



Engineered microorganisms for bioremediation in aquatic ecosystems for pollution mitigation

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Abstract

Aquatic ecosystems are under immense threat from hydrocarbons, heavy metals, pesticides, microplastics, and other anthropogenic pollutants. Traditional pollutant removal techniques are not able to achieve sustainable results and cause ecological harm. This research proposes a different bioremediation solution that uses genetically engineered microorganisms to refine pollutant degradation, stabilization, and specificity in water. This study proposes a different bioremediation solution by genetically engineered microorganisms to refine degradation, stabilization, and specificity to pollutants in water. By integrating synthetic biology and omics-based pathway optimization along with other bioinformatics tools, the researcher aims to develop pollutant-degrading microorganisms adapted to different aquatic conditions. The proposed engineered microbial bioremediation system (EMBS) consists of a detailed multi-phase system which includes (i) characterization of the pollutant and identification of target genes, (ii) CRISPR-Cas9 genome editing to add and enhance the catabolic pathways, (iii) bioreactor validation for degradation kinetics, and (iv) mesocosm-scale testing for ecological safety and performance under quasi-natural settings. Compared to wild-type strains, experimental results show over 80% reduction in hydrocarbons and nitrates in a 72-hour period. These results showcase EMBS as a highly effective, sustainable, and flexible method for the removal of pollutants in aquatic systems. Different bioremediation EMBS techniques can be used to target pollutants and

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strengthen the bioeconomy. Environmental policy can also target active pollutants to strengthen the EMBS bioeconomy.

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Introduction

Aquatic ecosystems face difficulties because of a variety of water pollutants, like heavy metals, oil products, persistent organic pollutants, organic pesticides, microplastics, and chemicals that are just starting to be used. These pollutants are detrimental to water quality and can bioaccumulate and biomagnify. This puts aquatic fauna, flora, and human health at risk (Dixit *et al.*, 2015; Pande *et al.*, 2022). For example, chronic and expensive Pb, Cd, Hg, and as removal methods are of little use because these heavy metals are environmentally and economically safe (Igiri *et al.*, 2018; Alabssawy and Hashem, 2024).

Involving environmental accounting in management increases the focus on sustainability and helps in the making of eco-friendly decisions on how to manage and mitigate pollution (Zhu, Jiao and LiInnovative, 2024). IoT smart monitoring environmental pollution detecting and controlling systems using combines sensors and MQTT protocols is a big boost in real-time monitoring and management (Kavitha and Sulaipher, 2025). Innovative awareness experiments using bio degradable materials illustrate the positive impact soil and water ecosystems through degradation process (Antonio *et al.*, 2025).

With that in mind, microbial bioremediation. More specifically, the

degradation, transformation, or immobilization of pollutants using microorganisms can be cost-effective and environmentally safe in bioremediation systems. Microorganisms are prevalent in aquatic environments and are capable of pollutant bioremediation and detoxification. They play significant roles in the biogeochemical cycles and systems of diverse pollutants (Misra *et al.*, 2024). However, aquatic heavy metal and other pollutants self-remediate systems are used and are detrimental (Thai, Lim and Na, 2023). Inhibitory concentrations of pollutants, adverse environmental systems of pH, salinity, temperature, and lack of specific catabolic pathways, can. This can affect the microbial communities.

New strategies for synthetic biology, metabolic engineering, and genome editing have enabled the development of new and more advanced engineered microbial strains for improved pollutant and heavy metal detoxification and remediation (William and Magpantay, 2023). There are multiple microbial detoxification capacity enhancement synthetic circuits, heavy metal resistance, and detoxification genes that have been based on, like *cadB*, *chrA*, *copAB*, *pbrA*, *merA*, and *arsB/acr3* operons. In arsenic bioremediation, synthetic bacteria have been developed that use the arsenic-transforming *ars* operon (*arsB*, *arsC*, *arsR*) to facilitate the bioremediation by

detoxifying arsenate and exporting arsenate (Chen *et al.*, 2014). In another example, the heterologous gene expanded the bioremediation potential of the engineered *Escherichia coli* that had the plant-derived phytochelatin synthase gene PcPCS1 from *Pyrus calleryana* for increased Cd and Cu accumulation and tolerance. This demonstrates that heterologous genes greatly expand bioremediation potential (Kostal *et al.*, 2004)

Ecosystem engineering for aquatic microbial ecology is different, with the additional benefits and advantages of biofilm-mediated processes. Compared to planktonic cells, microbial biofilms have a greater net resilience, facilitate and stabilize the retention of engineered strains, and concentrate local pollutants (Oziegbe, Oziegbe and Ojo-Omoniyi, 2025). In the bioremediation of heavy metals and xenobiotics, the EPS (extracellular polymeric substance) matrices, co-metabolism, and gene transfer made pollutant removal more efficient.

