation containing HPC in PAM printing. The results indicated that different infill patterns affected the film's mechanical properties and its drug-release kinetics [33].

**Process Workflow in 3D Printing Biopharmaceutics**

The 3d printing process work flow for pharmaceutical drugs involved a sequence of highly controlled and precise steps designed to produce customized drug delivery systems and dosage forms with unique properties such as tailored dosing complex drug release profiles and improved patient compliance. Here is a detailed explanation of the work flow.

**1. Design and Digital Modelling**

This stage involves creating a detailed 3digital model of the pharmaceutical dosage form using computer-aided design [CAD] software. The design captures the intended geometry, size, internal micro structure, and drug release features needed to meet therapeutic goals. The digital file is then converted into a printer specific format [commonly STL or OBJ] that includes the necessary spatial information for layer-wise printing. (34)

**2. Preparation of Pharmaceutical Formulations (Pharma- Ink)**

Pharmaceutical-grade formulations, are prepared to be compatible with the chosan 3D technology. These formulations mix active pharmaceutical ingredients (APIs) with excipients like polymers, binders and plasticizers to ensure stability, printability, and required mechanical and release properties. In methods like fused deposition modelling (FDM), hot-melt extrusion is used to produce drug loaded filaments. Other methods may use powder blends or semi-solid gels. (35)

**3. Printing Execution**

The pharma-ink or feed stock is loaded into the 3D printer, which fabricates the dosage form layer by layer

- **FDM, or Fused Deposition Modelling:** To create the printlet with exact control over shape and internal voids that impact drug release, drug-loaded filaments are melted and extruded through a nozzle.

- **Binder jetting**, also known as powder bed printing, involves spreading out layers of powder and selectively jetting liquid binder to bind the powder particles, resulting in a solid dosage form.
- **Digital Light Processing and Stereolithography:** Light cures layers of photopolymer resin combined with the medication to create intricate and precise structures.
- **Semi-Solid Extrusion:** Films, patches, or tablets are created by extruding semi-solid gels that contain APIs.

The printer optimizes structural integrity and medication distribution by controlling variables including temperature, print speed, and layer thickness. (36)

**4. Post-processing**

To solidify the dosage form and maximize mechanical strength, post-printing procedures include drying, curing, or cross-linking. Excess powder is eliminated for powder-bed printing. Sterilization may also be applied to some printed structures. This phase guarantees that the product is stable and prepared for additional testing.

**5. Testing and Quality Assurance**

To guarantee exact drug dosages, every batch is subjected to stringent quality control procedures, such as,

- Dose uniformity and content analysis.
- Testing for drug release characteristics using dissolution and release kinetics.
- Stability studies under simulated storage circumstances; mechanical tests, such as hardness and friability

Automated inline controls are being incorporated more frequently for real-time printing monitoring, which lowers human error and increases reproducibility. (37)

**Factors Affecting 3D Printing**

**1. Material Related Factors**

Strength, performance, and 3D printing are all significantly impacted by material-related aspects. Important factors include filament uniformity (uneven diameter impacts extrusion), quality and purity (impurities or moisture can create flaws), and material type (thermoplastics, resins, metals, composites). Strong circumstances stop moisture-related problems in hygroscopic materials, whereas thermal characteristics like melting temperature and stability affect layer bonding. Compatibility with the printer (nozzle,bed,extruder) and mechanical properties such as flexibility, haedness, and durability detemine the final part's

reliability and suitability for specific applications. (38)

**2. Process Parameters in 3D Printing**

Layer height (which affects details and print time), print speed (which affects precision and layer bonding), nozzle and bed temperature (which controls material melting and adhesion), infill density and pattern (which determine strength and weight), print orientation (which affects surface finish and load capacity), cooling rate and speed (which influence warping and layer adhesion), and extrusion flow rate (which prevents under or over extrusion) are important process parameters in 3D printing. A balance between quality, strength, and efficiency is ensured by maximizing these criteria.(39)

**3. Process and Post Processing Conditions**

Conditions during and after processing are critical to the strength, quality, and look of 3D prints. Temperature, print speed, layer thickness, bed leveling, and chamber environment all play a role in maintaining good layer adhesion and preventing warping during printing. Through support removal, surface finishing, heat treatment, UV curing (for resins), sintering or machining (for metals), coating, or painting, post processing enhances mechanical and aesthetic qualities. Managing both phases guarantees prints that are useful, long-lasting, and aesthetically and pleasing. (40)

**4. Regulatory and Quality Control Factors**

Regulations and quality control measures guarantee that 3D printed goods fulfill performance, safety, and dependability requirements. Regulatory compliance entails upholding material traceability and adhering to rules or standards such as ISO/ASTM 52900. Machine calibration, process monitoring, part inspection (3D scanning, testing), and confirming dimensional accuracy, surface finish, and strength are all included in quality control. Consistent, secure, and high-quality prints are ensured by the implementation of SOPs, documentation, operator training, and equipment/product validation. (41)

