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REVIEW ARTICLE

Advanced strategies for the development of synthetic microbiomes for effective pesticide biodegradation

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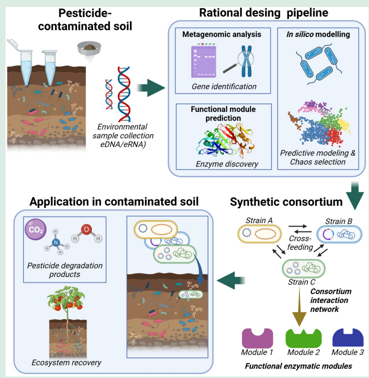
HIGHLIGHTS

- Synthetic microbial consortia are effective in pesticide degradation.
- Recent advancements in metagenomics are analysed.
- Synthetic biology methodologies for targeted construction are discussed.
- Critical aspects of biosafety, stability, and regulatory constraints are discussed.



ABSTRACT: Development of innovative strategies for detoxification of pesticides accumulated in ecosystems is of crucial importance for the global agricultural intensification and reducing their environmental footprint. One of the most promising approaches to the problem is the creation of synthetic microbial consortia possessing high catabolic activity and the ability to efficiently degrade persistent environmental pollutants. This review analyzes recent advancements in metagenomics that enable a detailed examination of the genetic diversity and functional potential of natural microbiomes. Special attention is given to the engineered modification of key genetic elements responsible for pesticide degradation, as well as synthetic biology methodologies aimed at the targeted construction of microbial consortia with predefined biodegradative properties. Additionally, the review discusses critical aspects of biosafety, biostability, and regulatory constraints associated with the introduction of genetically modified microbial systems into natural and agricultural ecosystems. The significance of an

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interdisciplinary approach is emphasized for the development of environmentally safe, adaptive, and highly effective biotechnological solutions. The implementation of such innovative strategies has the potential not only to minimize pesticide-related environmental burdens but also ensure the long-term sustainability of agricultural ecosystems. Furthermore, this review proposes conceptual models of semi-synthetic and synthetic microbial consortia designed for the sequential degradation of chlorpyrifos and methyl parathion. These models are based on recent experimental advancements in metagenomics and synthetic biology, underscoring their potential for the development of novel biodegradation systems.

KEYWORDS: Pesticide biodegradation, Metagenomics, Genetic engineering, Synthetic biology, Microbial consortia, Agroecosystem sustainability

1 Introduction

Pesticides play a crucial role in modern agricultural practices by providing effective protection for crops against pests and phytopathogens, thereby contributing to the preservation of yield and the resilience of food systems (Sharma et al., 2019). However, the intensified use of pesticides is associated with serious environmental and toxicological consequences due to their non-target distribution, low biodegradability, and persistence in the environment (Narayanan et al., 2022). According to published data, only about 1% of applied pesticides reach their intended targets, while the majority disperse into the environment, causing long-term alterations in biogeochemical cycles (Gangola et al., 2022; Bokade et al., 2023). Annually, approximately 2.4 million tons of these chemical compounds enter the global ecosystem. As a result of their accumulation in the soil, 74.8% of agricultural lands are at risk of contamination, with 31.4% classified as critical zones with high chemical loads (Zuo et al., 2024). Rapid land degradation leads to changes in land fertility and agricultural productivity, which negatively affects the global food supply and poses threats to food security (Sarsekeyeva et al., 2024). To mitigate the risks associated with pesticide pollution, international organizations develop regulatory standards that impose limits on the maximum allowable concentrations of these compounds in the environment and food products. However, even the stringent regulations adopted in developed countries are not always enforced on a global scale (Parra-Arroyo et al., 2022).

Monitoring of surface waters also reveals a severe extent of pesticide contamination, as confirmed by long-term research findings. Over a five-year period, a persistent presence of 221 pesticide compounds was detected at 74 river sites across the United States, exceeding their designated application zones and expected degradation periods (Stackpoole et al., 2021). A similar situation is observed in China, where pesticide usage reached 2.73 million tons in 2020 alone, accounting for 10.3% of global consumption (Li et al., 2024). Analysis of 184 samples collected from 16 river estuaries across seven major basins in eastern China identified 106 predominant pesticides, with herbicides, particularly triazines, being the most dominant (Li et al., 2023). Beyond their destructive impact on ecosystems, the prolonged presence of pesticides in the environment poses significant risks to human health (Zhou et al., 2025). This threat arises from the accumulation of pesticide residues in agricultural products, particularly in leafy crops, which frequently contain high concentrations of toxic compounds such as procymidone, chlorpyrifos, and sodium 4-chlorophenoxyacetate (Zhou et al., 2023). Chronic exposure to these compounds is associated with disruptions of the endocrine, nervous, and reproductive systems, as well as an increased risk of oncological diseases (Sharma et al., 2019; Nicolella and de Assis, 2022; Kaur et al., 2024).

The high toxicity of pesticides and their ability to accumulate within trophic chains necessitate the development of effective strategies for their removal from contaminated agroecosystems (Dhuldhaj et al., 2023). Existing approaches for pesticide removal include physicochemical and biological methods, which differ in their mode of action, efficiency, and adaptability to various environmental conditions (Ruomeng et al., 2023). Physicochemical methods, such as adsorption on activated carbon, photocatalysis, and oxidative reactions, are limited by high costs, implementation complexity, and the risk of generating toxic byproducts, underscoring the relevance of developing bioremediation strategies utilizing microorganisms (Raj et al., 2023; Singh et al., 2024). Microorganisms, due to their high biochemical adaptability, not only accelerate pollutant degradation but also utilize these compounds as a source of carbon and energy (Raffa and Chiampo, 2021; Guerrero Ramírez et al., 2023).

The effective implementation of microbial rehabilitation strategies requires the use of advanced technological solutions that enable a detailed study of microbial community dynamics and their adaptive mechanisms under adverse conditions. Breakthroughs in molecular biology, biotechnology, bioinformatics, and systems biology have opened new possibilities for controlling bioremediation processes at the genetic level, significantly enhancing the efficiency of contaminated site restoration (Mishra et al., 2021). Particular attention is given to microbial consortia, which exhibit high resilience and adaptability in dynamic and stressful environments. The complex intercellular interactions within these communities contribute to maintaining a dynamic equilibrium, ensuring stable metabolic activity even under abrupt fluctuations in external conditions (Cao et al., 2022).

The further advancement of bioremediation systems has been made possible through the integration of synthetic biology with multi-OMICS approaches (metagenomics, transcriptomics, proteomics, metabolomics) and cutting-edge genome editing technologies (CRISPR-Cas, ZFN, TALEN). The schematic in Fig. 1 illustrates the integration of synthetic biology, metagenomics, and genetic engineering in the development of synthetic microbial consortia to enhance pesticide degradation. These tools enable the targeted identification of key enzymes and regulatory mechanisms involved in biodegradation processes and allow for the modification of their activity to enhance the efficiency of contaminant breakdown (Tarfeen et al., 2022). The integration of modern biotechnological methods with microbial consortia engineering is leading to the development of environmentally safe, sustainable, and scalable bioremediation solutions capable of effectively decontaminating ecosystems from various pollutants, including pesticides.

In this work, we undertake a comprehensive review of current data on the application of metagenomic technologies, genetic engineering, and synthetic biology for the targeted design of synthetic microbial communities with high pesticide detoxification efficiency. Special attention is given to the development of an integrated model of the synthetic microbial consortia composed exclusively of genetically modified microorganisms (GMMs), each specialized in performing a specific sequential step in pesticide degradation. The review outlines key directions for

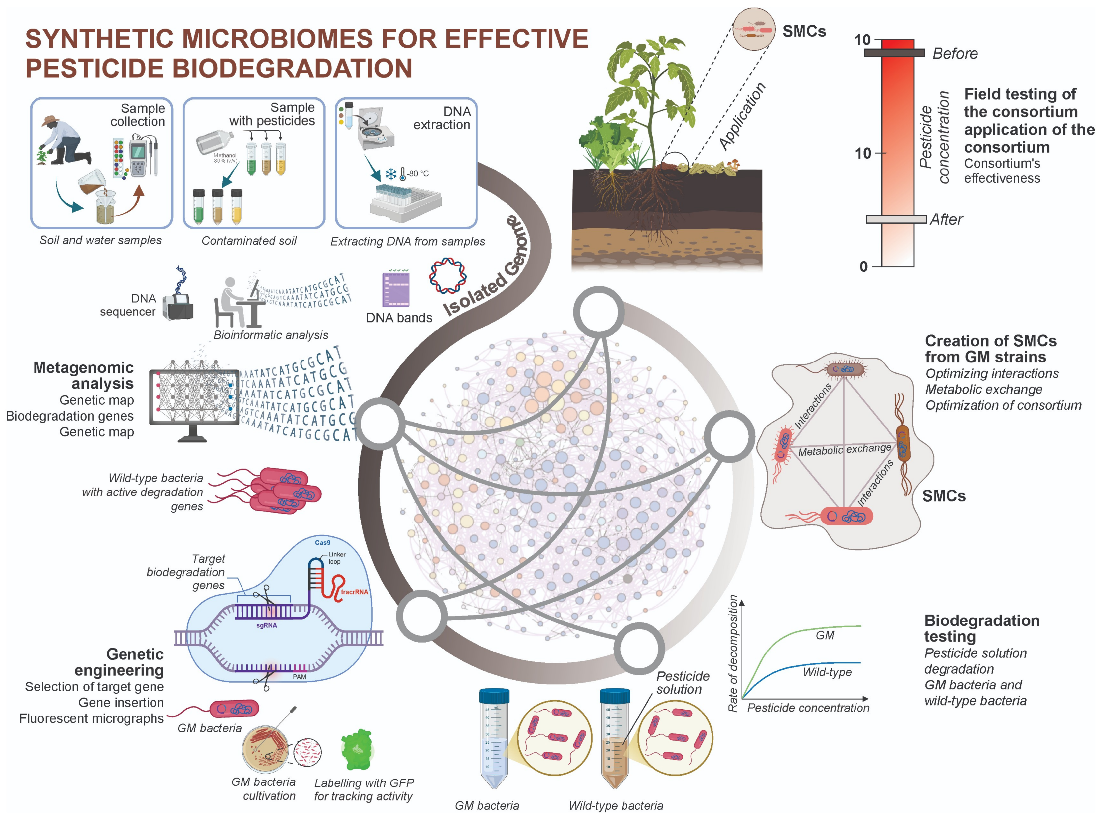

Fig. 1 Advanced strategies in engineering synthetic microbiomes for enhanced pesticide biodegradation.

future research required to translate fundamental knowledge into applied solutions that can be effectively implemented in agroecosystems. Further advancements in this field will significantly contribute to the development of innovative bioremediation strategies, facilitating the restoration of contaminated ecosystems and mitigating anthropogenic impacts on the biosphere.

