

Harnessing Microbial Plasmids for Plastic Biodegradation and Environmental Sustainability

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Abstract

This literature review examines the role of microbial plasmids in plastic biodegradation and their potential for environmental sustainability. Plastic pollution from polymers such as polyethylene (PE), polyethylene terephthalate (PET), and polypropylene (PP) persists globally, while conventional disposal methods, including landfilling and incineration, remain inefficient and environmentally harmful. Microbial biodegradation mediated by plasmids offers a promising alternative, as plasmids act as mobile genetic elements that transfer plastic-degrading genes and enhance microbial adaptability in contaminated environments. Key plasmid-encoded enzymes, including PETase and MHETase, are central to breaking down plastics, with horizontal gene transfer mechanisms such as conjugation, transformation, and transduction enabling their spread across microbial populations. Laboratory studies have demonstrated the successful use of engineered plasmids in *Escherichia coli* and *Pseudomonas* species, though challenges remain in biosafety, genetic containment, and large-scale application. Knowledge gaps include a limited understanding of plasmid stability in natural ecosystems, uncertainty regarding long-term degradation efficiency, and insufficient regulatory frameworks for the use of engineered plasmids. By synthesizing findings across microbiology, synthetic biology, and environmental policy, this review affirms that while plasmid-based strategies present considerable promise, their translation into scalable solutions will depend on integrated scientific validation and well-defined policy frameworks.

Keywords: Plastic Biodegradation, Microbial Plasmids, Synthetic Biology, Bioaugmentation, Circular Bioeconomy.

Introduction

The rise in plastic production and consumption has created one of the most complex environmental challenges in modern history. To date, nine billion tons of plastic have been produced, with over 75% ending up in landfills, oceans, and terrestrial environments (Geyer *et al.*, 2017). Plastics such as polyethylene (PE), polyethylene terephthalate (PET), polypropylene (PP), polystyrene (PS), and polyvinyl chloride (PVC) are designed to resist degradation and outlast natural processes. As a result, they accumulate in the environment, destabilize ecosystems, and contribute to the formation of microplastics. This poses health risks to humans and wildlife through food contamination (Jambeck *et al.*, 2015).

Globally, plastic waste primarily consists of packaging, disposable items, fishing nets, and other products (Sharma and Chatterjee, 2017). Improper handling, including illegal disposal and inadequate waste management systems, causes large quantities to enter water bodies. In the ocean, plastic debris breaks down into microplastics and nanoplastics, which are ingested by marine organisms and may affect their physiology and reproduction (Li *et al.*, 2020).

Plastic production continues to rise, with projections indicating that the global plastic burden will double by 2050 (UNEP, 2021). Figure 1 shows the global distribution of plastic marine debris and wastewater treatment levels in various countries. It highlights regions with inadequate treatment facilities and high levels of mismanaged waste, including Southeast Asia, South Asia, and Africa, areas that have become hotspots for plastic pollution. These regions are also key locations for isolating plastic-degrading microbes for bioremediation efforts.

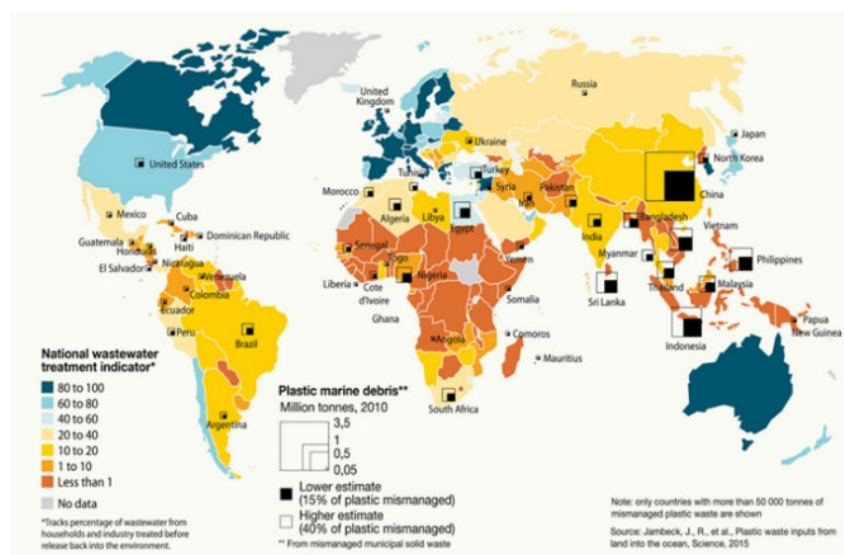


Figure 1: Global Distribution of Plastic Marine Debris and Wastewater Treatment Capacity. (Owen, 2020)

Problem Statement

Plastic pollution, particularly in regions with limited waste treatment infrastructure such as Southeast Asia, South Asia, and Africa, has become a critical environmental issue. These areas also serve as important sites for isolating plastic-degrading microorganisms for bioremediation purposes. The growing accumulation of plastic debris in these ecosystems underscores the urgent need for sustainable solutions. Traditional waste management methods, including incineration, landfilling, and mechanical recycling, have proven inadequate. Incineration generates harmful greenhouse gases and toxic by-products, while landfills occupy substantial land area and allow the slow leaching of residual plastics. Mechanical recycling is constrained by polymer contamination and degradation, making it economically unfeasible for many plastic types (Hopewell *et al.*, 2009). Chemical recycling technologies, although promising, face significant cost and environmental challenges.