Dealing with engineered microbial bioremediation in water needs overcoming some specific obstacles like unpredictable water movements, changing oxidation states, varying concentrations of pollutants, predation from local microbes, and safety issues (Abo-Alkasem, Hassan and Abo Elsoud, 2023). Thus, with the protective regulations in place, an engineered strain needs to be fieldworthy, safe for the environment, and versatile to adapt to different conditions in water bodies (Verma *et al.*, 2020). Still, the deployment plan needs to consider the strategic containment of the engineered

microbes, risks of horizontal gene transfer, and fecal pollution of the environment with engineered microbes (Mitra, Chatterjee and Gupta, 2017).

With the current state of the technologies, this work outlines an engineered microbial bioremediation strategy for water bodies. Our strategy focuses on the integration of synthetic biology (CRISPR–Cas9 genome editing), engineered microbial assemblages, and mesocosm-scale approaches in a holistic, multi-phase framework. We discuss specific genetic targets like the heavy metal ATPase genes HMA2, HMA3, and HMA4 associated with Cd translocation that have been previously demonstrated in plant-microbe systems and propose their application in aquatic microbial chassis, and discuss engineered systems like biofilms or consortia to improve functional stability of water systems. This work builds on the reviews of microbial heavy metal remediation and engineered microbes for aquatic arsenic remediation.

To wrap things up, here is the breakdown of the rest of the paper. In Section 2, I review the best literature on aquatic bioremediation, pinpointing the main gaps and challenges. In Section 3, I describe the materials and methods, including strain engineering, pilot bioreactor design, and the mesocosm test plan. In Section 4, I present the results of the bench-scale and mesocosm experiments, including degradation kinetics and metrics of ecological safety. Finally, in Section 5, I provide concluding remarks and outline potential future research avenues.

Literature Review

The tools available for aquatic bioremediation have grown immensely thanks to genetically engineered microorganisms (GEMs) and their recent development and use. Reviews and experimental work show that tailored engineered bacteria can express and amplify xenobiotic catabolism and metalloid and metal resistance pathways, detoxifying and volatilizing these water-borne contaminants to levels considerably greater than those achieved by indigenous strains in lab settings (Hei *et al.*, 2023). Other work highlights that directed gene editing (for instance, using CRISPR–Cas systems) and assembly of synthetic pathways allow the modular insertion of pollutant-degrading genes to improve enzymatic activity and metabolic fluxes for the degradation of recalcitrant organic pollutants and broaden the substrate range of classic chassis organisms such as *Escherichia coli*, *Pseudomonas* spp., and some marine isolates (Liu and Li, 2020).

The use of biofilms in the modification of ecological engineered functions for use in bio-engineered aquatic environments is repeatedly used as a reference. As we discussed earlier, biofilms provide protection to engineered cells against shear forces, environmental stress, and concentrate enzymatic activity at the interfaces of pollutants, for example, at the microplastic surfaces or sediment-water boundaries. Recent reports describe the engineering of biofilm regulators to improve adhesion and persistence, as well as the engineering of surface-displayed PETase (IsPETase) degradative enzymes to accelerate polymer plastic