**5. Printer & Hardware Limitations**

The performance, accuracy, and dependability of 3D printing are impacted by printer and device constraints. Print speed (balancing quality and production time), build volume (limiting object size), and printer resolution (affecting detail and surface finish) are important limitations. While material compatibility and exact temperature/curing control are crucial for some materials, hardware durability, calibration, and maintenance affect consistency. Efficiency and scalability are further impacted by support removal, post-processing software limitations, and replacement prices. (42)

**6. Environmental Conditions**

Environmental factors have a significant impact on the dependability and quality of 3D printing. While humidity affects hygroscopic materials like nylon or PAV, temperature impacts the behavior of materials and layer adhesion. Cooling and surface finish are affected by ventilation and airflow. Dust or contamination influences powder fusion in metal printing, while UV radiation can inadvertently cure materials in resin printing. Dimensional errors can be brought on by vibration. Consistent performance and print quality are ensured by regulating temperature, humidity, ventilation, cleanliness, and stability. (43)

**Advantages**

1. Makes it possible to customize dosage, size, shape, and medication combinations for specific patients, which is advantageous for cancer, pediatric, and geriatric care.
2. Enables the production of multilayered tablets and polypills with customized release profiles (pulsatile, sustained, and delayed), enhancing treatment results and adherence.
3. Reduces waste and logistical costs by facilitating rapid, on-demand medicine manufacture for clinical studies, emergencies, or remote locations.
4. Reduces waste of costly APIs, increasing the cost-effectiveness of customized and small-batch manufacturing.
5. By controlling drug loading and porosity, it improves the solubility and bioavailability of poorly soluble medications.
6. By combining several medications into a single, personalized polypill, it lowers polypharmacy errors.
7. Accelerates the development of formulations and testing in the early stages of drug research.

**Disadvantages**

1. Because there are no defined regulations for 3D printed dugs, regulatory approval, standardization, and quality control are still unclear.
2. 2. Exorbitant initial expenses and the requirement for qualified staff prior to widespread adaption and regular use.
3. There are fewer formulation alternatives due to limited material compatibility with APIs.
4. The lack of appropriate pharmaceutical grade polymers and excipients limits the flexibility of formulations.
5. Drug stability may be impacted and scale-up may be complicated by thermal deterioration during techniques such as FDM.
6. For large-scale mass production, current technologies are neither cost-effective nor efficient.

7. Heat-sensitive medications may be degraded by mechanical and thermal stresses during printing, which could reduce their effectiveness.

**Applications**

- Customized drug-loaded implants, microneedles, or oral films enable tailored dosage and targeted medication administration, increasing effectiveness and minimizing side effects.
- Spatial API distribution can produce complex medication release profiles, improving pharmacokinetics and patient adherence.
- Compared to 2D cultures or animal models, bioprinted tissue models predict drug toxicity and metabolism more accurately. (such as a tumor, liver, or kidney)
- Biodegradable scaffolds can support tissue regeneration with regulated release while delivering medications or growth hormones.
- Multiple-compartment polypills enable combination drugs with different release characteristics, lowering pill burden and increasing compliance.
- Disease models, such as tumors or heart tissues, allow for patient-specific therapy testing and real-time drug response monitoring.
- For high throughput and dynamic drug testing, an organ-on-a-chip system simulates interconnected organs, such as the heart, liver, and kidney.
- Quick iterations during formulation development are supported by rapid prototyping of DoSage forms.
- Personalized manufacture is made possible by on-demand drug printing at hospitals or remote locations, which lowers waste and speeds up delivery.
- Accurate dosing and acceptance are enhanced by customized pediatric and geriatric dose forms. (For instance, gummies or chewables)
- Localized treatment with less systemic exposure is offered via drug-eluting implants. (such as contraception and chemotherapy) with lower systemic exposure.
- The first 3D printed medication utilizing binder jetting with high dose quick disintegration is Spitam® (levetiracetam), which received FDA approval in 2015.

**Challenges and Limitations**

These challenges can be classified into three categories:

- Technical challenges
- Regulatory challenges
- Good manufacturing practice (GMP) challenges.

1. **Technical Challenges**

Further processing of this dosage forms can be challenging since printed products may have significant friability and inadequate mechanical characteristics depending on the printing technology used. Defects may arise, particularly during the packaging of printed tablets, which could result in the rejection of entire batches. After printing, a large amount of unprinted material builds up for several 3DP technologies, such as BJ or SLA. While it is necessary to find a technical solution to prevent an excessive amount of unprinted material, it is also necessary to clarify whether unprocessed material can be utilized for more printing. 3DP lacks process control techniques when compared to well-established pharmaceutical production procedures. In-process control (IPC) is used during traditional tablet production to closely monitor the process. IPC technologies, which evaluate printed tablets analytically after printing, are not yet widely used for 3DP operations. Furthermore, depending on the size of the tablet press, conventional tableting equipment may produce several hundred thousand tablets per hour, but 3DP is a time-consuming procedure.