2 Metagenomics as a key to unraveling the mechanisms of microbial pesticide biodegradation

2.1 Molecular and genetic foundations of microbial pesticide catabolism

After entering the agricultural environment, the distribution and movement of pesticides are determined by their chemical properties, soil characteristics, and the living organisms inhabiting it, which significantly influence microbiological processes and the productivity of agroecosystems. The impact of pesticide application on rhizosphere communities has been shown to result in a decline in abundance, diversity, and functional activity. This decline is accompanied by disruptions in the integrity of microbial cellular structures due to alterations in ultrastructure, membrane permeability, and fundamental biochemical processes (Shahid and Khan, 2022; Dhuldhaj et al., 2023). At the same time, soil, with its high level of genetic and metabolic microbial diversity, represents a dynamic ecosystem where horizontal gene transfer and metagenome diversification contribute to the emergence of specialized populations capable of utilizing pesticides as a source of carbon and energy (Castro-Gutiérrez et al., 2020). Given that genetic material determines an organism's functional potential, the biodegradative capacity of microbes is directly dependent on their genome (Kugarajah et al., 2023). It is evident that environmental heterogeneity exerts a selective pressure on a comprehensive array of enzymes, encompassing oxidative, reductive, and hydrolytic activities. This phenomenon facilitates the transformation of toxic compounds into less hazardous products (Maqsood et al., 2023). Consistent with convergent selection acting on function rather than strict taxonomic exclusivity, biodegradation-associated traits have been repeatedly reported across phylogenetically diverse taxa, including *Pseudomonas, Sphingomonas, Rhodococcus, Micro-coccus, Acetobacter, Burkholderia, Bacillus, Chlamy-domonas, Chlorella, Trichoderma,* and *Penicillium* (Nayak et al., 2018; Kumar et al., 2021; Sehrawat et al., 2021). At the mechanistic level, these transformations are mainly carried out by a limited set of enzyme classes, most commonly oxidoreductases (laccases, oxygenases, peroxidases) and hydrolases (including esterases), together with transferases that support downstream conversions (Sheng et al., 2022; Maqsood et al., 2023; Ebsa et al., 2024). Therefore, identifying genes encoding these enzymes, and interpreting them in their genomic and community context, provides a molecular basis to propose catabolic pathways and to connect microbial community structure with pesticide degradation potential (Guerrero Ramírez et al., 2023).

Current research indicates that effective pesticide mineralization is frequently driven by modular genetic units, such as operons or gene clusters, which facilitate the coordinated expression of enzymes required for sequential transformation steps. Organochlorine pesticides typically require dehalogenation-linked modules for efficient turnover, and consistent with this logic, the high efficiency of γ-hexachlorocyclohexane (lindane) degradation in *Bacillus cereus* SJPS-2 has been attributed to the activity of the *lin* degradation genes (*linA, linB, linC, linX, linD, linE*) (Jaiswal et al., 2023). Organophosphorus pesticides are often initiated by hydrolytic cleavage of P–O or P–S bonds followed by processing of aromatic or nitrophenolic intermediates. Hydrolysis of methyl parathion (MP) and its toxic product p-nitrophenol (PNP) by *Burkholderia cenocepacia* CEIB S5-2 occurs due to the activity of the *mpd* gene and the *pnpABA'E1E2FDC* gene cluster (Ortiz-Hernández et al., 2021). The strain *Arthrobacter* sp. HM01 is capable of degrading 99% of chlorpyrifos (CP) within 10 h, with the organophosphate hydrolase gene (*opdH*) playing a key role in pesticide catabolism (Mali et al., 2022a). Pymetrozine, a pyridine-based insecticide, is degraded by *Pseudomonas guariconensis* BYT-5, where the hydrolase gene *pyzH* and two nicotinic-acid catabolic clusters, *nic1* (*nicAB1R1X1-C1D1E1F1T1*) and *nic2* (*nicR2X2C2D2E2F2T2B2*), support conversion of metabolites into central intermediates such as nicotinic acid and fumarate (Zhang et al., 2025). Pyrethroid insecticides are likewise shaped by entry reactions that cleave ester linkages, and the strain *Rhodococcus pyridinivorans* Y6 efficiently metabolizes pyrethroids, such as prallethrin, through the expression of the *pnbA1564* gene encoding p-nitrophenyl esterase (Huang et al., 2023). The transformation of neonicotinoids may be contingent upon the selective cleavage of C–N bonds in the parent compound molecule. It is evident that the hydrolysis of the C–N bond in acetamiprid catalyzed by the amidase

AceAB, whose synthesis is regulated by *aceA* and *aceB*, yields intermediate metabolites IM 1–4 and enables *Pigmentiphaga* sp. D-2 to degrade this pesticide (Yang et al., 2020). Fungicides frequently require oxidative activation and aromatic-ring handling, which is reflected in both genomic signatures and inducible responses. Genomic annotation of *Acinetobacter johnsonii* LXL_C1 indicates the presence of genes potentially involved in cyprodinil transformation, including components of xenobiotic metabolism pathways associated with cytochrome P450-mediated reactions (frmA, ADH5, adhC), as well as genes for the degradation of aromatic compounds, including catA (Wang et al., 2019). In fungal systems, dichlorvos (DDVP) exposure induces multiple xenobiotic-related cytochrome P450 genes in *Trichoderma atroviride* T23, and deletion of the highly responsive *TaCyp548-2* decreases accumulation of the intermediate 2,2-dichloroethanol and alters its conversion to 2,2-dichloroethanol acetate via changes in low-molecular-weight organic acid production (Sun et al., 2022). Similarly, metolachlor degradation by *Penicillium oxalicum* MetF1 is associated with activation of oxidative phosphorylation and phenylacetic acid catabolism, upregulation of xenobiotic-response gene families encoding cytochrome P450s, peroxidases/oxygenases, and hydroxylases, and reinforcement of tolerance via ABC transporters. This indicates that the catabolic conversion and the adaptation to stress are mechanistically coupled in effective fungal degraders (Chen et al., 2025). More complex pesticide mixtures may be addressed by broader detoxification repertoires, as reported for *Bacillus brevis* 1B, where genes encoding aldehyde dehydrogenase and esterase activities are implicated in degradation of a multi-pesticide formulation, containing imidacloprid, fipronil, cypermethrin, and sulfosulfuron (Gangola et al., 2023).

In summary, the biodegradation of pesticides is governed by a modular genetic and metabolic architecture, in which a limited set of rate-controlling entry reactions is coupled to downstream pathway modules and supported by host tolerance functions. However, it should be noted that the catabolic potential of single isolates is an incomplete predictor of environmental performance. This is because realized activity is shaped by regulatory architecture and expression levels, cellular physiological state, substrate bioavailability, and community-level turnover of intermediates through cross-feeding and functional complementation. Addressing this discrepancy necessitates a transition from organism-centric portrayals to a community-resolved perspective on pathway organization. Metagenomics facilitates this transition by jointly capturing the taxonomic distribution, genomic context, and co-occurrence patterns of catabolic, stress-response, and mobility-associated functions across microbiomes. This process identifies module combinations and complementary taxa most likely to sustain complete degradation under field-relevant constraints, thereby providing an empirical basis for the rational design of stable synthetic consortia.

2.2 Metagenomics as a methodological framework for dissecting pesticide biodegradation in complex microbiomes

Metagenomics captures the genetic basis of pesticide biodegradation at the level at which transformation occurs in soils, because it profiles multispecies assemblages and includes taxa that remain inaccessible to routine cultivation (Bharagava et al., 2019; Dash and Osborne, 2023). By integrating taxonomic composition with functional gene repertoires under xenobiotic selection, metagenomic datasets support reconstruction of candidate pathway repertoires, attribution of key functions to likely degraders, and evaluation of the genomic organization of catabolic modules beyond what isolate-based surveys typically resolve. In parallel, approaches combining functional screening with nucleotide sequence analysis facilitate the characterization of novel taxa and gene products and support identification of metabolic pathways and operons relevant to pollutant removal (Zhang et al., 2021b). Often described as ecogenomics or environmental genomics, metagenomics relies on nucleic acid extraction directly from environmental matrices and thus represents both culturable and uncultured diversity (Bharagava et al., 2019). In monocrotophos-contaminated soils, sequencing resolved 20 bacterial phyla distributed across 119 families and 433 genera, with predominance of *Acidobacteria, Actinobacteria, Firmicutes, Proteobacteria, Bacteroidetes, Nitrospirae,* and *Verrucomicrobia*, illustrating the taxonomic complexity within which biodegradation-associated functions are selected and maintained (Borchetia et al., 2018).