Objectives of the Study

This study aims to examine the potential of microbial biodegradation, particularly the role of microbial plasmids, as an eco-friendly and sustainable alternative for plastic waste management. The specific objectives are:

1. To investigate microbial plasmids involved in plastic degradation.
2. To assess the role of horizontal gene transfer (HGT) in disseminating plastic-degrading genes across microbial communities.
3. To examine the potential of synthetic biology in enhancing plastic biodegradation through engineered plasmids.
4. To evaluate the feasibility of bioaugmentation for improving plastic degradation in polluted environments.

Literature Review

The Role of Microbes in Plastic Biodegradation

Microbial biodegradation of plastics has emerged as a promising eco-friendly strategy for mitigating the environmental burden of synthetic polymers. Certain microorganisms, including bacteria, fungi, and archaea possess natural or engineered enzymatic abilities to break down plastics into less harmful molecules through metabolic or cometabolic processes (Urbanek *et al.*, 2018; Danso *et al.*, 2019). However, biodegradation rates and efficiencies vary significantly depending on the type of plastic, microbial species, and prevailing environmental conditions.

a. Efficiency and Limitations of Microbial Species in Plastic Biodegradation

Bacteria have been the primary focus in plastic biodegradation, particularly genera such as *Pseudomonas*, *Bacillus*, *Rhodococcus*, and *Ideonella* (Ghosh *et al.*, 2013). For instance, *Ideonella sakaiensis* is well known for its ability to degrade PET through PETase and MHETase enzymes, which hydrolyze PET into its monomers: terephthalic acid and ethylene glycol. Similarly, *Pseudomonas putida* can degrade several types of plastics, including

polystyrene (PS), polyurethane (PU), and PET, using plasmid-encoded enzymes such as laccases and monooxygenases (Wilhelm *et al.*, 2021). *Rhodococcus ruber* is effective in degrading polyethylene (PE) via alkane hydroxylases and esterases, while *Bacillus subtilis* has been engineered to express recombinant PETase variants, enabling enhanced degradation of PET and polylactic acid (PLA) (Austin *et al.*, 2018).

Despite these successes, not all bacteria exhibit equal efficiency in plastic biodegradation. For example, while *I. sakaiensis* is highly effective in degrading PET, it does not naturally degrade other plastics such as PE or PS. Moreover, the degradation rate is often slow, and many bacteria require specific environmental conditions (e.g., optimal temperature or pH) to function effectively. This variability highlights the need to carefully select microbial strains tailored to particular bioremediation applications, such as bioaugmentation.

Fungi, including *Aspergillus*, *Penicillium*, and *Fusarium* also play a role in plastic biodegradation by releasing extracellular enzymes and organic acids (Russell *et al.*, 2011; Ali *et al.*, 2014). They have been shown to degrade a variety of plastics, including polyurethane and polyethylene, through mechanisms similar to those used by bacteria. However, fungal biodegradation typically requires longer incubation periods and specific growth conditions to achieve significant results. Additionally, some archaea, particularly halophiles and thermophiles, have demonstrated potential in degrading plastics under extreme environmental conditions (Kumar and Raut, 2015). Despite this potential, their application remains largely restricted to specialized industrial contexts.

b. Microbial Species, Plasmids, and Enzyme Efficiency

Table 1 summarizes microbial species involved in plastic degradation, their target polymers, and the plasmids that harbor the corresponding degradation genes. Recent studies highlight that plasmid-encoded pathways are central to enhancing the efficiency of bacterial plastic biodegradation, particularly in the case of polyethylene, with implications for climate change mitigation (Julius *et al.*, 2024). The table also distinguishes between plasmids whose roles in plastic degradation are experimentally validated and those that remain inferred.

Table 1: Summary of Plastic-Degrading Microbial Species and Associated Plasmids

Microbial Species	Target Plastic(s)	Plasmid Involved	Key Genes/Enzymes	Plasmid Details	Source
<i>Ideonella sakaiensis</i>	PET	pISF1	PETase, MHETase	Experimentally validated	Yoshida <i>et al.</i> , 2016; Owen <i>et al.</i> , 2020
<i>Pseudomonas putida</i>	PS, PU, PET	pWWo, pPZ81	Laccases, Monooxygenases	Experimentally validated	Wilhelm <i>et al.</i> , 2021; Ghosh <i>et al.</i> , 2013
<i>Rhodococcus ruber</i>	PE	Not specified	Alkane hydroxylase, Esterases	Inferred	Russell <i>et al.</i> , 2011; Ali <i>et al.</i> , 2014
<i>Bacillus subtilis</i>	PLA, PET	Engineered plasmid vectors	Recombinant PETase variants	Experimentally validated	Austin <i>et al.</i> , 2018; Tiso <i>et al.</i> , 2021
<i>Alcanivorax borkumensis</i>	HDPE, LDPE	Native plasmids	AlkB genes (alkane monooxygenases)	Inferred	Kumar Sen and Raut, 2015; Ghosh <i>et al.</i> , 2013
<i>Comamonas testosteroni</i>	PET intermediates	Megaplastids (uncharacterized)	Terephthalate dioxygenase	Inferred	Ojha <i>et al.</i> , 2017
<i>Cupriavidus necator</i>	PLA	pBBR1MCS (engineered)	PHA synthases	Experimentally validated	Danso <i>et al.</i> , 2019
<i>Escherichia coli</i> (engineered)	PET, PU	pET, pUC-derived plasmids	Engineered PETase, Cutinases	Experimentally validated	Pang <i>et al.</i> , 2014

c. Common Plastics Targeted

While microorganisms have shown the ability to biodegrade a wide range of plastics, the biodegradation rates differ significantly depending on the polymer type and microbial system. Polyethylene terephthalate (PET) is among the more biodegradable plastics, with multiple microorganisms and enzymes involved in its breakdown. In contrast, polyethylene (PE) and polypropylene (PP) are more resistant to degradation due to their high molecular weight and hydrophobicity. These plastics often require pretreatment or extended degradation times (Wei and Zimmermann, 2017). The varying efficiencies of plastic degradation underscore the need for targeted approaches in bioremediation, with specific microbes selected based on the plastic type and the desired degradation rate.