**2. Regulatory**

The same regulations that apply to traditionally manufactured dosage forms also apply to 3D-printed dosage forms. However, there is now a significant void in the regulatory system. Regulatory authorities have not provided any recommendations for the 3DP process, despite the fact that established processes have well-implemented and standardized criteria. Recognizing the dearth of advice, health authorities worldwide started the process of creating standards and practical instructions. To investigate the possibility of 3DP in pharmaceutics in the future, the FDA established two internal laboratories: the Laboratory for Solid Mechanics and the Functional Performance and Device Use Laboratory under the FDA's Office of Science and Engineering Laboratories (OSEL). These two units' activities should aid in first-step knowledge acquisition, standard development, and the identification of crucial factors influencing product safety. However, more work needs to be done by health authorities to define standard procedures and give pharmaceutical makers direction. Additionally, in the event of an occurrence, both culpability and accountability must be discussed. If it is intended to use 3DP as on-demand manufacturing method in community and hospital pharmacies, many possibilities for supply chain are available. In terms of FFF technology, the printing process must be carried out in the pharmacy itself, and drug-loaded filaments must be supplied by outside chemical or pharmaceutical companies. The situation calls into question who would be in charge of releasing the raw materials for production and how arriving items should be examined using the pharmacy's equipment. The clearance of Spritam® and recent presentations by regulatory officials showed that the regulatory and

required quality framework is the same regardless of the manufacturing process, even though the 3DP techniques for creating tablets may differ with 3D printing technology. (44)

### 3. GMP Challenges

In order to comply with GMP regulations, qualification standards for 3D printer manufacturers must be established. Especially, the subject of cleaning validation should be addressed to avoid cross contamination. Pharmaceutical firms are required to employ 3D printers as specialized equipment as long as cleaning concepts are not established and verified. To establish 3DP as a manufacturing process for pharmaceutical dosage forms, health authorities and pharmaceutical firms must work together to address the aforementioned regulatory and GMP obstacles. To produce 3D printed products, having a high degree of software expertise and proficiency is crucial. (45)

### Trends and Directions for Future Research

Innovative pharmaceutical dosage forms, such as topical patches, orodispersible forms, dissolving oral films, implantable devices, or multilayer pills, have been made possible by three-dimensional printing technology with the goal of improving patient compliance, improving drug efficacy, and offering new mechanisms for drug delivery. Individualized drugs that are tailored to each patient's specific needs, including precise dosages, drug combinations, and release patterns, can be produced thanks to three-dimensional printing. (46)

Pharmaceutical applications have benefited from the advancement of 3D printing, which has made it possible to create individualized drug administration and screening systems for each patient. Bioprinting offers the ability to print medications on demand in accordance with each patient's unique requirements, tailoring the shape, structure, and dosage to each patient's physical condition. For example, it is possible to print specific medications for controlled release rates, print porous tablets to lessen swallowing difficulties, create transdermal microneedle patches to lessen patient pain, and more. However, in place of animal testing and clinical trials, bioprinting can precisely regulate the distribution of cells and biomaterials to create organoids, or an Organ-on-a-Chip, for drug testing on printed organs that mimic certain disease characteristics. In order to expedite the launch of a pharmaceutical product, bioprinting plays a crucial role in preclinical and clinical drug testing. (47) When creating oral disintegrating tablets and modified release oral dose forms, three-dimensional printing is particularly helpful. Aprecia Pharmaceuticals' "Spritam" was the first FDA-approved medication made via 3D printing. It is made easier to take by using Zip Dose Technology, which produces a porous formulation that dissolves quickly with a sip of drink.

**Personalized Drug-Loaded Patches**

Three-dimensional printing can provide personalized drug-loaded patches for dermatological use, enabling the regulated release of pharmaceuticals. These patches can be modified to deliver antibiotics or anti-inflammatory drugs directly to the afflicted skin area, maximizing therapeutic results while reducing systemic side effects. They can also be made to conform to the specific contours of the application area on the patient's body, ensuring better contact and effectiveness.

**Customized Healthcare**

Another benefit is personalization, which enables customized needle lengths for better medication absorption in patients with skin disorders like psoriasis or diabetes. Customized implanted devices that can deliver medications to the targeted area of the body at a regulated rate are made using three-dimensional printing. These characteristics are crucial for therapies that call for long-term localized medication administration, including antibiotics or chemotherapy drugs. Implants used in periodontal therapy, for instance, include biodegradable frames made of 3D printing that gradually release antibiotics to cure infections. Expansion of bioprinting applications to produce medicine formulations customized to each patient's requirements.AI-driven design and patient data are used to optimize medication compositions and delivery systems.

**3D-Printed Microneedles**

Compared to oral or injectable methods, 3D-printed microneedles provide accurate, less invasive medication administration across the skin barrier, improving bioavailability and consistency. They can be designed to improve treatment efficacy by overcoming low skin permeability. Microneedles, which are customizable in terms of drug penetration and release, have demonstrated efficacy in administering analgesics, therapies for epilepsy, and drugs for Alzheimer's disease with sustained release, efficient skin penetration, and biocompatibility. A selective calcitonin gene related peptide, a neuropeptide secreted from sensory nerve terminals, can be trans dermally delivered via an analgesic microneedle patch made of dissolvable microneedles. (46)

**Combination Therapies**

3D pharmaceutical printing could be used to create multi-layered pills with distinct drugs in each layer that release each active ingredient at varying rates or periods. This technology greatly improves adherence by streamlining complicated medication regimes. To create sophisticated drug delivery systems that can distribute several medications in a regulated way, research is being done on multi-drug printing capabilities. Advances in the temporal and geographical regulation of medication release patterns.