Analytically, metagenomic evidence for pesticide biodegradation is commonly interpreted through three complementary levels of inference that differ in resolution and in the strength of mechanistic attribution. The first level is read-based functional profiling, in which shotgun reads are mapped to reference databases and functional models to quantify the relative abundance of gene families and selected functional markers. In comparative designs such as contaminated versus reference soils, exposure gradients, or time series, this strategy identifies functional categories enriched under pesticide pressure and evaluates their associations with measured environmental parameters, including pesticide concentrations and soil physico-chemical properties. Consistent with this logic, foliar application of glyphosate has been associated with shifts in community composition accompanied by redistribution of functional genes captured by meta-genomic profiling (Wang et al., 2023b). Read-based profiling therefore provides a statistically robust description of community-level enrichment patterns. However, because it relies on short reads without genomic linkage, it offers limited resolution for assigning functions to specific populations and, on its own, does not establish pathway completeness in complex soils.

These community-scale signals often take the form of coordinated taxonomic restructuring together with shifts in biodegradation-relevant gene repertoires. In the study by Regar et al. (2019), the microbiomes of pesticide-contaminated and uncontaminated soils were analyzed using samples collected from two industrial sites producing long-lasting pesticides. The results indicated that contaminated soil exhibited the lowest microbial diversity; however, *Proteobacteria, Actinobacteria, Firmicutes, Bacteroidetes,* and *Acidobacteria* were found to be dominant. Metagenomic analysis conducted by Malla et al. (2022) demonstrated that pesticide-contaminated soils (PCAS) exhibited lower microbial diversity while showing an increased relative abundance of *Proteobacteria, Actinobacteria, Firmicutes,* and *Bacteroidetes* compared to natural soils (NS). In soils contaminated with organochlorine pesticides (OCPs), bacterial diversity declined significantly, whereas viral diversity increased, accompanied by an enrichment of viral auxiliary metabolic genes (AMGs) associated with pesticide degradation. A higher abundance of taxa such as *Streptomyces* and *Nocardiodes,* known for their role in organic pesticide degradation, was also observed. Jokhakar et al. (2022) reported that the pesticide-contaminated Amlakhadi region was dominated by the taxa *Proteobacteria, Bacteroidetes, Firmicutes, Chlorobi,* and *Actinobacteria*. Maharana et al. (2025) observed that high concentrations of residual pesticides, including chlorpyrifos, hexachlorobenzene, and dieldrin, stimulated the active growth of biodegrading bacteria from the genera *Streptomyces, Xanthomonas, Cupriavidus,* and *Pseudomonas.* Additionally, genes encoding enzymes involved in pesticide degradation (cytochrome P450, organophosphate hydrolase, aldehyde dehydrogenase, and oxidase) were predominant in the microbial community, with their expression positively correlated with the presence of *Bacillus, Sphingobium,* and *Burkholderia.* While these observations provide a coherent community-scale picture of selection under pesticide pressure, they remain inferential with respect to realize *in situ* catabolic flux. It is important to note that changes in microbial taxa or gene abundance do not necessarily indicate active pesticide mineralization in the environment. Similar enrichment patterns can arise from increased tolerance, competitive release, altered nutrient and substrate availability, or co-selection by other co-occurring contaminants. The mechanistic interpretation is thus reinforced when enrichment patterns are complemented by pathway-level reconstruction and corroborated by independent evidence of pesticide transformation in the same system, such as metabolite profiles or concordant expression patterns reported in the primary studies.

The second level is assembly-based, genome-resolved metagenomics, in which longer contigs and metagenome-assembled genomes preserve local genomic context and link catabolic genes to specific populations. This enables inference of pathway organization, co-localized accessory traits, and candidate division-of-labour architectures in which multi-step transformations may be distributed across community members. Li et al. (2021a) conducted an extensive metagenomic analysis of 49 contaminated soil samples, identifying 201 metagenome-assembled genomes (MAGs) associated with xenobiotic degradation. The obtained data allowed for the identification of key microbial taxa, catabolic mechanisms, and inter-microbial interactions underlying adaptive bioremediation strategies. However, genome-resolved reconstruction is constrained by factors such as assembly fragmentation, incomplete genome recovery, and strain-level microdiversity. These factors can disrupt operon structure and complicate the assignment of multi-step modules to single genomes, particularly in heterogeneous soils.

The third level is functional metagenomics, in which environmental DNA libraries are screened for catalytic activities, followed by sequencing of active inserts and validation by heterologous expression. This strategy increases causal specificity by prioritizing genes based on measured activity rather than annotation alone. In this broader context, metagenomic analysis is widely applied for identification of functional genes and enzymes in environmental samples, and shotgun sequencing facilitates broad coverage of community DNA and functional annotation of recovered sequences (Zhang et al., 2021b; Liu et al., 2022b; Wani et al., 2022). Within activity-guided discovery, Sun et al. (2024) isolated a novel amidase gene from a soil metagenomic library and heterologously expressed it in *E. coli* BL21(DE3), where the recombinant enzyme exhibited dual esterase and amidase activity, achieving the highest possible degradation rate of amide herbicides. Similarly, a metagenomic library was used to isolate the *est804* gene encoding the Est804 esterase, which can catalyze the hydrolysis of ester bonds, making it a promising candidate for the biodegradation of pyrethroids (Chen et al., 2022). Shang et al. (2022) applied shotgun sequencing in the study of *Bacillus* sp. YS-1, which enabled the elucidation of the lactofen degradation pathway and the identification of two key esterase genes (*rhoE* and *rapE*) directly involved in the catabolism of this pesticide. Han et al. (2022) identified eight dominant bacterial genera associated with the degradation of S-enantiomers of chloroacetamide herbicides (acetochlor and S-metolachlor) in soils repeatedly exposed to these compounds and analyzed the role of key genes (*ppah, alkb, benA, p450*) in herbicide degradation. In the analysis of rhizosphere soils from *Inga striata* and *Caesalpinia ferrea* plants, metagenomic methods enabled the identification of key microorganisms and the genes *atzD*, *atzE*, and *atzF,* which are involved in the degradation of atrazine (Aguiar et al., 2020). In aggregate, concordant evidence across these three analytical levels – enrichment patterns, genome-resolved context, and activity-based validation – provides a more robust and defensible basis for linking sequence-derived inference to pesticide transformation capacity than any single level considered in isolation.

A further determinant of community biodegradation potential is the genomic mobility of catabolic functions. Genetic studies of pesticide-degrading microorganisms indicate that catabolic genes can be in both bacterial chromosomes and on mobile genetic elements (MGEs), including plasmids and transposons (Shahid et al., 2023). MGEs play a crucial role in horizontal gene transfer (HGT), enabling bacteria to exchange adaptive traits, including the ability to degrade pesticides (Horne et al., 2023). Plasmids, which commonly harbor genes responsible for the synthesis of various enzymes, provide microorganisms with the ability to break down a wide range of toxic compounds either through the transfer of catabolic genes or by modifying existing enzymes (Bhatt et al., 2021a). Metagenomic analysis can resolve conserved gene cassettes, flanking insertion sequences, and co-occurrence patterns indicative of mobility. Through metagenomic analysis of a microbial community exposed to isoproturon, Storck et al. (2020) reported that the *pdmAB* genes, encoding an N-demethylase responsible for the initial demethylation of isoproturon, are localized within a highly conserved cassette flanked by insertion sequences IS*6* and IS*256*, primarily transferred via sphingomonad plasmids. It was demonstrated that the abundance and expression of *pdmA* increased simultaneously with isoproturon mineralization, confirming the critical role of this gene in herbicide transformation. The observed high mobility of the *pdmAB* cassette highlights the broad evolutionary potential of such biosystems, enabling them to acquire new catabolic properties. In the complementary observations by Dunon et al. (2018), the key element facilitating the dissemination of pesticide degradation genes was the insertion sequence IS*1071*, which, according to metagenomic analyses, serves as a major component in the transfer of bacterial catabolic clusters under prolonged pesticide contamination, thereby enabling microbial communities to adapt to xenobiotic stress.

Beyond bacterial MGEs, organochlorine pesticide-contaminated soils have been reported to show reduced bacterial diversity alongside increased viral diversity and enrichment of viral auxiliary metabolic genes linked to pesticide biodegradation. In this setting, Zheng et al. (2022) proposed that a virus-encoded dehalogenase (L-DEX) can enhance bacterial growth under subinhibitory contaminant concentrations. Collectively, these findings indicate that biodegradation capacity may be distributed across taxa and genetic compartments and may be reshaped through genetic exchange processes over ecologically relevant timescales.

The expansion of metagenomic profiling capabilities has also facilitated discovery of genes involved in degradation of hazardous compounds, including pesticides, thereby widening opportunities for integration with genetic modification strategies aimed at enhanced bioremediation potential (Liu et al., 2019; Sarker et al., 2021). Microbial engineering provides routes for targeted enhancement of catabolic pathways to increase the rate and efficiency of persistent organic pollutant degradation (Sehrawat et al., 2021), and metagenomic data have been positioned as a basis for constructing microbial consortia with predefined functions and for monitoring bioremediation using functional markers alongside community dynamics (Bharagava et al., 2019; Rana et al., 2019; Wani et al., 2022).