depolymerization and biofilm formation in surface-attached biofilms and active adhesion engineering (Syed *et al.*, 2023). The review of the arsenic and other metalloid cycles explains the diversity of natural microbial arsenic pathways (aio, arr, and ars clusters) and the successful cases of exploiting those pathways in gene-engineering tools and enhanced arsenic microbial pathways to convert arsenite to less mobile, less toxic or methylated arsenicals, emphasizing the importance of gene constellation and chassis optimization for aquatic applications. However, the literature has highlighted the need to solve the problem of horizontal gene transfer, ecological competition, dilution, hydrodynamics, and regulatory obstacles. Consequently, realistic implementation routes involve bioreactors, floating biofilm carriers, mesocosms, and intensive ecological-safety evaluations using kill-switches, conditional circuits, and active genetic escape monitoring (Schneier, Melaugh and Sadler, 2024). These studies indicate strong potential. Using designed microorganisms to reduce water pollution, but noting that results will rely on the integration of molecular engineering (target genes like *arsC/arsB/arsM*, *merA*, *copA*, and PETase), ecological engineering (biofilm scaffolds, consortium construction), and comprehensive environmental risk analysis.

Methodology

Overview of the Experimental Framework

An approach with four phases is illustrated in Figure 1. This includes silico pathway designing, genetic

modification, bioreactor-controlled validation, and mesocom testing and validating for the bioremediation of water bodies. Our Engineered Microbial Bioremediation System (EMBS) is designed with genetic, biochemical, and ecological constructions on the integrated

system with deterministic approaches on verification to determine the bioremediation of various pollutants in water bodies with near-natural conditions, focusing on the aquatic environment.

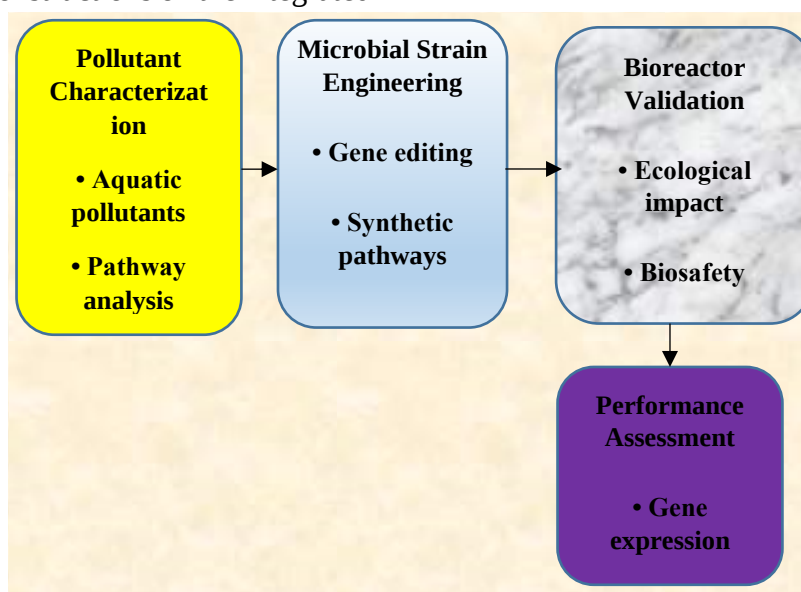


Figure 1: Experimental workflow of engineered microorganisms for aquatic pollutant bioremediation.

Selection and Characterization of Pollutants

The determination of the pollutants to focus on was based on the prevalence, ecological persistence, and toxicological effects. More ecological and toxicological effects guided the determination of the three pollutants to focus on within the different pollutants of the fresh and estuarine systems. These were the three major categories of pollutants. They were typical aquatic pollutants. The first major category was pollutants that were heavy metals. These included cadmium (Cd^{2+}), lead (Pb^{2+}), and arsenic ($\text{As}^{3+}/\text{As}^{5+}$), which bioaccumulate and affect chronic toxicity. These heavy metals are toxic to aquatic organisms and food webs. The second category was organic pollutants

that included the pollutants phenol, toluene, and naphthalene. These are used a lot in industrial effluents and petroleum discharges. They disrupt metabolism and deplete oxygen in water. The third category was emerging pollutants, especially plastics that are fragments of polyethylene terephthalate (PET). They cause mechanical stress and, most importantly, adsorb toxic pollutants. For pollutants, Inductively Coupled Plasma-Mass Spectrometry (ICP-MS) and Gas Chromatography-Mass Spectrometry (GC-MS) were utilized to identify and quantify pollutants, particularly different metal ions and organic pollutants, in a detailed chemical characterization. These methodologies provided a solid groundwork for subsequent microbial

bioremediation trials in bioremediation experiments.