**Sustainability**

Investigating eco-friendly materials and methods to lessen bioprinting's negative environmental effects. creation of biodegradable or reusable bioprinting materials. Environmentally beneficial substitute for traditional production. Benefits include reduced waste, energy efficiency, and prevention of overproduction.

**Regulatory and Quality Control**

To guarantee the safety and effectiveness of bio printed medications, thorough regulatory criteria are developed. Cooperation to develop standardized procedures and validation techniques amongst business, academia, and regulatory organizations. MP-compliant printers and particular FDA/EMA regulations are required. Prioritize safety and reproducibility while creating customized medications.

**Innovations in Technology**

advancements in printing technology to increase scalability, accuracy, and resolution. Real-time monitoring and feedback systems are integrated to guarantee quality control throughout the printing process. creation of novel biocompatible and bioactive materials to enhance the stability and performance of bio printed medications. Investigation of hydrogels, composite materials, and synthetic and natural polymers to improve medication delivery systems. (47)

**Clinical Translation**

Carrying out additional clinical trials to confirm the safety and effectiveness of bio printed medications in practical settings. working together with medical professionals to incorporate bioprinting technologies into clinical settings.

***Reference***

1. Sen K, West TG, Chaudhuri B. History and present scenario of additive manufacturing in pharmaceuticals. In Additive Manufacturing in Pharmaceuticals 2023 Sep 14 (pp. 1-44). Singapore: Springer Nature Singapore.
2. Serrano DR, Kara A, Yuste I, Luciano FC, Ongoren B, Anaya BJ, Molina G, Diez L, Ramirez BI, Ramirez IO, Sánchez-Guirales SA. 3D printing technologies in personalized medicine, nanomedicines, and biopharmaceuticals. Pharmaceutics. 2023 Jan 17;15(2):313.
3. Pravin S, Sudhir A. Integration of 3D printing with dosage forms: A new perspective for modern healthcare. Biomedicine & Pharmacotherapy. 2018 Nov 1; 107:146-54.

4. Trenfield SJ, Awad A, Goyanes A, Gaisford S, Basit AW. 3D printing pharmaceuticals: drug development to frontline care. Trends in pharmacological sciences. 2018 May 1;39(5):440-51.
5. Basit AW, Trenfield SJ. 3D printing of pharmaceuticals and the role of pharmacy. Pharmaceutical Journal. 2022 Mar 30;308(7959).
6. Vaz VM, Kumar L. 3D printing as a promising tool in personalized medicine. Aaps Pharmscitech. 2021 Jan 17;22(1):49.
7. McDonagh T, Belton P, Qi S. Manipulating drug release from 3D printed dual-drug loaded polypills using challenging polymer compositions. International Journal of Pharmaceutics. 2023 Apr 25; 637:122895.
8. Macdonald E, Salas R, Espalin D, Perez M, Aguilera E, Muse D, Wicker RB. 3D printing for the rapid prototyping of structural electronics. IEEE access. 2014 Mar 13; 2:234-42.
9. Pillai A, Bhande D, Pardhi V. Controlled drug delivery system. InAdvanced drug delivery: Methods and applications 2023 Oct 25 (pp. 267-289). Singapore: Springer Nature Singapore.
10. Maulvi FA, Shah MJ, Solanki BS, Patel AS, Soni TG, Shah DO. Application of 3D printing technology in the development of novel drug delivery systems. Int J Drug Dev Res. 2017;9(1):44-9.
11. Sousa AC, Alvites R, Lopes B, Sousa P, Moreira A, Coelho A, Santos JD, Atayde L, Alves N, Maurício AC. Three-dimensional printing/bioprinting and cellular therapies for regenerative medicine: current advances. Journal of Functional Biomaterials. 2025 Jan 16;16(1):28.
12. Iftekar SF, Aabid A, Amir A, Baig M. Advancements and limitations in 3D printing materials and technologies: a critical review. Polymers. 2023 May 30;15(11):2519.
13. Sharma S, Wilberforce K, Patel S. Regulatory Challenges, Quality Standards, and Compliances for 3D Printed Pharmaceuticals. InInnovations in Drug Manufacturing: FDM 3D Printing for Pharmaceuticals 2025 Sep 1 (pp. 77-99). Singapore: Springer Nature Singapore.
14. Dabbagh, S.R.; Sarabi, M.R.; Rahbarghazi, R.; Sokullu, E.; Yetisen, A.K.; Tasoglu, S. 3D-Printed Microneedles in Biomedical Applications. IScience 2020, 24, 102012.
15. Jabbar Qasim Al-maliki, Alaa Jabbar Qasim Al-Maliki (2015), The Processes and Technologies of 3D Printing,International Journal of Advances in Computer Science and Technology, pp. 161–165.
16. Kumar, S. Additive Manufacturing Processes; Springer Nature: Cham, Switzerland, 2020.
17. Gardan, J. Additive manufacturing technologies: State of the art and trends. Int. J. Prod. Res. 2016, 54, 3118–3132.