d. Natural Enzymatic Pathways

The enzymatic breakdown of plastics typically follows a two-step process: depolymerization of the plastic into smaller molecules (oligomers or monomers), followed by assimilation and mineralization by microorganisms (Russell *et al.*, 2011). Key hydrolytic enzymes involved include PETase, cutinase, lipase, and esterases, which break ester and urethane bonds in synthetic polymers (Danso *et al.*, 2019; Tokiwa *et al.*, 2009). Oxidative enzymes such as peroxidases, laccases, and monooxygenases play a crucial role in degrading complex plastics like PE and PVC (Sabev *et al.*, 2006). Many of these enzymes are plasmid-encoded, facilitating their horizontal transfer and expression within microbial populations, and thereby promoting adaptation to synthetic polymers in polluted environments (Yoshida *et al.*, 2016; Urbanek *et al.*, 2018).

The role of plasmid-encoded enzymes in plastic biodegradation is critical, as it accelerates the degradation process within microbial communities. Figure 2 illustrates the microbial enzymatic pathways involved in plastic biodegradation, showing both aerobic and anaerobic processes. Under aerobic conditions, environmentally friendly byproducts such as H_2O and CO_2 are produced, whereas under anaerobic conditions, H_2O , CH_4 , and H_2S are generated. This diagram visually summarizes the two-step biodegradation process, linking enzymatic action to the corresponding byproducts.

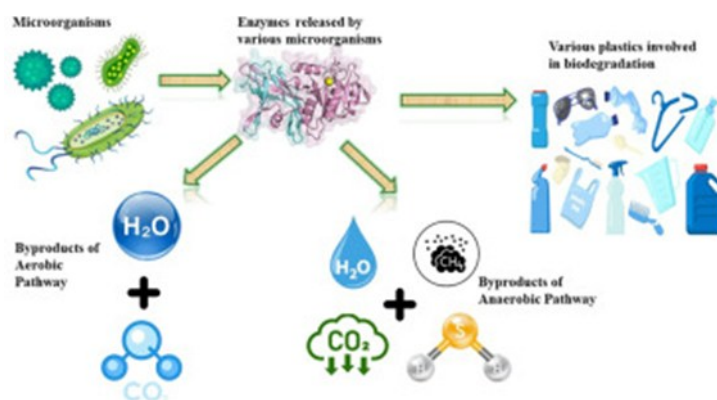


Figure 2: Microbial Enzymatic Pathways for Plastic Biodegradation. (Dhali *et al.* (2024))

Conceptual Framework

1. Plasmids: Structure, Functions, and Environmental Relevance

Microorganisms can biodegrade various groups of plastics, with biodegradation rates differing depending on the polymer and microbial system. Common plastics such as PET, PE, PP, PS, and PVC are among the most frequently biodegraded (Shah *et al.*, 2008). PET is more readily biodegradable, with multiple microorganisms and enzymes identified for its degradation (Wei and Zimmermann, 2017). In contrast, PE and PP are more recalcitrant due to their high molecular weight and hydrophobic nature, often requiring pretreatment for effective degradation (Ali *et al.*, 2014).

(a) Structure and Functional Role of Plasmids

Plasmids are small, circular DNA molecules that exist independently of the chromosome. They are classified as conjugative or non-conjugative based on their ability to mediate horizontal gene transfer (HGT) (Figure 3). Conjugative plasmids, such as F (fertility) and R (resistance) plasmids, contain *tra* genes that form pili to transfer DNA between cells. These plasmids play a significant role in spreading genes involved in antibiotic resistance, xenobiotic degradation, and virulence (Heuer and Smalla, 2012). Figure 3a illustrates the process of conjugation, where conjugative plasmids (e.g., F and R plasmids) transfer genetic material from a donor cell to a recipient cell via the formation of a sex pilus.

Degradative plasmids, for example, carry enzymes capable of breaking down complex materials such as hydrocarbons and plastics. Figure 3b shows the mobilization of mobilizable plasmids by conjugative plasmids, highlighting the transfer of genetic material

even when the plasmids themselves lack conjugation machinery. Non-conjugative plasmids, such as Col plasmids and metabolic plasmids, may not possess the necessary transfer machinery but can persist through vertical inheritance or coexist with conjugative plasmids. Cryptic plasmids, on the other hand, enhance microbial resilience under stress. In addition, industrially engineered plasmids are widely applied in gene expression and cloning (Wilhelm *et al.*, 2021).

Both conjugative and non-conjugative plasmids significantly enhance microbial survival and ecological success in plastic-contaminated environments. They frequently harbor catabolic enzymes (e.g., PETase and MHETase), efflux pumps, and stress-response proteins that contribute to plastic degradation (Wong *et al.*, 2021). The diverse functions associated with these plasmids underscore their role as carriers of environmental adaptability, particularly in the bioremediation of plastic waste. This significance is summarized in Table 2.