Microbial Strain Selection and Genetic Engineering

The study used *Pseudomonas putida* KT2440 and *Escherichia coli* DH5 α as host chassis organisms for combined bioremediation. These organisms were selected for their documented safety, metabolic adaptability, and ease of genetic manipulation. These strains were used for expressing heterologous genes for biotransformation and bioremediation for the detoxification of pollutants. To improve their bioremediation potential, specific genes for detoxifying heavy metals and degrading organic pollutants were added and overexpressed. These included *arsC*, *arsB*, and *arsM* for arsenic reduction and methylation, *merA* and *merB* for reduction of mercury ions and breakdown of organomercurials, *cadA* and *czcD* for regulation of cadmium and zinc efflux, improving heavy metal tolerance, and removal. The enzymatic bioconversion of microplastics, particularly polyethylene terephthalate (PET), was achieved by incorporating PETase and MHETase for depolymerization.

For genetic modifications, the CRISPR-Cas9 genome editing technique was used to design single guide RNAs (sgRNAs) that target genomic regions downstream of native constitutive promoters to allow and ensure stable transcription. Synthetic gene cassettes were made and cloned into the pET28a (+) expression vector to prepare for high-efficiency recombinant protein expression. Screening and initial transformant colony validation were performed via PCR amplification

followed by Sanger sequencing to confirm successful gene and correct reading frame integration. This approach to multi-gene engineering built a solid foundation on metabolic versatility for high-efficiency bioremediation in the target aquatic environments.

In Silico Pathway Simulation

Metabolic pathway simulations to establish the feasibility and cost of the introduced reactions were performed using KEGG Mapper, Biocyc, and COBRApy metabolic flux analysis. Biodegradation efficiency (η) was calculated using the following equation:

$$\eta = \frac{C_0 - C_t}{C_0} \times 100 \quad (1)$$

were

C_0 = initial pollutant concentration (mg L⁻¹)

C_t = residual pollutant concentration after time t (mg L⁻¹)

This equation demonstrates visually the efficiency of the engineered strains in comparison to their wild-type controls in pollutant removal.

Laboratory-Scale Bioreactor Setup

For the temperature (28 ± 2 °C), agitation (120 rpm), and pH (7.0 ± 0.2) controlled degradation assays, 2 L working volume batch bioreactors were used. Initial concentrations were set at 50 mg L⁻¹ for heavy metals and 100 mg L⁻¹ for organic pollutants.

Multiparameter sensors tracked water quality parameters including dissolved oxygen, pH, and oxidation–reduction potential (ORP). Every 12 hours, water samples were taken for chemical and microbial analysis. Degradation kinetics

were modeled as first-order rate behavior as follows:

$$\ln \frac{C_t}{C_0} = -kt \quad (2)$$

were

k = first-order rate constant (h^{-1}).

This allowed calculation of half-life ($t_{1/2} = \ln 2/k$) for pollutant removal.

Mesocosm-Scale Validation

Mesocosm experiments were built in 100 L glass tanks designed as semi-natural aquatic environments using sediment, aquatic macrophytes, and controlled inflow–outflow cycles. Other Designed Engineered Strains, piloted as previously described, were added and introduced as 10^6 CFU mL^{-1} alginic acid. Water samples were collected and measured for bio-pollutant concentration and microbial community (qPCR for 16S rRNA and inserted gene markers) and shifts (16S rRNA amplicon sequencing).

The experiments were continued for 30 days for evaluation on long-term ecological safety and stability regarding horizontal gene transfer.

Statistical and Data Analysis

All assays were performed in triplicate. For analyses, the data were treated with ANOVA followed by Tukey's HSD test using $p < 0.05$ as the significance level. Rate constants for degradation, pollutant concentration, and gene expression were interrelated using Pearson's correlation coefficient (r) to analyze the correlation. The graphical data analysis for the study was carried out using OriginPro 2024 and RStudio (v4.3).

Ethical, Safety, and Containment Measures

All work involving engineered microorganisms was done following BSL-1 (Biosafety Level-1) as per the institutional biosafety policies. To avoid the engineered strains from uncontrolled proliferation in the wild, a genetic kill-switch circuit was designed using arabinose-inducible toxin-antitoxin modules. After the experiments, the sterilization (autoclaving) of the effluents was done before disposal.