18. Elkaseer, A.; Schneider, S.; Scholz, S.G. Experiment-Based Process Modeling and Optimization for High-Quality and Resource-Efficient FFF 3D Printing. Appl. Sci. 2020, 10, 2899.
19. Gross, B. C.; Erkal, J. L.; Lockwood, S. Y.; Chen, C.; Spence, D. M. Anal. Chem. 2014, 86, 3240– 3253 DOI: 10.1021/ac403397r
20. Chua, C. K., Leong, K. F., & Lim, C. S. (2010).
21. Rapid Prototyping: Principles and Applications (3rd Edition). World Scientific Publishing.
22. Gibson, I., Rosen, D., & Stucker, B. (2021).
23. Additive Manufacturing Technologies: 3D Printing, Rapid Prototyping, and Direct Digital Manufacturing (3rd Edition). Springer.
24. Gibson, I., Rosen, D., & Stucker, B. (2021). Additive Manufacturing Technologies: 3D Printing, Rapid Prototyping, and Direct Digital Manufacturing (3rd Edition). Springer.
25. Chua, C. K., Leong, K. F., & Lim, C. S. (2010). Rapid Prototyping: Principles and Applications (3rd Edition). World Scientific Publishing.
26. Firth, J.; Basit, A.W.; Gaisford, S. The Role of Semi-Solid Extrusion Printing in Clinical Practice. In 3D Printing of Pharmaceuticals; Springer International Publishing: Cham, Switzerland, 2018; Volume 31, pp. 133–151. ISBN 978-3-319-90754-3. [Google Scholar]
27. Seoane-Viaño, I.; Januskaite, P.; Alvarez-Lorenzo, C.; Basit, A.W.; Goyanes, A. Semi-Solid Extrusion 3D Printing in Drug Delivery and Biomedicine: Personalised Solutions for Healthcare Challenges. J. Control. Release Off. J. Control. Release Soc. 2021, 332, 367–389. [Google Scholar]
28. Ngo, T. D., Kashani, A., Imbalzano, G., Nguyen, K. T. Q., & Hui, D. (2018). "Additive Manufacturing (3D Printing): A Review of Materials, Methods, Applications and Challenges." Composites Part B: Engineering, 143, 172–196.
29. Yang D. Recent advances in hydrogels. Chemistry of Materials. 2022 Mar 8;34(5):1987-9.
30. Nolan MC, Caparrós AM, Dietrich B, Barrow M, Cross ER, Bleuel M, King SM, Adams DJ. Optimising low molecular weight hydrogels for automated 3D printing. Soft Matter. 2017;13(45):8426-32.
31. Shahrubudin N, Lee TC, Ramlan RJ. An overview on 3D printing technology: Technological, materials, and applications. Procedia manufacturing. 2019 Jan 1; 35:1286-96.
32. Al-Zaidi AA, Al-Gawhari FJ. Types of polymers using in 3D printing and their applications: a brief review. EJTAS. 2023; 1:978-85.
33. Quan H, Zhang T, Xu H, Luo S, Nie J, Zhu X. Photo-curing 3D printing technique and its challenges. Bioactive materials. 2020 Mar 1;5(1):110-5.

34. Fontes AB, Marcomini RF. 3D bioprinting: a review of materials, processes and bioink properties. The Journal of Engineering and Exact Sciences. 2020 Dec 12;6(5):0617-39.
35. Ochoa E, Morelli L, Salvioni L, Giustra M, De Santes B, Spena F, Barbieri L, Garbujo S, Vigano M, Novati B, Tomaino G. Co-processed materials testing as excipients to produce Orally Disintegrating Tablets (ODT) using binder jet 3D-printing technology. European Journal of Pharmaceutics and Biopharmaceutics. 2024 Jan 1; 194:85-94.
36. Grof Z, Štěpánek F. Artificial intelligence-based design of 3D-printed tablets for personalised medicine. Computers & Chemical Engineering. 2021 Nov 1; 154:107492.
37. Wang S, Chen X, Han X, Hong X, Li X, Zhang H, Li M, Wang Z, Zheng A. A review of 3D printing technology in pharmaceutics: technology and applications, now and future. Pharmaceutics. 2023 Jan 26;15(2):416.
38. Jiang Z, Diggle B, Tan ML, Viktorova J, Bennett CW, Connal LA. Extrusion 3D printing of polymeric materials with advanced properties. Advanced Science. 2020 Sep;7(17):2001379.
39. Gampa TR, Katakam P, Adiki SK, Sriram N, Satapathy S. Quality Control Methods for Three-Dimensional Printed Pharmaceuticals. InHandbook of 3D Printing in Pharmaceutics 2024 Dec 10 (pp. 195-210). CRC Press.
40. Muehlenfeld C, Duffy P, Yang F, Zermeño Pérez D, El-Saleh F, Durig T. Excipients in Pharmaceutical Additive Manufacturing: A Comprehensive Exploration of Polymeric Material Selection for Enhanced 3D Printing. Pharmaceutics. 2024 Feb 24;16(3):317.
41. Alzhrani RF, Fitahi RA, Majrashi MA, Zhang Y, Maniruzzaman M. Toward a harmonized regulatory framework for 3D-printed pharmaceutical products: the role of critical feedstock materials and process parameters. Drug Delivery and Translational Research. 2025 Sep 16:1-8.
42. Tian Y, Chen C, Xu X, Wang J, Hou X, Li K, Lu X, Shi H, Lee ES, Jiang HB. A review of 3D printing in dentistry: Technologies, affecting factors, and applications. Scanning. 2021;2021(1):9950131.
43. Narayan CV, Saini M, Verma S, Grewal AS, Nepali K. Regulatory and Quality Considerations in 3D Printing Technology. Precision 3D Printing in Pharmaceutical Sciences: A Transformative Shift in Drug Manufacturing and Delivery Systems. 2025 Nov 20:323-44.
44. .Iftekar SF, Aabid A, Amir A, Baig M. Advancements and limitations in 3D printing materials and technologies: a critical review. Polymers. 2023 May 30;15(11):2519.
45. Nadagouda MN, Ginn M, Rastogi V. A review of 3D printing techniques for environmental applications. Current opinion in chemical engineering. 2020