In summary, metagenomics characterizes pesticide biodegradation at the community scale by resolving how catabolic functions are organized across microbiomes in contaminated soils. It delineates degradation modules repeatedly enriched under pesticide pressure, links them to the microbial lineages that carry them, and places these modules in genomic context alongside tolerance and mobility determinants. Functional targets relevant to consortia construction are prioritized through three complementary approaches: read-based profiling of gene-family enrichment across exposure contrasts, genome-resolved reconstruction that assigns modules to specific populations and preserves operon context, and functional metagenomics that recovers and validates active enzymes from environmental DNA. At the same time, metagenomic results primarily describe genetic potential and remain constrained by assembly fragmentation and annotation uncertainty, particularly for oxygenases and hydrolases. Accordingly, the most robust interpretation is obtained when enrichment patterns are consistent with genome-context pathway reconstruction and supported by activity-based evidence, thereby strengthening the link between sequence-derived inference and pesticide transformation capacity *in situ* and supporting rational design of synthetic microbial consortia.

3 Application of genetically engineered microbial strains in the development of synthetic microbiomes for pesticide biodegradation

3.1 Genetic engineering of model and non-model microorganisms for the implementation of pesticide catabolism pathways

Microorganisms from natural ecosystems often exhibit insufficient degradation rates and incomplete breakdown of persistent compounds, questioning their efficiency for the purpose of bioremediation (Pant et al., 2021). Consequently, genetic engineering tools play a crucial role in the development of microorganisms with enhanced metabolic capabilities (Saxena et al., 2020; Wu et al., 2021). Genetic engineering involves modifying the genetic machinery of microorganisms to improve their biodegradation potential (Maglione et al., 2024). One of the most promising approaches is the development of microorganisms capable of complete degradation of toxic compounds through recombinant DNA technologies (Maqsood et al., 2024). The use of recombinant DNA allows for the creation of microorganisms with an increased number of genetic copies responsible for pollutant degradation. This is achieved through amplification, modification, and integration of exogenous fragments, eliminating weak points in biochemical pathways, activating redox reactions, and enhancing energy supply, ultimately improving biodegradation efficiency (Anode and Onguso, 2021; Sharma et al., 2024).

Model microorganisms, particularly *Escherichia coli*, remain indispensable for controlled heterologous expression and reconstruction of catabolic pathways because of their high genetic tractability and compatibility with high-density cultivation (Ishwarya et al., 2024). Within this framework, model hosts are primarily used to assemble multi-enzyme routes, verify reaction order, and optimize expression architecture (e.g., promoter-terminator design and gene dosage), thereby enabling systematic identification of rate-limiting steps and metabolic coupling of xenobiotic conversion to central carbon metabolism. As demonstrated by Wang et al. (2023c), a model example was presented through the engineering of a modified *E. coli* strain that possesses a reconstructed 2,4-dichlorophenoxyacetic acid (2,4-D) degradation pathway. Fluorescent PCR confirmed the synthesis of nine genes (*tfdA*, *tfdB*, *tfdC*, *tfdD*, *tfdE*, *tfdF*, *pcaI*, *pcaJ*,and *pcaF*), responsible for the sequential oxidative breakdown of 2,4-D. This strain efficiently degraded 0.5 mmol/L 2,4-D within 6 h and was able to utilize the compound as its sole carbon source. Additionally, coordinated gene regulation through T7 promoters and terminators facilitated the conversion of 2,4-D into acetyl-CoA and succinyl-CoA, which were subsequently integrated into the tricarboxylic acid cycle (TCA cycle). Hu et al. (2019) reported that the carboxylesterase EstA from *Bacillus cereus* BCC01, expressed in *E. coli* BL21(DE3), retained high stability for pyrethroid degradation across a temperature range of 15–50 °C and pH 6.5–9. Zhang et al. (2024b) cloned the *phnA* and *mhpC* genes from *Brevibacillus parabrevis* BCP-09, responsible for the enzymatic breakdown of deltamethrin, into recombinant plasmids and expressed them in *E. coli* BL21(DE3) using L-arabinose induction, leading to efficient pyrethroid degradation. Zhang et al. (2024) expressed *est10* and *est13* from *Bacillus subtilis* in *E. coli* with Est13 showing the highest activity and enabling removal of *β*-cypermethrin from complex matrices, including milk, meat, vegetables, and fruits, indicating applicability to residue mitigation in food products. Zang et al. (2020) further demonstrated the value of model hosts for functional validation: genome mining of *Rhodococcus erythropolis* D310-1 identified the *carE* carboxylesterase gene, and heterologous expression in *E. coli* BL21 confirmed catalytic de-esterification of chlorimuron-ethyl. Finally, Elarabi et al. (2023) showed that increasing copy number and hyperexpression of catechol 1,2-dioxygenase (*catA*) from *Pseudomonas putida* in *E. coli* M15 accelerated isoproturon degradation, illustrating how gene dosage can be used to relieve pathway constraints.

Despite these advantages, evidence accumulated across studies indicates that laboratory-optimized constructs in model organisms may not directly translate into reliable function in environmental matrices. High expression burden, toxicity of intermediates, plasmid instability, and competitive pressures in mixed microbial communities can substantially reduce persistence and net degradation outcomes outside controlled cultivation. These constraints have driven increasing emphasis on non-model microorganisms that provide ecological robustness, including tolerance to salinity, oxygen limitation, and fluctuating nutrient regimes (Aminian-Dehkordi et al., 2023). In such systems, engineering objectives extend beyond catalytic activity to include genetic stability and operational controllability under field-relevant stresses. Xiong et al. (2024) successfully integrated three pesticide hydrolase genes directly into the chromosome of *Halomonas cupida* J9, resulting in the development of the multipesticide degrader J9U-PVG, which is capable of simultaneously degrading chlorpyrifos, carbaryl, and α-cypermethrin under conditions of 60 g/L NaCl and 4 g/L glucose. The additional insertion of the *VHb* and *GFP* genes improved oxygen supply and enabled *in situ* monitoring of strain activity. Similarly, Liu et al. (2025c) introduced a heterologous pesticide degradation pathway into the halotolerant strain *Halomonas cupida* J9 by incorporating seven genes (*mpd/pnpABCDEF*) required for the conversion of PNP-based pesticides into β-oxoadipate, along with *VHb* and *GFP*. This resulted in the development of the halotolerant degrader J9U-MP, which completely degraded 50 mg/L of methyl parathion within 12 h and converted 25 mg/L of $^{13}C_6$-PNP into $^{13}CO_2$ within three days under 60 g/L NaCl conditions. The genetic stability, resistance to low oxygen concentrations, and the ability to monitor activity via GFP highlight the potential of J9U-MP for *in situ* bioaugmentation of wastewater containing organophosphorus pesticides. In parallel, Zhang et al. (2023a) developed a genetically modified *Bacillus subtilis* WB800-ipaH strain with stable extracellular transcription of *ipaH* that eliminated over 90% of iprodione (5–20 mg/kg) under real-world conditions within 9-15 days, demonstrating that ecological performance can be treated as a primary engineering endpoint. Bridging strategies further extend this logic by combining rapid module validation in model hosts with subsequent deployment in environmentally adapted bacteria: Guo et al. (2024a) integrated the fungal *cyp57A1* gene into *E. coli* BL21(DE3) and transferred it to an adapted *Pseudomonas* strain, enabling fomesafen reduction from 5 to 500 mg/L and achieving 82.65% degradation at 100 mg/L.

Overall, model microorganisms such as *E. coli* continue to play an important role in rapid pathway assembly and fine-tuning of expression architecture, while non-model, stress-tolerant degraders are increasingly becoming the priority when the main requirement is resistance to salinization, oxygen limitation, and fluctuations in feeding regimes, especially when catabolic modules are stabilized through chromosomal integration. In both types of systems, the dominant constraints are expression load, intermediate toxicity, and loss of engineered traits. These can reduce competitiveness and impair net degradation outside of controlled cultivation. A key challenge in the field of pesticide biodegradation engineering is to achieve a balance between pathway completeness, genetic stability, and physiologically sustainable flow in hosts that is compatible with environmental constraints.

Taken together, the evidence indicates that engineering success is determined not only by catalytic potential but also by long-term trait stability and pathway flux balance under environmental selection. Therefore, strain choice and genetic design should be evaluated against field-relevant constraints, with particular attention to intermediate fate and the capacity to sustain degradation beyond controlled cultivation.

3.2 Engineering approaches at the genome level and recombination strategies for pesticide biodegradation

In addition to plasmid-based expression of catabolic genes, modern engineering increasingly relies on genome-level and recombination strategies to improve trait stability and establish causal relationships between genetic determinants and pesticide degradation phenotypes. These include recombination-based approaches (e.g., protoplast fusion), transformation systems for non-bacterial groups of microorganisms, and targeted editing tools used to establish the role of specific genes in degradation.

Protoplast fusion represents a recombination-based approach capable of generating recombinant strains with combined or enhanced degradative capacity (Zaynab et al., 2021). Liu et al. (2025b) fused parental cultures of *Klebsiella varicola* FH-1 (an atrazine degrader) and *Bacillus aryabhatti* LY-4 (an acetochlor degrader) through protoplast fusion, generating the recombinant strain RH-92. In this novel organism, the degradation rates of atrazine and acetochlor increased by 63.1% and 68.5%, respectively, while their halflives in non-sterilized soil were reduced from 4.9 and 7.6 days to 1.6 and 1.8 days, respectively. The application of RH-92 also contributed to the restoration of bacterial diversity and a reduction in the phytotoxic effects on soybean seedlings.