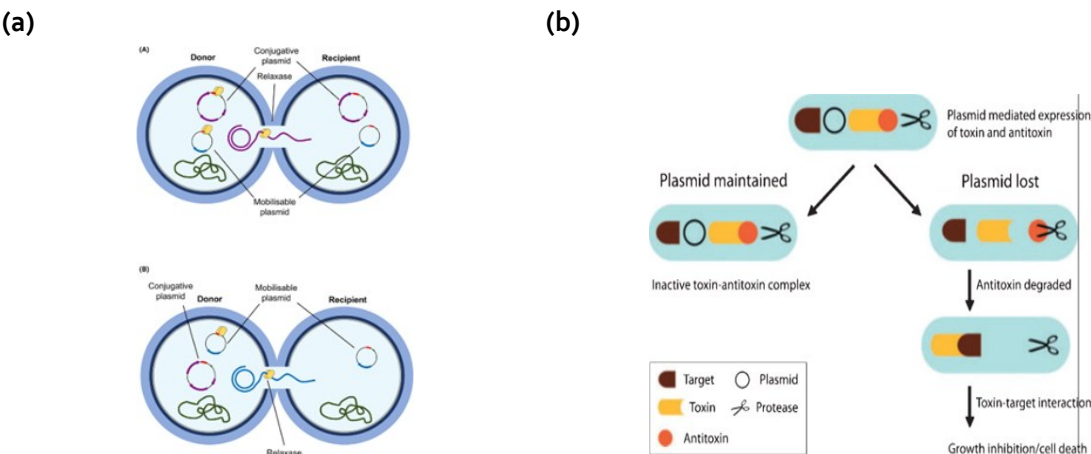


Figure 3: Conjugative and Mobilizable Plasmid-Mediated Horizontal Gene Transfer in Microbial Populations (Daniel *et al.*, 2015)

Table 2: The primary functions of Plasmids

Plasmid Type	Functions
Conjugative Plasmids	
F (Fertility) Plasmids	Enable horizontal gene transfer through conjugation by forming sex pili.
R (Resistance) Plasmids	Carry antibiotic resistance genes and can spread resistance among bacteria.
Degradative Plasmids	Encode enzymes that allow degradation of toxic compounds (e.g., toluene, plastics).
Virulence Plasmids	Enhance pathogenicity by encoding toxins or adhesion factors.

Non-Conjugative Plasmids	
Cryptic Plasmids	Maintain plasmid stability, replication control, or stress resistance; do not carry obvious phenotypic traits.
Col Plasmids	Produce bacteriocins, which kill or inhibit closely related bacterial strains.
Metabolic Plasmids	Carry genes for specialized metabolic functions, like sugar or pollutant breakdown.
Biotechnological Vectors	Engineered plasmids used for gene cloning, expression, and synthetic biology.

(b) Ecological and Evolutionary Implications

The ecological implications of plasmid-mediated biodegradation are substantial. Plasmids, by facilitating the transfer of plastic-degrading enzymes among microbial populations, enable microbes to adapt and survive in polluted environments. The evolutionary implications are equally important, as plasmid-mediated HGT accelerates the spread of plastic-degrading genes across different species. This adaptability and the evolution of biodegradation capabilities suggest that microbial communities are not static but are continuously evolving in response to anthropogenic plastic pollution (Brito *et al.*, 2016). The continuous adaptation of plasmids in microbial populations could also lead to new plastic degradation pathways, thereby aiding future biotechnological advancements in plastic waste management.

(c) Horizontal Gene Transfer and Evolution of Plastic-Degrading Traits

HGT is a key driver of microbial evolution, enabling bacteria to acquire genes for plastic degradation via conjugation, transformation, and transduction (Allen and Waclaw, 2016). Genes for plastic-degrading enzymes, such as PETase and laccases, are frequently located on mobile genetic elements, making them transferable across species (Albert *et al.*, 2022). As shown in Figure 4, microplastics and nanoplastics serve as surfaces for biofilm formation, which enhances HGT. While microplastics can increase gene transfer, nanoplastics may inhibit it by damaging bacterial membranes (Li *et al.*, 2022; Liu *et al.*, 2023). This gene exchange not only spreads plastic-degrading traits but may also contribute to the dissemination of antibiotic resistance, particularly when both types of genes are carried on the same plasmid (Yu *et al.*, 2023).

(d) Environmental Selection and Plasmid Retention

The role of environmental selection is critical in plasmid retention. HGT facilitates the rapid spread of plastic-degrading traits across microbial populations, allowing them to persist in plastic-contaminated environments (Dantas and Sommer, 2012). The retention of these plasmids is shaped by environmental factors that select for plastic-degrading genes, thereby fostering microbial communities capable of degrading plastics in polluted ecosystems.

(e) Case Studies in Polluted Ecosystems

Horizontal gene transfer plays a central role in the dissemination of degradative traits within microbial communities in plastic-rich environments such as landfills, ocean plastic debris, and wastewater treatment plants. For instance, the degradation of aromatic compounds in *Pseudomonas resinovorans* was linked to plasmid pCAR₁, isolated from soil polluted with plastic additives (Maeda *et al.*, 2003). Similarly, plasmids carrying hydrolase- and esterase-related genes were found in bacteria from polyethylene-contaminated landfills in India, demonstrating microbial adaptation to plastic pollution (Raghul Subin *et al.*, 2022). Other strains, such as *Alcanivorax* and *Marinobacter*, have been shown to degrade hydrocarbons and plastics via plasmids encoding alkane hydroxylase and monooxygenase genes (Zettler *et al.*, 2013). These plasmid-based systems hold significant potential for genetic engineering approaches aimed at enhancing microbial degradation of plastics under diverse environmental conditions.