Jun 1; 28:173-8.
46. Tracy T, Wu L, Liu X, Cheng S, Li X. 3D printing: Innovative solutions for patients and pharmaceutical industry. International Journal of Pharmaceutics. 2023 Jan 25; 631:122480.
47. Shahrubudin N, Koshy P, Alipal J, Kadir MH, Lee TC. Challenges of 3D printing technology for manufacturing biomedical products: A case study of Malaysian manufacturing firms. Heliyon. 2020 Apr 1;6(4).
48. Bernatoniene J, Stabrauskiene J, Kazlauskaite JA, Bernatonyte U, Kopustinskiene DM. The Future of Medicine: How 3D Printing Is Transforming Pharmaceuticals. Pharmaceutics. 2025 Mar 19;17(3):390.
49. Mihaylova A, Shopova D, Parahuleva N, Yaneva A, Bakova D. (3D) Bioprinting-Next Dimension of the Pharmaceutical Sector. Pharmaceuticals (Basel). 2024;17(6):797. Published 2024 Jun 17. doi:10.3390/ph17060797

# Fluid Dynamics of Xylem and Phloem Transport in Plants: A Physical and Mathematical Perspective

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## Abstract

The vascular transport systems of plants—xylem and phloem—represent a remarkable fusion of biological design and physical law. This chapter provides a comprehensive narrative synthesis of the fluid dynamics underlying long-distance transport in higher plants, emphasizing recent advances in biophysical modeling and mathematical formulation. Xylem water ascent, governed by the cohesion–tension mechanism, is reinterpreted through the lens of capillary physics, negative pressure potential, and the Hagen–Poiseuille equation. Modern imaging and computational fluid dynamics (CFD) approaches reveal that cavitation and embolism formation are not random failures but predictable phase transitions governed by vessel geometry and surface tension. Complementarily, phloem transport, traditionally explained by the Munch pressure-flow hypothesis, is modeled as a laminar, pressure-driven flow arising from osmotic loading at source tissues. New research elucidates the dynamic coupling between xylem and phloem via radial water exchange, described mathematically using Darcy's law and osmotic potential gradients. These findings redefine plant hydraulics as a

coupled, self-regulating system rather than independent conduits. Integration of physical theory, experimental visualization, and computational modeling has thus advanced the quantitative understanding of plant vascular function, providing predictive insights into plant drought responses, carbon allocation, and climate resilience. Ultimately, this synthesis demonstrates how fundamental principles of physics—hydrodynamics, thermodynamics, and osmosis—govern the living architecture of plants and inspire emerging biomimetic designs in engineering.

***Keywords:*** Xylem hydraulics, pressure flow, Plant biophysics, Cohesion–tension theory, Mathematical modeling, Darcy's law, Poiseuille flow, Source–sink dynamics.

## Introduction

The transport of water, minerals, and photoassimilates in vascular plants represents one of the most elegant natural examples of applied physics in biological systems. Unlike animals, plants lack a centralized pump; instead, they rely on finely tuned physical principles—fluid mechanics, thermodynamics, and osmotic theory—to sustain long-distance transport across meters of vertical height. Recent advances in experimental imaging, computational modeling, and theoretical biophysics have substantially refined classical concepts of xylem and phloem transport, revealing them as dynamically coupled hydraulic systems rather than independent conduits (Mai, 2025).

## Fluid Transport in Plants

At the core of plant fluid transport lies the xylem, responsible for the ascent of sap, and the phloem, which distributes sugars and signaling molecules. These systems operate under contrasting but complementary physical regimes: xylem transport is dominated by negative pressure and cohesion–tension, whereas phloem transport is governed by positive pressure gradients arising from osmotic loading. The integration of these two flows is now recognized as a central determinant of whole-plant function, drought resilience, and carbon allocation (Meteignier et al., 2023; Nakad et al., 2025).