Engineering has also been extended to photosynthetic microorganisms when the target compound and application context support such platforms. Liu et al. (2025a) highlighted the effectiveness of *Agrobacterium*-mediated transformation as a genetic modification approach, specifically for generating *Chlorella sorokiniana* expressing the gene for purple acid phosphatase (*PAP*) from *Phaeodactylum tricornutum.* This genetically engineered strain was capable of completely utilizing glyphosate at concentrations below 10 ppm, a capability attributed to improved photosynthetic, energetic, and antioxidant parameters.

Targeted genome editing provides a complementary strategy for establishing causality between candidate genes and pesticide-degradation phenotypes and for generating genetically defined variants for subsequent functional characterization. Techniques such as CRISPR-Cas, ZFN, and TALEN enable precise modifications in microbial genomes that can support optimization of catabolic activity and stress adaptation (Jaiswal et al., 2019; Rafeeq et al., 2023). Of particular interest is the CRISPR-Cas system, recognized as an effective tool for precise gene modification (Bala et al., 2022). Zhang et al. (2023b) introduced a dual-plasmid genome editing approach that combined CRISPR/Cas9 with the Red homologous recombination mechanism. By constructing plasmids pACasN (carrying *Cas9* and Red recombinase) and pDCRH (harboring a dual guide RNA for precise deletion of the *opdB* gene), they achieved a high homologous recombination efficiency of over 30%. The resulting mutant, $X1^T$-Δ*opdB*, lost its ability to degrade organophosphorus insecticides, highlighting the critical role of *opdB* in the catabolism of these compounds. This study is the first to demonstrate the potential of combining CRISPR/Cas9 with the Red system for precise and efficient genome editing in *Cupriavidus* species.

Comparative consideration of these tools suggests that recombination-based approaches such as protoplast fusion are primarily useful for generating multi-trait recombinants that integrate degradative capacities from different parental lineages, whereas CRISPR-enabled genome editing provides superior precision for constructing and optimizing genetically defined strains with targeted catabolic functions. However, an unresolved and often under-addressed constraint across engineering strategies is the metabolic fate of degradation intermediates. Accordingly, tool effectiveness should be evaluated not only by the decline of the parent compound, but also by evidence that intermediate products are further processed and assimilated into central metabolism without accumulation of toxic residues. Even when genome-level stabilization of catabolic modules is achieved, the integration of extended multi-step pathways within a single strain can reach a physiological limit due to expression burden and flux imbalance. This provides a mechanistic rationale for transitioning toward synthetic consortia, where catabolic modules are distributed among specialized community members to reduce single-strain burden, promote sequential cross-feeding, and improve robustness in heterogeneous environmental matrices while preserving the controllability offered by modern genetic tools.

3.3 Synthetic consortia and functional modularization

Despite significant advances in genetic engineering, the use of individual microbial strains in bioremediation remains limited. One of the main challenges is the narrow specificity of monocultures, which are often unable to efficiently degrade complex mixtures of pollutants characteristic of contaminated soils. Consequently, increasing attention is being directed toward the development of microbial consortia capable of facilitating comprehensive xenobiotic degradation through interactions among different microorganisms (Bhatt et al., 2021c; Sharma et al., 2022; Chaudhary et al., 2023; Sadvakasova et al., 2023). Co-cultivation of microorganisms not only accelerates pollutant degradation but also enhances system adaptability to environmental changes. The interaction between different strains promotes a more balanced distribution of resources and reduces the metabolic burden on individual organisms, making consortia more resilient to stress conditions (Xu and Yu, 2021; Guo et al., 2024b). Additionally, some degradation byproducts serve as nutrients for other microorganisms within the community, stimulating their growth and improving overall system productivity (Jiménez-Díaz et al., 2022; Zhang and Zhang, 2022).

Microbial communities can be classified into three types: natural, artificial, and synthetic. Natural communities are obtained directly from the environment without isolating individual members and often exhibit high ecological resilience. However, their composition and functional output can be difficult to predict and reproduce across sites. Artificial consortia are assembled deliberately from cultivable wild strains, regardless of whether these taxa co-occur in nature

(Ibrahim et al., 2021; Chen et al., 2024). Empirical studies summarized in Table 1 confirm that artificial communities can accelerate degradation of diverse pesticides, supporting their practical relevance. Nevertheless, artificial consortia may remain vulnerable to compositional drift driven by competitive exclusion and changing substrate regimes, which can erode long-term functional stability.

Existing natural and artificial microbial communities have already demonstrated high efficiency in decomposing various pollutants. At the same time, in recent years, there has been growing interest in the rational design of improved strains and communities using synthetic biology, metabolic engineering, and genetic modification tools (Efremenko et al., 2024). Synthetic biology views living systems as sets of functional modules that can be reconstructed and reconfigured to impart new properties, including targeted degradation functions (Dvořák et al., 2017). Within this approach, the efficiency and stability of microbial consortia are enhanced by distributing metabolic functions among community members, which reduces the load on individual cells and increases the system's resistance to stressful conditions (Tsoi et al., 2019; Che and Men, 2019). Accordingly, synthetic consortia containing genetically modified cells with high catabolic activity can ensure more complete and stable degradation of xenobiotics even under unfavourable conditions (Huang and Lu, 2021; Yaashikaa et al., 2022). If a constructed community contains both wild-type strains and genetically modified components, such a system is usually referred to as a semi-synthetic consortia (Bernstein and Carlson, 2012). The comparative features of natural, artificial, and synthetic consortia, as well as their key advantages and limitations in bioremediation, are presented in Fig. 2.

Designing stable synthetic communities requires careful selection of strains, spatial distribution, population control mechanisms, and intercellular interactions (Cao et al., 2022; Liu et al., 2024). The division of biochemical functions among species reduces the metabolic burden on individual cells, increasing overall resilience and efficiency while minimizing the need for extensive genetic modifications (Aminian-Dehkordi et al., 2023). In a synthetic consortia, biosynthetic pathways can be distributed among different microorganisms in a stepwise manner, facilitating the sequential stages of

Table 1 Application of artificial microbial consortia for pesticide degradation

Consortium composition	Pollutant type	Degradation pathway	Degradation efficiency (%) and treatment time	Reference
Chlorella sp., *Scenedesmus* sp.	Chlorpyrifos (35%), Cypermethrin (14%), Oxadiazon (55%)	Combination of sorption and biodegradation	Removal up to 35%, 14%, 55% in 7 d	Avila et al., 2021
Consortium MF0904 (*Pandoraea, Stenotrophomonas, Paracoccus*)	Methomyl (25 mg/L)	Hydrolysis, C–S bond cleavage, subsequent metabolism	100% in 96 h	Pang et al., 2023
Bacterial-algal consortium (*Paenibacillus* sp. ISTP10, *Scenedesmus* sp. ISTGA1)	Lindane (50 ppm)	Degradation producing penta- and trichlorocyclohexanes and phenols	100% in 120 h; toxicity reduction by 1.89-fold (LC50) and EROD induction decrease by 3.1-fold after 168 h	Kumari et al., 2020
Consortium YS622 (*Azospirillum, Cloacibacterium, Ochrobactrum*)	Glyphosate (50 mg/L)	Degradation via the aminomethylphosphonic acid pathway with C–N bond cleavage	100% in 36 h; over 97% degradation in water-sediment systems	Zhang et al., 2024a
Arthrobacter sp., *Sphingomonas* sp., *Stenotrophomonas* sp., *Pseudomonas* sp., *Bacillus* sp.	2,4-D (100 mg/L)	Decomposition into metabolites including 2,4-dichlorophenol and 4-chlorophenol	98%–100% in 9 d at 10% inoculation; 85%–90% degradation in 4 d for consortium R3 (*Arthrobacter* sp., *Sphingomonas* sp.)	Vanitha et al., 2023
Rhodococcus sp. JQ-L, *Comamonas* sp. A-3	Cypermethrin (100 mg/L)	JQ-L converts cypermethrin into 3-phenoxybenzoic acid (3-PBA), A-3 degrades 3-PBA	100% cypermethrin degradation in 24 h; complete 3-PBA degradation in 15 h	He et al., 2022
Bacillus sp. CSA-1 strain and a consortium of 10 bacterial strains	Cypermethrin (CYP)	Biodegradation of CYP producing 29 metabolites, including amide and amine derivatives	71% in 24 h	Birolli et al., 2022
Enterobacter, Microbacterium, Ochrobactrum, Pseudomonas, Achromobacter	*β*-Cyfluthrin	Ether hydrolysis and oxidation via carboxylesterase and oxidoreductase	Half-life from 4.16 to 22.73 d (depending on concentration)	Li et al., 2022
Pseudomonas stutzeri, Comamonas odontotermitis, Sinomonas atrocyane, Chlorella protothecoides	Glyphosate	Degradation without aminomethylphosphonic acid formation	Up to 79% glyphosate removal from wastewater	Borella et al., 2023