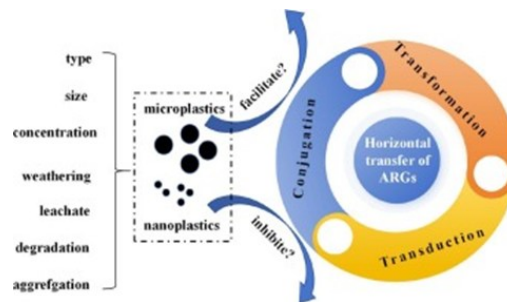


Figure 4: Influence of plastic characteristics (type, size, concentration, weathering, leachate, degradation, aggregation) on the horizontal transfer of antibiotic resistance genes (ARGs). (Wang *et al.*, 2024).

Figure 5 illustrates the conceptual flow of genetic material, where plasmid types serve as carriers for genes encoding degradative enzymes. These genes are exchanged between bacteria through horizontal gene transfer (HGT) mechanisms such as conjugation, transformation, and transduction. Once acquired, plasmid-borne enzymes enhance microbial capacity to degrade complex pollutants, including hydrocarbons, plastics, and chemicals. The interaction between plasmid types and HGT mechanisms accelerates the dissemination of biodegradation-related genes across microbial populations, strengthening their collective ability to break down pollutants. This process leads to effective detoxification and mineralization of harmful substances, contributing both to environmental cleanup and to the adaptive evolution of bacterial communities in contaminated ecosystems.

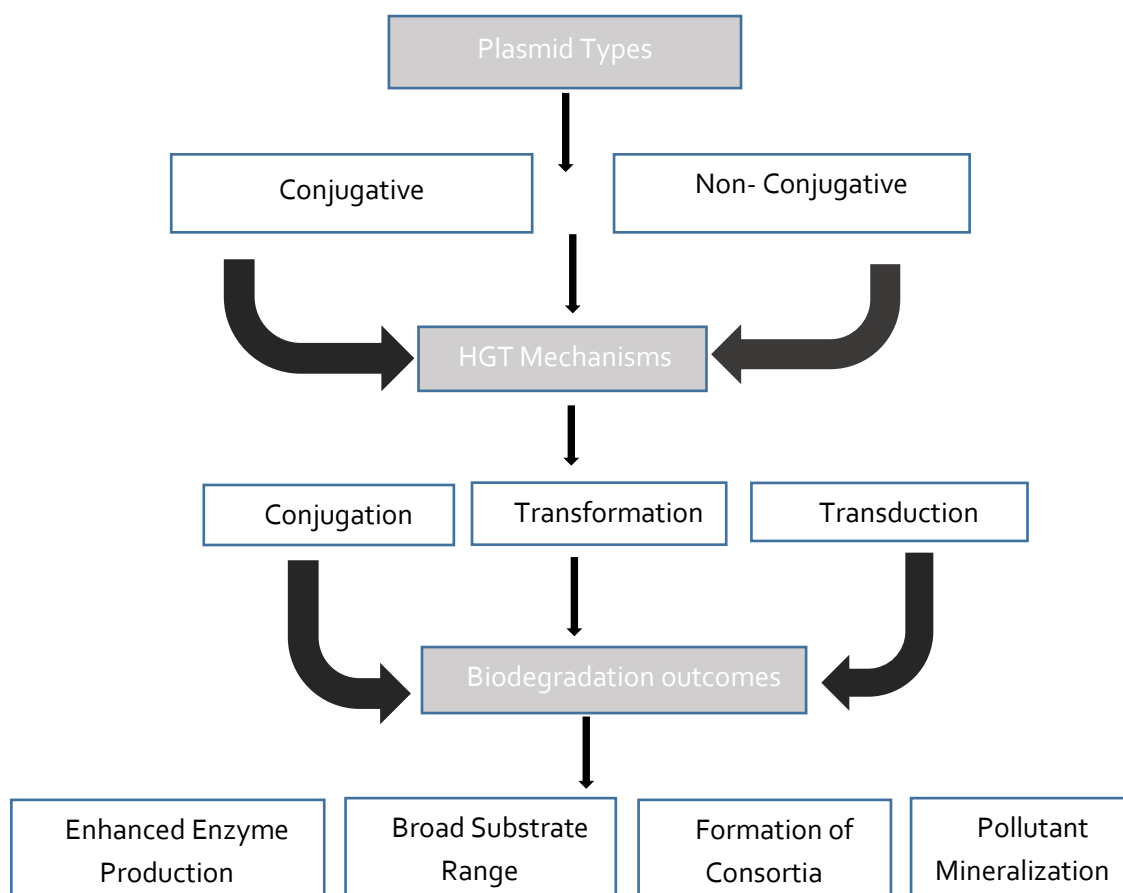


Figure 5: Conceptual diagram linking plasmid types, HGT mechanisms, enzyme production, and biodegradation outcomes. (Zhu and Zhang, 2018).

Methodology

As a review article, this study does not present original experimental methods; instead, the methodology focuses on the systematic process by which relevant literature was identified, selected, and included in the review. The aim is to ensure transparency and reproducibility in the review process.