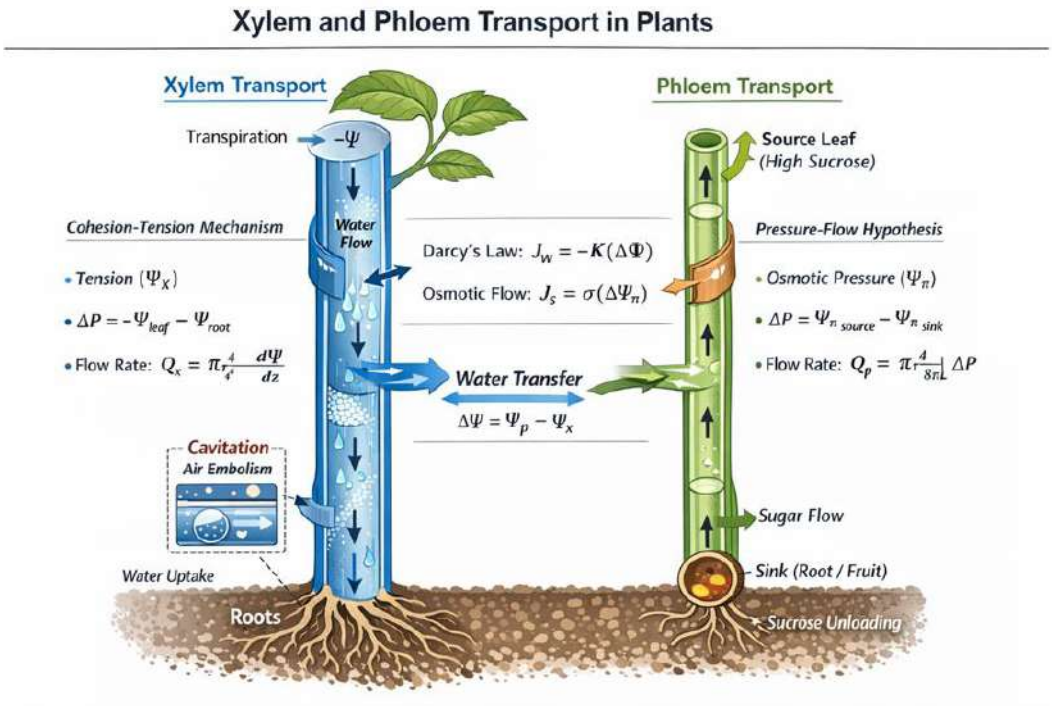


***Fig.1 Scientific schematic modal illustrating xylem and phloem transport in plants.***

From a physical standpoint, xylem flow is a remarkable case of metastable liquid water under tension. Transpiration at the leaf surface lowers the water potential (Ψ) of mesophyll cells, generating a gradient that propagates downward through the xylem continuum to the roots. The water potential gradient driving xylem flow may be expressed as:

$$\Delta\Psi = \Psi leaf - \Psi root$$

While the equation $\Delta\ \Psi = \Psi$ leaf - $\Psi$ root represents the mathematical difference in water potential between two points in a plant, it is important to understand the direction of flow and the typical values involved:

**Direction of Flow:** Water moves from areas of higher (less negative) water potential to areas of lower (more negative) water potential. In a transpiring plant, the gradient follows the order:

$$\Psi \text{ soil} > \Psi \text{ root} > \Psi \text{ stem} > \Psi \text{ leaf} > \Psi \text{ atmosphere.}$$

Sign of ΔΨ: Because water moves "downhill" toward more negative potentials, the value of Ψ leaf (Ψleaf is more negative than Ψroot ) will result in a negative value, which indicates the potential "drops" as water moves upward.

The absolute magnitude of this difference ($\Delta\ \Psi$) determines the strength of the driving force for xylem flow. A larger difference between the roots and leaves leads to a steeper gradient and faster flow, provided the xylem's hydraulic conductance remains constant.

Because xylem conduits behave approximately as cylindrical capillaries, sap flow can be modeled using the Hagen–Poiseuille equation, adapted for plant hydraulics:

$$\mathbf{Qx = (\pi r^4 / 8\eta)\ (dP/dz)}$$

where Qx is the volumetric flow rate, r is vessel radius, η is sap viscosity, and (dP/dz) represents the pressure gradient along the conduit (Zimmermann, 2013). This formulation highlights the extraordinary sensitivity of hydraulic conductivity to vessel diameter, explaining evolutionary trade-offs between efficiency and vulnerability to cavitation.

Recent studies using micro-computed tomography and high-resolution magnetic resonance imaging have demonstrated that xylem flow deviates from ideal laminar assumptions under stress conditions. During drought, negative pressures intensify, increasing the probability of cavitation, where dissolved gases nucleate into embolisms that disrupt hydraulic continuity (Vincent, 2022). Cavitation is now modeled as a thermodynamically driven phase transition, dependent on conduit geometry, pit membrane properties, and surface tension.