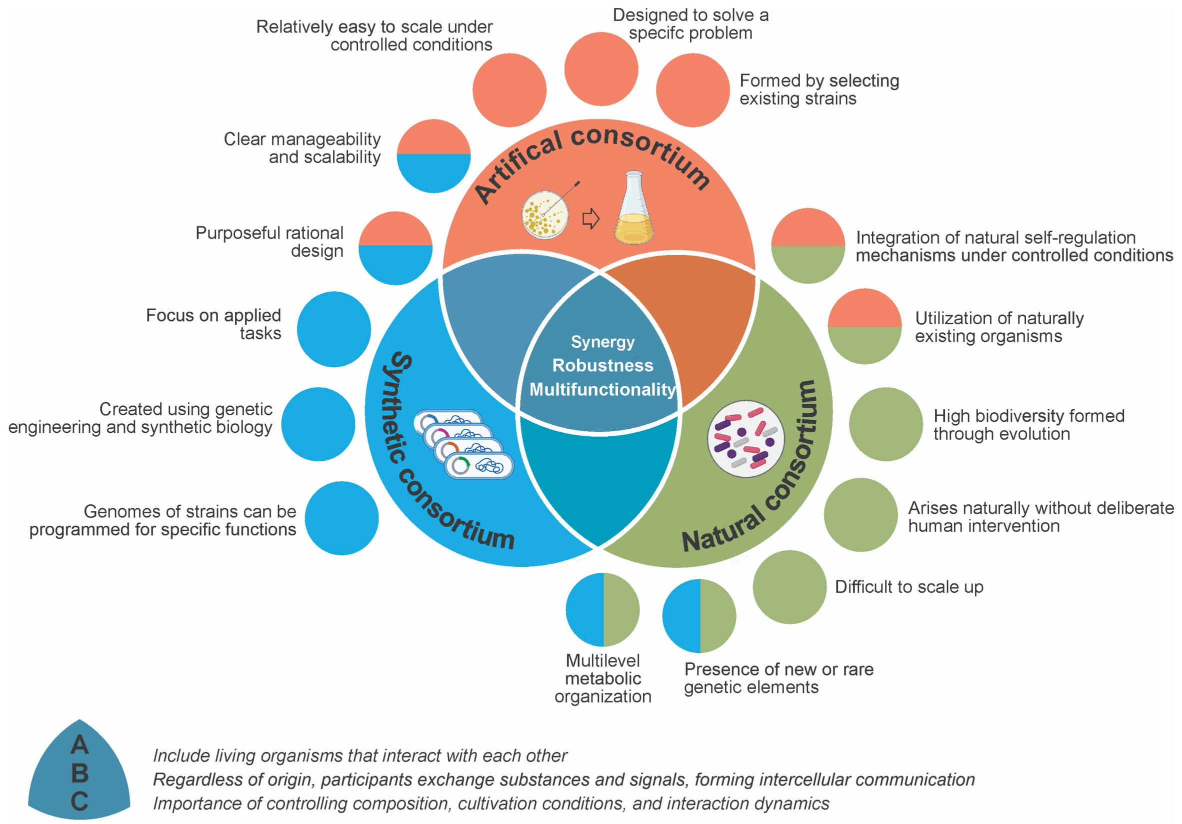

Fig. 2 Comparative analysis of natural and artificially created microbial consortia.

degradation. Additionally, adjusting species proportions within the community allows for precise control over the expression levels of each functional module (Qian et al., 2020). Horejs (2024) developed a framework for constructing synthetic microbiomes optimized for herbicide biodegradation, which includes selecting and simplifying the functional microbiome, modeling with SuperCC to optimize strain composition and metabolic interactions, and demonstrating that metabolite exchange and cross-feeding significantly enhance bioremediation efficiency. The bottom-up engineering approach leverages metabolic process analysis and interspecies interactions to predict and regulate microbial consortia. Advances in multi-omics and automation enable effective modeling of microbial dynamics, considering key parameters of intercellular metabolic exchange (Li et al., 2021b).

Beyond pathway partitioning, functional stability depends on coordination of activity across members. Quorum sensing (QS) systems provide a regulatory layer that links gene expression to population density and environmental cues, supporting synchronized metabolic activity and improving the resilience of consortia under fluctuating conditions (Bhatt et al., 2021b; Duncker et al., 2021; Zeng et al., 2023). QS-associated traits can also influence mass transfer and substrate bioavailability. A representative example is biosurfactant production, which enhances solubilization of hydrophobic pesticides and can increase uptake by the broader community. Singh et al. (2016) reported that the addition of a microbial rhamnolipid biosurfactant increased chlorpyrifos degradation in soil to 82.3% compared with 52.3% without biosurfactant in a consortium comprising *Pseudomonas*, *Klebsiella*, *Stenotrophomonas*, *Ochrobactrum*, and *Bacillus*. This observation supports the view that consortia performance is determined not only by enzymatic capability but also by community-level traits that improve access to poorly bioavailable substrates.

Even when cooperative interactions are present, maintaining high cell density and sustained activity in operational contexts often requires additional stabilization measures. Immobilization on carriers can improve functional persistence, facilitate biomass recovery and reuse, and reduce operational costs relative to free-living forms (Gong et al., 2022). The most common immobilization techniques include hydrogel matrix entrapment, adsorption, and covalent binding onto solid substrates (Mehrotra et al., 2021). An artificial microbial consortium composed of

Pseudomonas esterophilus and *Rhodococcus ruber*, optimized for biomass ratio, cell density, and granule size within a polyvinyl alcohol cryogel, demonstrated a 225% increase in the degradation efficiency of organophosphorus pesticides. Moreover, the observed enhancement of lactonase activity underscored the importance of QS in coordinated intercellular interactions (Efremenko et al., 2022). A binary culture of *Bacillus thuringiensis* SG4 and *Bacillus* sp. SG2, immobilized in agar granules supplemented with nitrogen and organic substrates, significantly accelerated the breakdown of cypermethrin, reducing its half-life to 4.5 d (Bhatt et al., 2022). Similarly, the immobilization of the MB3R consortium on biochar in potato-cultivated soil enhanced metribuzin degradation to 96%, compared to 29.3% in the control, shortened the half-life of the herbicide, restored soil microbiome structure, and promoted plant growth (Wahla et al., 2020). In another study, bacterial cultures of *Providencia stuartii* JD and *Brevundimonas naejangsanensis* J3, immobilized on wood charcoal, alginate, and chitosan, efficiently degraded dicarboximide fungicides in brunisolic soils, achieving 96.7% decomposition of dimethachlon, 95.0% of iprodione, and 96.3% of procymidone, with a significantly reduced half-life compared to free-living strains (Zhang et al., 2021a). The integration of microbial communities with materials science advancements, utilizing both non-living carriers (such as sodium alginate-chitosan composites) and living carriers (*Myxococcus xanthus* DK1622), enabled the formation of a robust and reusable system. Specifically, the acetochlor-degrading T3 consortium exhibited an optimized structural organization, stable degradation performance, and the potential for multiple reuse cycles (Liu et al., 2022a).

Across studies, the main advantage of consortia-based biodegradation is the distribution of pathway steps and complementary functions among community members, which can broaden substrate coverage and reduce the physiological burden on any single strain. However, increasing community complexity also introduces vulnerabilities, including compositional drift, dependence on keystone populations, and destabilization of cross-feeding under competition with indigenous microbiota in non-sterile matrices. Moreover, a substantial fraction of published validations is performed in controlled or semi-controlled systems, which may not fully capture the selection pressures operating in real soils and wastewaters. These observations indicate that, alongside strain selection and pathway definition, consortia engineering benefits from a clearly articulated framework for how functional modules are partitioned, coordinated, and maintained, which motivates the conceptual schemes presented in the subsequent section.

3.4 Proposed conceptual models for semi-synthetic and synthetic microbiomes targeting organophosphorus insecticides

Based on a synthesis of published research, we propose theoretical models of semi-synthetic and synthetic microbial consortia, where each component is specialized in performing a sequential stage of chlorpyrifos and methyl parathion degradation, respectively. Organophosphorus insecticides, which account for approximately 40% of the total pesticide production, are of particular interest in the context of bioremediation model development. Their widespread use in agriculture and continuous environmental presence make them critical targets for evaluating the efficacy of microbial consortia designed for their safe decomposition. Furthermore, these compounds were selected due to the challenges associated with conventional detoxification methods and the potential risk of their accumulation in food chains (Kaushal et al., 2021; Raj et al., 2023). The selection of specialized bacterial strains with enzymatic activity against organophosphates, optimization of their interactions, and the integration of synthetic microbial complexes with metabolic cooperation present new avenues for the environmentally safe and efficient removal of these contaminants.

A single-strain solution can simplify deployment, yet organophosphate conversion often involves reaction sequences that are difficult to optimize within one host for three recurrent reasons. First, prolonged expression of multiple heterologous enzymes imposes resource and expression burdens, which can reduce fitness and accelerate loss of engineered traits. Second, upstream hydrolysis may outpace downstream turnover, creating a kinetic imbalance and accumulation of inhibitory intermediates. Third, the required catalytic steps may differ in optimal cellular context, cofactor supply, and regulatory tuning, making simultaneous optimization within one organism non-trivial. A consortium architecture can mitigate these constraints by distributing steps among specialists and enabling sequential substrate transfer (cross-feeding), if community composition and module coupling remain sufficiently stable (Che and Men, 2019).

The proposed consortia are conceptual in nature and have not been tested experimentally. However, being based on literature data on the functional activity of individual microorganisms and their interactions within microbial communities, this approach allows for the integration of empirically validated characteristics of different strains into a unified configuration, expanding the scope for developing effective biotechnological solutions for remediating contaminated ecosystems. Figure 3 shows the key functional stages of the proposed consortia's work on the degradation of the organophosphorus pesticides methyl parathion and chlorpyrifos.