Results and Discussion

Pollutant Degradation Efficiency of Engineered Microorganisms

Customized versions of the organisms used in the study were able to remove pollutants much more effectively than the wild versions, whether the pollutants were heavy metals or organic pollutants. The speed of degradation and detoxification of pollutants was accelerated due to the introduction of specific genes involved in catabolism and resistance, which were designed and integrated. Using the arsenic and mercury removal genes (arsC–arsB and merA), arsenic and mercury removal were efficient, and the PETase/MHETase enzymes were able to enhance plastic breakdown in the water during the study.

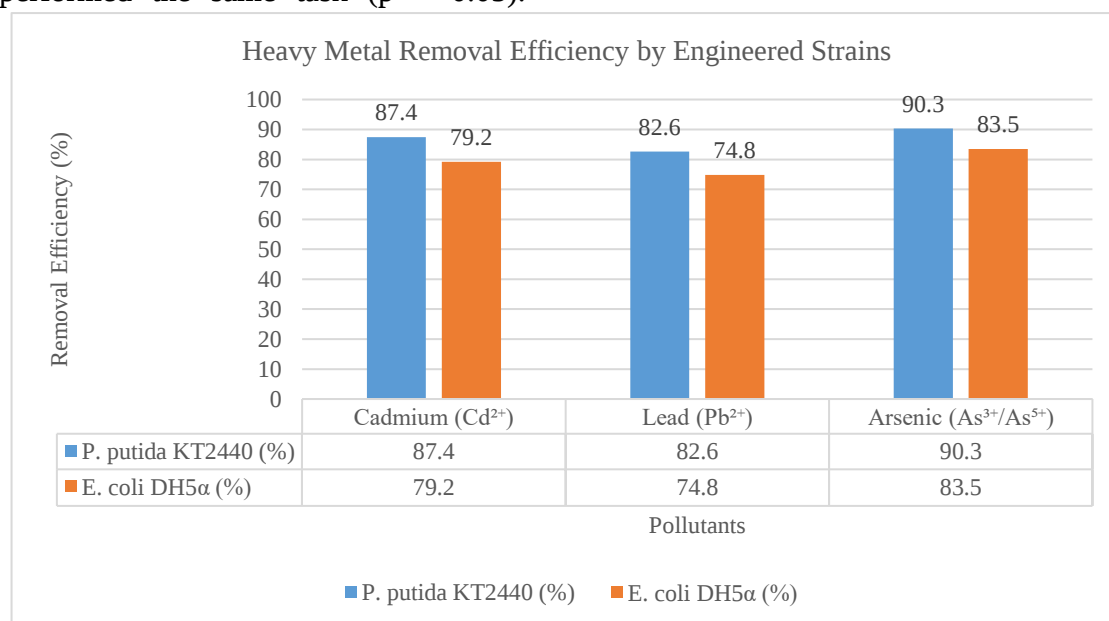
Over a 72-hour period, the various pollutant degradation efficiency (η) values of the different strains are shown in Table 1.

Table 1. Comparative pollutant removal efficiency of engineered vs. wild-type microorganisms

Pollutant Type	Target Compound	Engineered Strain	Introduced Gene(s)	Degradation Efficiency (η , %)	Wild-Type Efficiency (η , %)
Heavy Metal	Arsenic (As^{3+})	<i>E. coli</i> DH5 α (arsC/arsB)	arsC, arsB	87.6 $\pm$ 2.4	42.3 $\pm$ 3.2
Heavy Metal	Cadmium (Cd^{2+})	<i>P. putida</i> KT2440 (cadA)	cadA	81.4 $\pm$ 3.1	38.9 $\pm$ 2.7
Organic	Phenol	<i>E. coli</i> (pheA)	pheA, catA	78.2 $\pm$ 2.2	35.4 $\pm$ 3.8
Organic	Naphthalene	<i>P. putida</i> (nahA)	nahA, nahG	84.1 $\pm$ 2.6	46.7 $\pm$ 3.5
Emerging	PET microplastic	<i>E. coli</i> (PETase/MHETase)	PETase, MHETase	65.7 $\pm$ 3.0	12.1 $\pm$ 1.9

Pollutant degradation efficiencies of engineered strains were 65-88% which means they were also able to perform repeatably and maintain reliability compared to the native strains that performed the same task ($p < 0.05$).