1. Literature Selection Criteria

a. Database Search

A comprehensive search was conducted in the following databases: PubMed, Web of Science, Google Scholar, Scopus, and Springer Link. The search was limited to peer-reviewed articles, conference proceedings, and book chapters published in English.

b. Keywords and Search Terms

Search terms included "plastic biodegradation," "microbial plasmids," "synthetic biology," "bioaugmentation," and "circular bioeconomy," which were used in various combinations to identify studies on plasmid-mediated biodegradation of plastics.

c. Inclusion Criteria

1. Study Type: Only original research articles, reviews, and meta-analyses published from 2000 to the present were included.
2. Microbial Focus: Studies that demonstrated plastic biodegradation by microbial species (including bacteria, fungi, and archaea) or involvement of plasmids in this process were prioritized.
3. Plasmid Details: Articles describing plasmid types (conjugative or non-conjugative) and their role in plastic biodegradation were included, with special attention to enzymes encoded by plasmids.
4. Geographical Scope: Studies from plastic-contaminated environments (e.g., landfills, oceans, wastewater treatment plants) were included to understand plasmid adaptability and ecological relevance.

d. Exclusion Criteria

1. Studies lacking plasmid-related data or focused exclusively on non-microbial degradation methods (e.g., chemical or mechanical processes) were excluded.
2. Studies with limited or no experimental data on microbial plasmids involved in plastic degradation were also excluded.

e. Data Extraction

For each selected study, the following data were extracted:

1. Microbial species involved in plastic degradation
2. Plasmid types identified
3. Key genes and enzymes responsible for biodegradation
4. Mechanisms of horizontal gene transfer (HGT)
5. Ecological implications and potential for bio-augmentation or synthetic biology applications

f. Assessment of Literature Quality

1. Articles were assessed for methodological rigor, including experimental design, sample size, reproducibility, and clear reporting of plasmid-related findings.
2. The quality of plasmid data, whether experimentally validated or inferred was carefully evaluated.

2. Synthesis and Analysis

1. A narrative synthesis approach was employed to critically compare and contrast findings across the selected studies.
2. Emphasis was placed on understanding mechanisms of plasmid-mediated biodegradation and their contributions to ecological adaptability and plastic waste management.

- Findings were grouped by plasmid type, microbial species, and plastic type to identify trends and gaps in the literature.

Results and Findings

a. Engineered Plasmids for Enhanced Plastic Degradation

The field of synthetic biology has opened new possibilities for improving plastic biodegradation through engineered plasmids. Tools such as CRISPR-Cas systems, modular plasmid constructs, and standardized genetic parts (BioBricks) enable the redesign of microbial metabolism to efficiently degrade polymers like PET, polystyrene, and polyethylene (Brophy and Voigt, 2014).

(i) Synthetic Biology and CRISPR Tools

CRISPR-Cas systems, combined with modular plasmid assemblies and standard genetic components (BioBricks), enable precise genome editing, gene insertion, and transcriptional control in environmental and model microbes (Barrangou and Doudna, 2016). For example, CRISPR has been used to introduce PETase and MHETase genes into *Escherichia coli* and *Bacillus subtilis*, enhancing their plastic-degrading abilities and making them more environmentally feasible (Lu *et al.*, 2022).

(ii) Engineering *E. coli*, *Pseudomonas*, and *Ideonella sakaiensis*

- Escherichia coli* is widely used as a chassis due to its rapid growth and genetic tractability. Researchers have introduced PETase genes to enable it to depolymerize PET into terephthalic acid (TPA) and ethylene glycol (Tournier *et al.*, 2020).
- Pseudomonas putida* has been engineered with PETase and MHETase genes to metabolize degradation by-products (e.g., TPA) into biomass or bioplastics like polyhydroxyalkanoates (PHAs) (Knott *et al.*, 2020).
- Ideonella sakaiensis*, a natural PET degrader, has been further modified to increase PETase production, broaden substrate range, and improve enzyme secretion (Taniguchi *et al.*, 2019).

(iii) BioBricks and Modular Plasmid Systems

BioBricks standardized DNA sequences consisting of promoters, ribosome binding sites, enzyme genes (e.g., PETase), secretion signals, and recycling peptides enable modular plasmid systems for plastic degradation (Canton *et al.*, 2008). Cloning strategies like Golden Gate and Gibson Assembly allow combinatorial testing of genetic modules to optimize enzyme expression under various environmental conditions. Recent work has engineered plasmids with multiple degradation pathways (e.g., PET, polystyrene, polyurethane), enabling microbial consortia to synergistically degrade complex plastics (Wei *et al.*, 2020).

Table 3: Comparison of Natural vs. Engineered Plasmids in Biodegradation Performance

Feature	Natural Plasmids	Engineered Plasmids
Origin	Naturally occurring in environmental microbes	Artificially designed or modified in the lab
Gene Content	Limited to naturally selected catabolic genes	Can include optimized, multiple, or synthetic degradation genes
Host Range	Often narrow, host-specific	Can be tailored for broad-host range compatibility
Expression Levels	Moderate; regulated by native promoters	Tuned for high expression via strong, synthetic promoters
Enzymatic Efficiency	Moderate activity; evolutionarily conserved	Enhanced via directed evolution or rational design
Plastic Types Targeted	Usually specific (e.g., PET, PE, or PU)	Can be designed to degrade multiple plastic types simultaneously
Stability in Host	Stable under natural conditions	May require stabilization mechanisms (e.g., addiction systems)
Horizontal Gene Transfer Risk	High; can spread resistance genes	Can be minimized using containment or kill-switch strategies
Environmental Adaptability	Naturally evolved for ecological niches	May require optimization for in-situ performance
Biosafety Concerns	Lower, as naturally selected	Higher; requires regulatory oversight for field release
Biodegradation Speed	Slower; dependent on natural enzyme kinetics	Faster; enzymes engineered for improved kinetics

Environmental Applications and Biotechnological Potential

The use of plasmid-bearing microbial (PBM) strains in natural and constructed environments provides solutions to alleviate plastic pollution and promote sustainable waste management. These microbes are widely used in biotechnological applications in environments such as landfills, marine systems, and industrial wastewater, playing a crucial role in the circular bioeconomy (Sentchilo *et al.*, 2013).