Here, we propose a three-module semi-synthetic microbial consortium for the biodegradation of chlorpyrifos (CP). The first step is assigned to a recombinant *Escherichia coli* expressing *cpd* from *Paracoccus* sp. TRP, reported to cleave the P–O bond and yield 3,5,6-trichloro-2-pyridinol (TCP) and diethyl thiophosphoric acid (DETP) (Fan et al., 2018). Positioning rapid hydrolysis upstream is justified because parent-compound detoxification frequently determines whether downstream modules can operate without inhibitory carry-over. A second recombinant module expressing *opdH* from *Arthrobacter* sp. HM01 is proposed to transform phosphorus-containing transformation products and thereby reduce their persistence and potential inhibitory effects (Mali et al., 2022b). Importantly, the contribution of this module should be supported by product-oriented analytics for the relevant phosphorus-containing species rather than by CP disappearance alone. The third step is assigned to a native degrader, *Cupriavidus nantongensis* X1^{T}, reported to carry out sequential TCP dechlorination followed by ring cleavage and routing of downstream products toward central metabolism (Shi et al., 2019). The rationale for including a wild-type member at this stage is that late steps often depend on broader catabolic networks that are challenging to reconstruct and balance in an engineered host. At the same time,

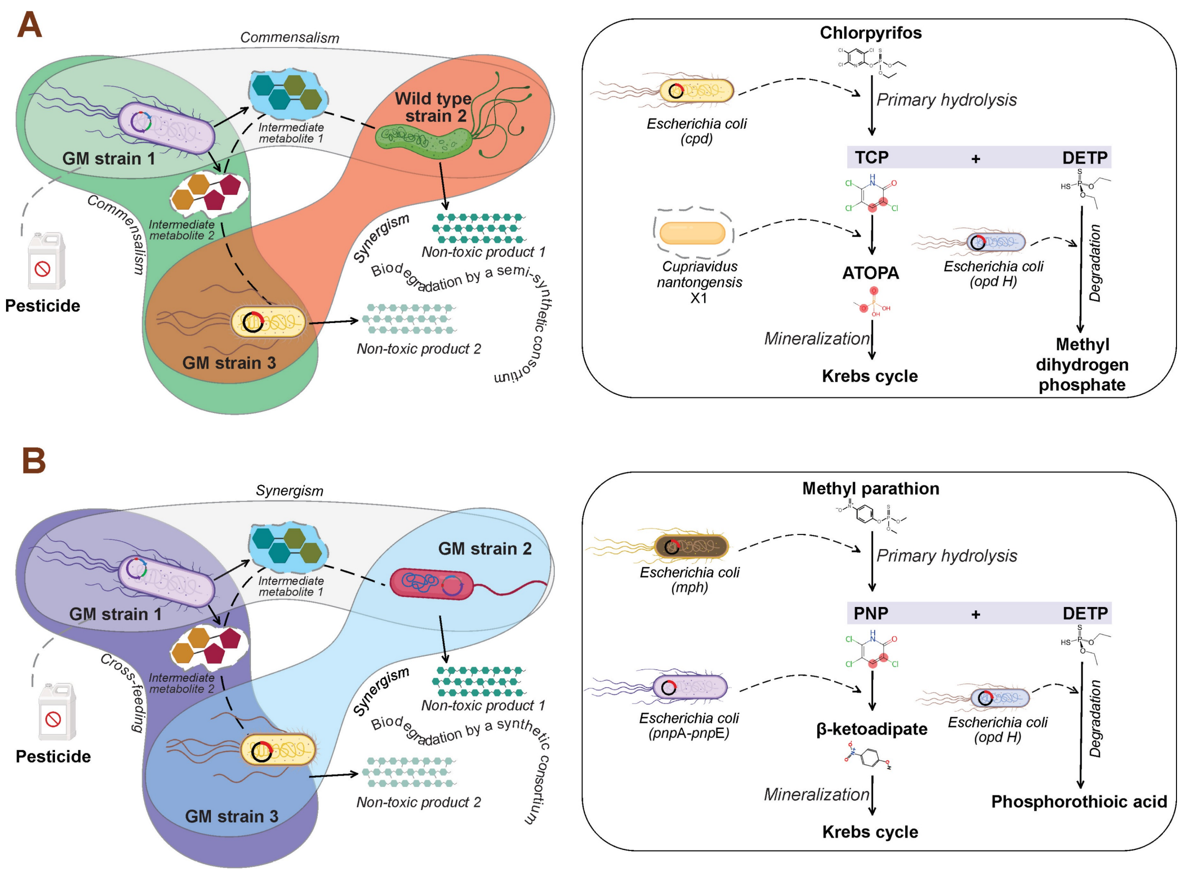

Fig. 3 Consortia models for pesticide biodegradation. Panel A illustrates the model of a semi-synthetic consortium comprising genetically modified (GM) and wild-type strains of microorganisms interacting to degrade the pesticide chlorpyrifos. In this consortium, GM strains and wild type complement each other, metabolizing the intermediate degradation products into non-toxic substances, which then enter the Krebs cycle, facilitating mineralization. Panel B represents a synthetic consortium, consisting exclusively of genetically modified strains for the biodegradation of methyl parathion. This model demonstrates synergism between the strains during primary hydrolysis and subsequent mineralization, resulting in the formation of phosphorothioic acid and other metabolites, which are also integrated into the Krebs cycle.

endpoint claims should be stated conservatively: TCP-derived intermediates can persist if any downstream step becomes limiting, and therefore evidence of completion requires intermediate profiling and, where feasible, mass-balance-type indicators (e.g., chloride release and disappearance of chlorinated aromatics). This design is semi-synthetic because it combines engineered and wild-type members and relies on sequential substrate transfer as the principal coupling mechanism. The most probable failure modes are resource competition**,** growth-rate mismatch**,** and divergent nutritional/physiological requirements, which can shift module ratios and reduce reproducibility. Accordingly, evaluation should track stability of engineered functions, TCP/DETP dynamics, and persistence of the downstream module under non-sterile conditions.

For methyl parathion, it is also appropriate to consider a modular degradation organization, since the rate and thermodynamic-kinetic consistency of individual stages, as well as the toxicological profile of intermediate products, generally determine the overall reproducibility of the process. The proposed synthetic consortia model for the enzymatic degradation of methyl parathion (MP) is conventionally divided into three key stages, each carried out by specialized enzyme systems. In the first stage, the P–O bond in the MP molecule is cleaved, resulting in the formation of p-nitrophenol (PNP) and O,O-dimethyl phosphorothioate (DMPT). This process is catalyzed by methyl parathion hydrolases (MPH), which can be derived, for example, from *Serratia marcescens* subsp. *marcescens* (gene *mphGM004539Δsp*) (Wang et al., 2018) or *Azohydromonas australica* (Zhao et al., 2021). Experimental data confirm that the enzyme from *S. marcescens* exhibits optimal activity at 35 °C and pH ~11, with Co^{2+} and Fe^{2+} further enhancing its activity. In contrast, the enzyme from *A. australica* demonstrates its optimum at a higher temperature (50 °C) and pH 9.5. Significant differences in MPH operating optimums (pH/temperature) and ion-metal regulation preclude their interpretation as interchangeable: the choice of a specific enzyme must be parameterized by the intended technological regime and matrix constraints. The purpose of the unit is to ensure a high-speed reduction in the concentration of the starting compound and to form a deterministic PNP flow for subsequent processing. The next critical stage involves the degradation of the phosphorothioate residue (DMPT/DEPT) formed after the initial hydrolysis. For this purpose, an *E. coli* strain (DH5α, BL21) expressing the organophosphate hydrolase gene *opdH* from *Arthrobacter* sp. HM01 is utilized (Mali et al., 2022b). This strain can cleave O,O-dimethyl phosphorothioate, converting it into less toxic derivatives such as O-dimethyl-O-hydrogen phosphorothioate, significantly reducing the toxicity of phosphorus-containing compounds. This facilitates their subsequent mineralization into inorganic phosphorus or further oxidation in the environment. It is essential that the effectiveness of this block be confirmed by the kinetics of phosphorus-containing intermediates and their derivatives, i.e. by-product-oriented markers, since a decrease in the concentration of the initial MP does not exclude the preservation of persistent and potentially toxic organophosphorus residues. In the third stage, PNP utilization is hypothesized to be achieved using an engineered *E. coli* BL-MP strain carrying integrated *pnpA-pnpE* gene clusters, which enable stepwise degradation of PNP to β-ketoadipate, a central intermediate that can be routed into the TCA cycle (Xu et al., 2022). Within the synthetic consortia, different recombinant *E. coli* strains (harboring genes for methyl parathion hydrolase *mph,* organophosphate hydrolase *opdH*, and the *pnpA-pnpE* cluster) form a «metabolite supplier-consumer» chain, sequentially breaking down the original substrate and its derivatives. The literature emphasizes that such stepwise substrate transfer within a single community (cross-feeding) fosters cooperative interactions, where metabolites synthesized by one strain serve as resources for another, thereby maintaining high metabolic activity across the entire consortium (Smith et al., 2019). The selection of the *mph, opdH*, and *pnpA-pnpE* genes is due to their location in toxicologically and kinetically critical nodes. However, it should be noted that for each block, there are alternative enzymes and pathways that differ in kinetics, stability, and regulatory compatibility. Therefore, the proposed configuration should be interpreted as a plausible architectural example rather than the only possible solution (Wang et al., 2018; Zhao et al., 2021; Mali et al., 2022b; Xu et al., 2022). When co-cultured, limitations are expected due to differences in growth rates, substrate preferences, and sensitivity to intermediate products. This can lead to composition drift and functional disruption of the sequential conversion chain. Mitigating these risks typically requires controlled inoculation ratios and feeding regimes, spatial structuring (including immobilization where necessary), and stabilization of key functions at the genome level to reduce the likelihood of loss of engineered traits. Therefore, the effectiveness of the system should be correctly assessed in several ways. Firstly, by the disappearance of MP. Secondly, by the reproducible depletion of PNP and phosphorus-containing intermediates. Thirdly, by the stability of composition and functions over time.