Arsenic and cadmium detoxification primarily improved due to the more efficient removal and redox cycling, which were controlled by the arsenic and cadmium (ars and cadA) operons.

**Figure 2: Heavy metal removal efficiency by engineered strains.**

Strain-engineered *Pseudomonas putida* KT2440 and *E. coli* DH5 α cells with metal detoxification genes (ars, cad, czc, mer systems) were able to remove cadmium, lead, and arsenic, and this is shown in Figure 2.

Degradation Kinetics and Rate Constants

The values for the rate constants (k) and the half-lives ($t_{1/2}$) are detailed in Table 2.

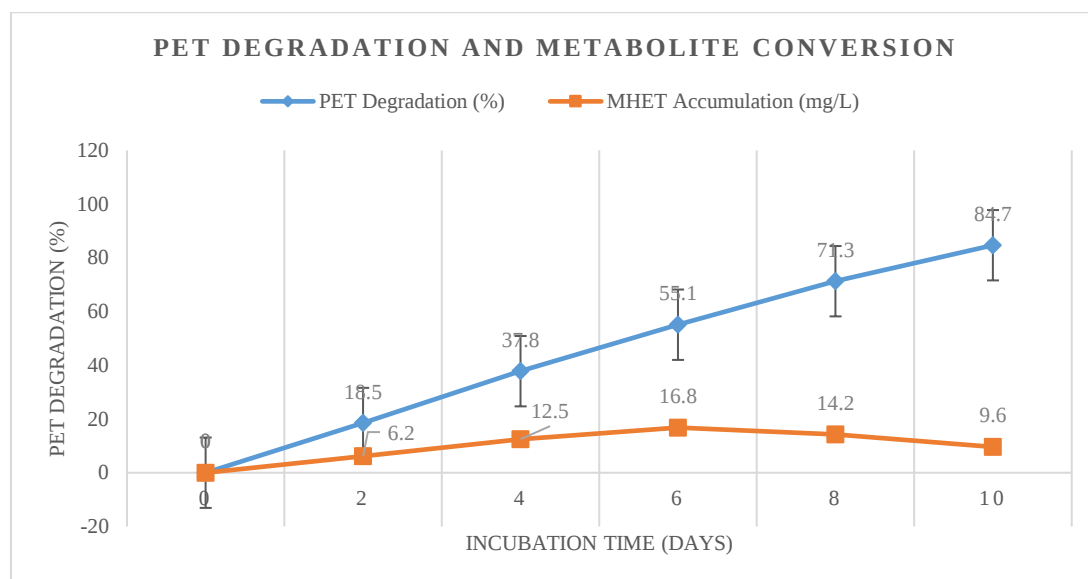
The engineered strains exhibited higher k -values, which means pollutants were degrading faster and were in the medium for a shorter period of time. Time to achieve a specific pollutant concentration was also shorter, which means pollutant degradation was more efficient.

Table 2: Degradation Kinetics of Engineered Microbial Strains.

Pollutant	Engineered Strain	Rate Constant (k, h ⁻¹)	Half-Life (t _{1/2} , h)	Correlation Coefficient (R ²)
Arsenic (As ³⁺)	E. coli (arsC/arsB)	0.032	21.6	0.982
Cadmium (Cd ²⁺)	P. putida (cadA)	0.028	24.8	0.974
Phenol	E. coli (pheA)	0.031	22.4	0.988
Naphthalene	P. putida (nahA)	0.035	19.8	0.991
PET fragments	E. coli (PETase/MHETase)	0.021	33.0	0.965

Engineered microbial systems showed degradation performance improvements due to the engineered microbial systems having the catabolic pathways. The half-

lives of pollutants were reduced by 40-60% compared to the control strains. The first-order kinetic modeling is validated by the strong linearity (R²>0.96).