While xylem transport is fundamentally passive, phloem transport requires metabolic energy for solute loading, yet the bulk flow itself obeys classical fluid mechanics. According to the Munch pressure-flow hypothesis, sucrose loading at source tissues lowers osmotic potential (Ψπ), drawing water from adjacent xylem and increasing turgor pressure. The resulting pressure gradient drives mass flow toward sink tissues, where sugars are unloaded:

$$\mathbf{\Delta P = \Psi\pi source - \Psi\pi sink}$$

Phloem sap flow is likewise described using a Poiseuille-type relationship:

$$\mathbf{Qp = (\pi r^4 / 8\eta L)\ \Delta P}$$

One of the most significant advances in recent years has been the recognition of dynamic xylem–phloem coupling. Traditionally treated as independent systems, xylem and phloem are now understood to exchange water continuously through radial pathways. Nakad et al. (2015) demonstrated using coupled partial differential equations, that spatial variation in sink strength reshapes pressure profiles along the phloem, inducing localized water fluxes from xylem to phloem. This coupling can be mathematically represented using Darcy's law for radial water movement:

$$Jw = -K(\Delta\Psi)$$

where Jw is radial water flux, K is membrane hydraulic conductivity, and ΔΨ is the water potential difference between tissues. Such coupling becomes particularly critical under drought, when declining xylem water potential constrains phloem turgor, leading to carbon starvation despite adequate photosynthesis.

Recent modelling frameworks integrate xylem tension, phloem osmotic loading, and tissue elasticity into unified systems of equations. These models reveal that phloem transport can fail before xylem under severe drought, challenging earlier assumptions that hydraulic failure is always the primary cause of plant mortality (Li, 2022). Theoretical analyses suggest that maintaining minimal phloem pressure gradients is essential for survival, even when xylem conductance is partially compromised.

Advances in computational fluid dynamics (CFD) have further enhanced our understanding of plant vascular transport. High-resolution anatomical data are now incorporated into three-dimensional simulations that solve Navier–Stokes equations under biologically realistic boundary conditions. These models capture heterogeneous flow patterns, embolism spread, and refilling dynamics, offering unprecedented predictive power (Chen, 2023). When combined with machine learning, such models are increasingly used to forecast plant responses to heat waves and drought events.

From an ecological and applied perspective, the physics of xylem and phloem transport has profound implications. Crop productivity, forest carbon sequestration, and ecosystem resilience all depend on efficient vascular function (Imran, 2025). Breeding strategies now explicitly consider hydraulic traits such as vessel diameter distribution, pit membrane porosity, and phloem loading capacity. Understanding the physical limits imposed by fluid dynamics thus provides a mechanistic foundation for climate-smart agriculture and sustainable forestry.

## Conclusion

The fluid dynamics of xylem and phloem transport exemplify how fundamental physical laws govern biological function across scales. Modern plant science increasingly relies on mathematical modelling, experimental biophysics, and integrative theory to move beyond descriptive physiology. As recent findings demonstrate, plants are not merely passive conduits of water and sugar but dynamic hydraulic systems optimized through evolution to operate at the edge of physical possibility.

## *References*

1. Chen, Z., Zhu, L. T., & Luo, Z. H. (2023). Characterizing flow and

transport in biological vascular systems: A review from physiological and chemical engineering perspectives. Industrial & Engineering Chemistry Research, 63(1), 4-36.

2. Imran. (2025). Carbon cultivation for sustainable agriculture, ecosystem resilience, and climate change mitigation. Communications in Soil Science and Plant Analysis, 56(9), 1430-1456.
3. Li, X., Xi, B., Wu, X., Choat, B., Feng, J., Jiang, M., & Tissue, D. (2022). Unlocking drought-induced tree mortality: physiological mechanisms to modeling. Frontiers in Plant Science, 13, 835921.
4. Mai, M. H. (2025). Architectural Solutions for Hydraulically-Coupled Material Transport in Plants (Doctoral dissertation, Harvard University).
5. Méteignier, L. V., Nützmann, H. W., Papon, N., Osbourn, A., & Courdavault, V. (2023). Emerging mechanistic insights into the regulation of specialized metabolism in plants. Nature plants, 9(1), 22–30. https://doi.org/10.1038/s41477-022-01288-7
6. Nakad, Mazen & Weng, Ensheng & Gentine, Pierre. (2025). Impact of sucrose sinks on phloem transport. 10.1101/2025.01.28.635320.
7. Vincent, O. (2022). Negative pressure and cavitation dynamics in plant-like structures.
8. Zimmermann, M. H. (2013). Xylem structure and the ascent of sap. Springer Science & Business Media.

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Ms. Anu Chaudhary is a motivated and academically driven postgraduate in Zoology with a strong foundation in life sciences. She completed her M.Sc. in Zoology with First Division and qualified the GATE examination in 2023, also currently pursuing a Ph.D. in Dr. Ram Manohar Lohia Avadh University, Ayodhya. She has actively participated in national and international conferences, presenting oral papers and posters. Her academic interests include molecular biology, integrative biology, and environmental sciences. She has undergone an online E-Workshop training program on in silico tools for molecular cloning, which provided theoretical knowledge and practical exposure to computational approaches used in molecular biology. She has also successfully completed the Malaviya Mission Teacher Training Programme (MMTTP) on NEP 2020 Orientation and Sensitization, organized by UGC at Jamia Millia Islamia (Central University), New Delhi. She aims to grow professionally in an environment that values research, learning, and innovation.

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