(a) Bioaugmentation Strategies Using Plasmid-Bearing Microbes (PBM)

Bioaugmentation involves the introduction of genetically engineered microorganisms carrying plasmid-encoded pathways to degrade plastics in contaminated environments. These microbes are selected based on their ability to produce plastic-degrading enzymes such as PETase and MHETase, which are encoded on mobile plasmids capable of horizontal transfer (Tiso *et al.*, 2021). Strains of *Pseudomonas*, *Ideonella sakaiensis*, and *Bacillus* harboring plasmids have been tested for the biodegradation of PET, polystyrene, and PVC under controlled conditions (Restrepo-Ruiz *et al.*, 2014). These microbes demonstrate adaptability and potential for gene transfer, making them promising candidates for field applications.

(b) Applications in Landfills, Oceans, and Industrial Wastewater

In landfills, where plastics accumulate under anaerobic conditions, PMB consortia can enhance degradation rates and reduce long-term persistence (Zrimec *et al.*, 2021). For example, pilot-scale tests using *Rhodococcus* and *Pseudomonas* strains containing hydrocarbon-degrading plasmids have shown promise in improving polymer breakdown in biocells (Wilkes, 2017). In marine systems, plastic-degrading enzymes from marine metagenomes have been detected colonizing plastics, allowing the plastisphere to serve as a microbial habitat for plastic-degrading communities (Amaral-Zettler *et al.*, 2020).

In industrial wastewater, where plastics are introduced through textile, packaging, and cosmetic industries, bioreactors with plasmid-enhanced microbial consortia are being developed. Genetically modified *Escherichia coli* and *Bacillus subtilis* harboring PET-degrading plasmids have shown enzymatic activity under wastewater-like conditions, offering scalable solutions for plastic treatment (Knott *et al.*, 2020).

(c) Integration into Circular Bioeconomy and Waste Management

PMB can be integrated into circular bioeconomy models that emphasize resource efficiency, waste minimization, and valorization of biowaste. Plastic-degrading microbial systems may be applied in closed-loop recycling, converting waste into bioplastics, biofuels, or other valuable products (Wei *et al.*, 2020). Moreover, microbial degradation of plastics can be combined with mechanical pretreatment and chemical valorization to enhance overall waste management (Zrimec *et al.*, 2021). These biotechnological advances provide scalable and eco-friendly options for plastic waste management, contributing to the global effort to reduce plastic pollution.

Discussion

1. Critical Analysis and Knowledge Gaps

Although significant progress has been made in using microbial plasmids for plastic biodegradation, several scientific, technological, and regulatory challenges remain. This section critically reviews existing knowledge, identifies gaps, and highlights urgent areas requiring further investigation to improve the real-world application of plasmid-based degradation strategies (Restrepo-Ruiz *et al.*, 2014). Research demonstrates that microbes such as *Pseudomonas*, *Bacillus*, and *Ideonella sakaiensis* can degrade synthetic plastics particularly PET and PU through plasmid-encoded enzymes such as PETase and MHETase (Tiso *et al.*, 2021). While mobile catabolic genes on plasmids facilitate plastic degradation under laboratory conditions, the mechanisms of these pathways in complex, heterogeneous environments such as landfills, oceans, and wastewater systems remain poorly understood. The interactions among plasmid stability, microbial cell fitness, and environmental stressors are also unclear (Yoshida *et al.*, 2016).

(a) Challenges in Gene Transfer, Containment, and Field Efficiency

A major challenge in applying PMB is the horizontal gene transfer (HGT). Although conjugation and transformation facilitate gene exchange within microbial populations, predicting and controlling these processes in dynamic environments is difficult (Top *et al.*, 2002). Additionally, plasmid loss due to metabolic burden or lack of selective pressure can reduce microbial degradation efficiency in situ (Domingues *et al.*, 2012). Many studies rely on laboratory-optimized strains, which may not reflect the physicochemical variations of natural environments, including fluctuations in pH, salinity, temperature, and microbial competition. This creates uncertainty regarding plasmid stability and enzyme performance in field applications (Knott *et al.*, 2020).

(b) Risks of Engineered Plasmids: Biosafety and Gene Flow

The release of genetically engineered plasmids into the environment raises biosafety concerns. The unintended transfer of synthetic genes to pathogenic bacteria could provide them with novel metabolic capabilities or selective advantages (Heuer and Smalla, 2012). This may result in genetic contamination, where engineered constructs spread uncontrollably among natural microbial populations. Current regulatory frameworks often lag behind advances in synthetic biology, leaving insufficient guidance on assessing and controlling the ecological risks of releasing engineered microbes (Schmidt *et al.*, 2009). Although strategies such as kill switches, auxotrophy, and gene flow barriers have been proposed, their practical use in open-field conditions remains limited (Chan *et al.*, 2016).