Considered together, the proposed conceptual models illustrate how enzyme- and gene-level knowledge on organophosphate insecticide degradation can be translated into testable architectures of semi-synthetic and synthetic consortia with stepwise transfer of substrates and intermediates across modules. The underlying rationale is to alleviate single-strain burden by partitioning catabolic functions, to reduce the likelihood of toxic intermediate accumulation (e.g., TCP or PNP), and to improve reproducibility by assigning key transformations to dedicated biological components. Importantly, these designs should be viewed as plausible exemplars rather than unique solutions: for each module, alternative enzymes and host organisms exist with distinct origins, kinetic properties, and operational optima, and the final choice should be supported by comparative evaluation of performance and compatibility within the intended process window.

At the same time, the literature indicates that the practical value of modular consortia is governed not only by the disappearance of the parent pesticide, but primarily by intermediate fate and functional stability during co-cultivation in non-sterile matrices. Accordingly, further development and validation should rely on product-oriented readouts (TCP/DETP/PNP profiles and phosphorus-containing derivatives, mineralization and mass balance indicators where feasible), alongside monitoring of genetic cassette stability, compositional drift, and inter-strain antagonism. In this sense, the main value of the proposed models lies in the fact that they fix the boundaries of modules, «docking» metabolites, and likely points of failure in advance, forming a clear experimental agenda for subsequent optimization and verification in conditions as close as possible to applied ones.

4 Challenges and prospects in the development of synthetic microbiomes for pesticide biodegradation

Currently, research in the field of pesticide bioremediation has made significant progress due to the application of molecular biology, genetic engineering, and omics technologies (Malla et al., 2018; Rawat and Rangarajan, 2019). However, the transition from laboratory experiments to real-world applications presents numerous challenges.

One of the major constraints for translating pesticide-degrading synthetic microbiomes from controlled systems to applied environmental matrices is biosafety, together with the genetic and physiological stability of engineered functions and the limited predictability of community dynamics. A key biosafety concern is horizontal gene transfer, which can redistribute engineered cassettes within indigenous communities and facilitate dissemination of resistance determinants, underscoring the need for robust containment and control measures (Jaiswal and Shukla, 2020; Saxena et al., 2020). Accordingly, increasing attention is being directed to biosafety systems—most notably genetic containment mechanisms and related safeguards—designed to reduce the probability of genetic leakage and persistence outside the intended operational window (Rebello et al., 2021; Perera and Hemamali, 2022). Importantly, recent kill-switch concepts aim to improve genetic stability by reducing the selective advantage of loss-of-function escape mutants through coupling host viability to the same module that mediates conditional killing. In the dual-function genetic containment design described by Kato and Mori (2024), an essential gene (*tyrS*) is deleted from the chromosome and supplied by a single expression cassette that supports viability at a low basal level in the OFF state but triggers lethality upon inducible overexpression in the ON state; the authors reported that this configuration maintained conditional suicidality over 300 generations in *Escherichia coli*.

Technical limitations are largely rooted in how catabolic pathways are encoded. Plasmid-based overexpression of degradation genes can speed up pesticide removal, but high copy number and large inserts increase metabolic burden and destabilize engineered strains. In addition, the cargo capacity of standard plasmids and incompatibilities between different replication origins make it difficult to assemble long, multi-step catabolic pathways that span tens of kilobases and contain multiple regulatory modules (Liu et al., 2019; Leskovac and Petrović, 2023). The implementation of subsequent strategies is currently underway, with the overarching aim of addressing the obstacles. First, catabolic operons are integrated directly into well-characterized chromosomal loci, which has enabled the stable installation of complete pathways for γ-hexachlorocyclohexane and 3-chloro-1,2-propanediol degradation in *Pseudomonas putida* without relying on multicopy plasmids (Liang et al., 2020). Second, naturally occurring catabolic mobile genetic elements and genomic islands, capable of carrying over 100 kb of coordinated degradation functions, are being repurposed as high-capacity scaffolds for synthetic pathway installation in xenobiotic-degrading bacteria (Miguel et al., 2020; Fujihara et al., 2023; Tokuda and Shintani, 2024). Third, modern modular vector toolboxes for Gram-positive and Gram-negative chassis, such as ProUSER2.0 and SubtiToolKit, combine integrative and low-copy replicative modules so that copy number and expression strength can be tuned to minimize metabolic load while still accommodating moderately large synthetic operons (Falkenberg et al., 2021; Caro-Astorga et al., 2024). In parallel, CRISPR-based multiplex genome editing and base-editing systems make it possible to distribute different pathway segments across several chromosomal loci and genomic islands within a single engineering campaign, enabling extended catabolic networks for complex pesticide mixtures without exceeding the capacity of any individual plasmid vector (Allemailem et al., 2024; Xin et al., 2025).

Another significant challenge is the insufficient understanding of microbial community dynamics and the impact of environmental variability on their functionality, which still hampers the rational design and deployment of synthetic microbiomes (Hu et al., 2022). The application of omics approaches (metagenomics, transcriptomics, proteomics, metabolomics) combined with machine-learning methods enables more accurate prediction of community responses under fluctuating conditions (Yaashikaa et al., 2022; Wang et al., 2023a). Recent work in environmental microbiology shows that graph neural networks and other time-series architectures can forecast species-level trajectories in complex wastewater communities several months ahead using only historical compositional data, providing a template for anticipating how engineered degraders will behave *in situ* (McElhinney et al., 2022; Andersen et al., 2025). In parallel, ML-guided design frameworks trained on multi-omics and degradation-performance datasets are beginning to optimize synthetic consortia by selecting minimal community cores, inoculation ratios, and environmental regimes that sustain the breakdown of recalcitrant pollutants, including persistent pesticides and plasticizers (Alidoosti et al., 2024; De la Vega-Camarillo et al., 2025; Renganathan and Gaysina, 2025). At the enzyme and pathway level, reaction-class predictors and web-based ML platforms for xenobiotic biodegradation can now infer candidate enzymes and complete catabolic routes for specific pesticides directly from sequence and molecular structure, thereby narrowing the search space for pathway engineering and synthetic microbiome construction (Aljabri, 2025; Mahalle et al., 2025).

The development of regulatory frameworks is also of paramount importance to ensure the transparent and responsible environmental release of genetically modified microorganisms. Stringent ecological risk assessments, including analysis of potential toxic metabolite formation, together with harmonized international standards, highlight the need for continuous dialogue among scientists, regulators, and the public (Li and Jiong, 2024; Ishwarya et al., 2024). Finally, the scalability and practical implementation of synthetic microbiomes remain pressing issues. While laboratory tests yield promising results, maintaining sufficient cell density and metabolic activity in real ecosystems requires the selection of robust, adaptive associations capable of competing with native microbiota (Ruomeng et al., 2023; Chaudhary et al., 2024; Contreras-Salgado et al., 2024). In the future, efforts should focus on optimizing genetic constructs to minimize cellular stress, strengthening biosafety measures, and systematically exploiting multi-omics and machine-learning pipelines to design stable, predictable communities. The coordinated integration of technological advances, rigorous ecological monitoring, and regulatory improvements will enable the safe and effective application of synthetic microbiomes for pesticide degradation, which is crucial for environmental sustainability and responsible development (Malla et al., 2018; Rawat and Rangarajan, 2019).

Overall, we envisage that overcoming these interconnected biosafety, engineering and regulatory barriers will require sustained, long-term collaboration across microbial ecology, synthetic biology, environmental engineering, and policy. By jointly advancing mechanistic understanding, computational design, and field-scale validation, the community will be able to move synthetic microbiomes for pesticide biodegradation from promising concepts to reliable tools for safeguarding agroecosystems and human health.

5 Conclusions

In conclusion, the latest advancements in synthetic biology, genetic engineering, and multi-omics have significantly expanded the potential for developing microbial systems capable of pesticide degradation. Despite the accumulated knowledge, the transition from laboratory models to real-world applications remains hindered by technical, environmental, and regulatory challenges. Key issues include preventing horizontal gene transfer, improving constructs for overexpression of catabolic genes, utilizing omics-based approaches for modeling microbial interactions, and establishing standardized biosafety regulations.

The primary direction for future research is the formation of adaptive, stable, and functional microbial communities that can withstand competition from native microorganisms while ensuring the safe degradation of complex pesticides. The integration of optimized genetic assemblies, containment measures for recombinant elements, and systems-level analysis will facilitate the implementation of these technologies in real-world ecosystems. Interdisciplinary collaboration and active engagement with the public provide a solid foundation for the development of reliable and scalable bioremediation systems, which are crucial for environmental conservation and sustainable development in the face of increasing anthropogenic pressures.

Abbreviations
MP, Methyl Parathion; CP, Chlorpyrifos; PNP, p-Nitrophenol; DMPT, O,O-Dimethyl Phosphorothioate; DEPT, Diethyl Phosphorothioate; MPH, Methyl Parathion Hydrolase; GMM, Genetically Modified Microorganisms; QS, Quorum Sensing; TCA Cycle, Tricarboxylic Acid Cycle; CYP, Cypermethrin; 2,4-D, 2,4-Dichlorophenoxyacetic Acid; MAG, Metagenome-Assembled Genomes; HGT, Horizontal Gene Transfer; CRISPR-Cas, Clustered Regularly Interspaced Short Palindromic Repeats-CRISPR-Associated System; ZFN, Zinc Finger Nucleases; TALEN, Transcription Activator-Like Effector Nucleases.

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