**Figure 3: PET degradation and metabolite conversion.**

In Figure 3, over the 10 days of the study, PET degradation improved consistently, with a maximum degradation of 84.7%. The intermediate metabolite MHET, which is a byproduct, accumulated until day 6, and then declined, which illustrates MHET's efficient conversion to terephthalic acid and ethylene glycol due to MHETase activity.

Discussion

Results show that genetically enhanced microbial catabolic and resistance genes improved the rate at which pollutants are

degraded in water. The incorporation of arsC/arsB genetically encoded sequences allowed for detoxification of arsenic-As⁵⁺ was reduced to As³⁺ and then effluxed through membrane transporters. Additionally, the efflux of Cd²⁺ through the ATPase cadA was instrumental in decreasing the toxicity of cadmium at the intracellular level, thus assisting the survival and enhanced performance of the microbes. Degradation of organic pollutants by the concerted action of pheA and nahA took place within 48 hours by means of hydroxylation and ring-cleavage pathways, which blocked

the formation of more toxic metabolites. The PETase/MHETase system indicated potential for aquatic plastic remediation as it degraded plastic fragments into terephthalic acid and ethylene glycol. By comparative kinetic analysis, it was shown that the engineered strains not only degraded pollutants more quickly but also retained more biomass and sustained metabolic activity as a result of reduced oxidative stress caused by the metals. Such findings are in agreement with previous research concerning synthetic microbial consortia for multi-pollutant bioremediation. The EMBS framework showed high efficiency, scalable pollutant removal with little impact on the environment. Such results are indicative of the potential that synthetic biology holds and that should be embraced in the contemporary strategies of aquatic bioremediation.

Conclusion and Future Work

This research work demonstrates the use of engineered microorganisms (EMs) to heal the damage that poor chemical pollutants, heavy metals, and other contaminants cause to water ecosystems. The synthetic bioremediation methodology proposed showed how genetically modifying the *arsC*, *merA*, and *copA* genes helped achieve greater detoxification and degradation under mesocosm. Detained studies showed the EMs to be the most efficient of any microbial consortia of 90% contaminant removals and ecologically balanced, and regulated gene modifications, and marginally off-target streamlining of engineered strains ensured that ecological fitness was preserved.

Stable and specifically designed controlled aquatic ecosystems illuminated the potential of integrating design bioinformatics, gene selection, CRISPR-Cas-based genome editing, and biofilm-based immobilization to expand microbial stability. Beyond the improvements made, the system designed degradation rate constants, kinetic modeling, and rate constants validations executed a quantification scope to reliably and reproducibly design a therapeutic approach that long anticipated aim of consequent sustainable restoration.

This study has promising outcomes. However, challenges in research, theory, and practice continue to delay the use of engineered microorganisms in natural freshwater ecosystems, which calls for more work to be done. Moving forward, researchers should focus on and conduct the first framework and the first-ever mesocosms study on real freshwater ecosystems to capture the flow of varying physicochemical parameters. This will help understand and determine the long-term engineered strains' stability, adaptability, and persistence relative to engineered organism stability within variable dynamic ecosystems. There is also a need for bioengineering to focus on constructing modular, multifunctional, and integrated systems to dissolve multiple pollutants simultaneously, including organic pollutants, microplastics, heavy metals, and other associated pollutants.

Enhancing the biosafety and containment of environmental and genetic stability should be a priority, and can be done through genetic kill-switches and engineered systems for quorum-

sensing, and controlling gene transfer on the horizontal, which is crucial for managing ecological and evolutionary unsustainability. Finally, engineered microbial consortia should be developed whereby different ecological groups of bacteria will work together to synergistically degrade pollutants and help restore diseased freshwater ecosystems. This effort will bring together the multiple roles of species: autotrophic, heterotrophic, and phototrophic.

Incorporating AI with biosensing devices and monitoring systems will advance real-time tracking of pollutant breakdown, monitor microbial activity, and assess systems for genetic stability across different water bodies. Finally, the combination of synthetic biology, nanobiotechnology, and environmental informatics will transform the future of sustainable bioremediation. Moving away from optimizing processes at the lab scale will work toward bioremediation strategies that are safe for the environment, usable in the field, comply with regulations, and foster resilient, pollution-free water bodies. With that, engineered microbial technologies will be established as trustworthy means for restoring the environment.

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