2. Limited Real-World Application Data

While plasmid-based plastic degradation shows promise in laboratory studies, large-scale field applications are still rare. Most findings come from proof-of-concept or microcosm experiments, which fail to capture the complexity of natural ecosystems. Environmental fluctuations, competition, and nutrient limitations often hinder the success of plasmid-carrying microbes in real-world conditions (Wei *et al.*, 2020).

Recent findings suggest that bacterial plasmids may contribute to polyethylene biodegradation, offering a potential strategy for mitigating climate change (Julius *et al.*, 2024). However, the long-term ecological consequences of introducing plasmid-bearing microbes remain poorly understood. Addressing these uncertainties will require longitudinal studies, environmental monitoring, and cost-benefit analyses to assess both feasibility and public acceptance (Timmis *et al.*, 2019).

Future Research Directions**(a) Field Testing and Long-Term Ecological Studies**

The urgent need to address global plastic waste highlights plasmid-based biodegradation as an attractive solution. Translating laboratory results into practical applications will require rigorous field testing. Real-world environments such as landfills, marine sediments, and wastewater treatment systems are highly variable, influencing microbial colonization

and degradation dynamics (Napper and Thompson, 2023). Long-term studies are necessary to evaluate microbial interactions, HGT events, and the persistence of engineered traits. Such work will also help clarify biosafety concerns and ecological risks (Danso *et al.*, 2018).

(b) Comparison with Other Biodegradation Approaches

Comparisons with alternative plastic degradation strategies are essential for assessing the advantages and limitations of plasmid-based approaches. For instance, enzymatic treatments with natural or recombinant enzymes can degrade plastics without relying on plasmid-mediated gene transfer. However, these methods often lack the scalability and adaptability of plasmid-enhanced systems (Daniel *et al.*, 2015). Chemical recycling, while effective in breaking plastics into monomers, is typically energy-intensive and produces harmful by-products. By contrast, plasmid-driven biodegradation provides a potentially more sustainable and environmentally friendly strategy, provided that plasmid stability and gene flow are carefully managed in diverse environments (Restrepo-Ruiz *et al.*, 2014).

(c) Trade-Off between Broad-Host-Range Engineered Plasmids and Containment Risks

One of the major challenges of engineered plasmids is balancing broad-host-range compatibility with containment risks. While engineered plasmids can be designed to function across a wide range of hosts, this broad compatibility increases the risk of unintended gene transfer to non-target organisms, including pathogenic bacteria. The trade-off between increased biodegradation efficiency and the biosafety risks of uncontrolled gene flow remains a critical concern. Containment strategies, such as kill switches or gene flow barriers, are being explored, but their practical implementation in environmental settings has yet to be fully realized (Stirling *et al.*, 2017).

(d) Engineering More Efficient and Stable Enzymes

Enhancing the activity and stability of plastic-degrading enzymes is crucial for their success in industrial and environmental applications. Current enzymes, such as PETase and MHETase, may lack the necessary thermal stability or substrate specificity for large-scale use (Knott *et al.*, 2020). However, advances in protein engineering, machine learning-guided design, and structure-based mutagenesis provide opportunities to develop more efficient and versatile enzymes capable of degrading a broader range of plastics under diverse environmental conditions (Lu *et al.*, 2022).

(e) Cross-Disciplinary Collaboration

Addressing plastic pollution requires a multidisciplinary approach. Close collaboration among microbiologists, synthetic biologists, environmental engineers, and policy experts is essential to bridge the gap between scientific innovation and societal needs. Engineers can design bioreactors or in situ delivery systems tailored to specific environments, while policy analysts can develop regulatory frameworks that ensure the safe deployment of

these technologies (Wilhelm *et al.*, 2021). Environmental ecologists can monitor the ecological impact of these systems. Funding for integrative research consortia is critical to facilitate bench-to-field innovations and accelerate the development of sustainable plastic degradation technologies (Tournier *et al.*, 2020).

Conclusion

The growing crisis of plastic pollution demands sustainable, scalable, and innovative solutions that can be implemented globally. This review highlights the potential of microbial plasmids to enhance plastic biodegradation, emphasizing their capacity to transfer catabolic genes across microbial populations in diverse environments. By leveraging both naturally occurring plasmid-encoded enzymes (e.g., PETase and MHETase) and engineered microbial systems enhanced through synthetic biology and CRISPR technologies, plasmids represent a cutting-edge approach to bio-based plastic waste management.

While laboratory-based results are promising, translating these findings into real-world applications remains challenging. Key obstacles include gene stability, environmental variability, biosafety concerns, and regulatory uncertainties. The introduction of engineered plasmids, particularly those incorporating kill switches or self-limiting circuits, must be carefully managed with robust scientific oversight and ethical considerations.

This review advances understanding by synthesizing current research on plasmid-mediated biodegradation, comparing it with alternative methods such as enzymatic treatments and chemical recycling, and addressing practical challenges of field implementation. Unlike previous reviews, it critically examines the feasibility of scaling these approaches and highlights trade-offs between efficiency and containment risks.

Future research should prioritize long-term ecological testing, enzyme optimization, and the development of effective biosafety mechanisms. A coordinated approach involving scientists, policymakers, and investors is essential to bridge the gap between laboratory innovation and environmental regulation. By strategically advancing plasmid-based biodegradation systems, there is potential to revolutionize plastic waste management and contribute to a circular bioeconomy that is both sustainable and environmentally responsible.

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