



GENETICALLY ENGINEERED MICROBES FOR HYDROCARBON BIOREMEDIATION: ADVANCES AND APPLICATIONS, RISKS, GLOBAL CASE STUDIES, AND PROSPECTS FOR OIL-POLLUTED AFRICAN ECOSYSTEMS

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ABSTRACT

Hydrocarbon contamination from petroleum activities poses a persistent global environmental threat, with particularly severe impacts in Africa's oil-producing regions. This review explores the advances in genetically engineered microbes (GEMs) as a powerful bioremediation strategy, detailing molecular tools (e.g., CRISPR-Cas9, synthetic biology), metabolic pathway optimizations, resilience enhancements, and innovative gene delivery systems that enable superior degradation of aliphatic hydrocarbons, polycyclic aromatic hydrocarbons (PAHs), and total petroleum hydrocarbons (TPHs) compared to native consortia. Applications span laboratory demonstrations, pilot trials, and synergistic integrations with biostimulation, nanomaterials, and phytoremediation, yielding efficiency gains of up to 95% in controlled settings. However, deployment is tempered by ecological risks (e.g., community shifts, biodiversity loss), biosafety concerns (e.g., horizontal gene transfer, antibiotic resistance spread), human health implications, and stringent regulatory frameworks. Global case studies highlight contained successes in North America and Asia, contrasted with Europe's precautionary stance and limited field data. For Africa's hotspots, including the Niger Delta, Libya, Angola, Algeria, and emerging producers, GEMs offer tailored prospects for tropical, saline, and heterogeneous environments, promising community empowerment, alignment with Sustainable Development Goals, and cost-effective restoration. Overarching challenges encompass technical stability, monitoring, and equitable partnerships, bridged by interdisciplinary AI, metagenomics, and climate modeling. Ultimately, realizing GEM potential in Africa demands ethical, collaborative innovation to transform hydrocarbon legacies into sustainable renewal, balancing bold advancement with rigorous precaution.

Keywords: Genetically engineered microbes, Hydrocarbon bioremediation, Oil-polluted African ecosystems, Biosafety risks, Sustainable remediation

Introduction

Hydrocarbon pollution is a widespread environmental problem caused by human activities and threatens ecosystems globally (Alao et al., 2025). This study reviews the global impact of this pollution, introduces genetically engineered microbes (GEMs) as a promising solution, and details the goals of this review, emphasizing how international advances can be adapted to African settings.



Global Burden of Hydrocarbon Contamination: Sources, Impacts, and Conventional Remediation Limitations

Hydrocarbon contamination mainly originates from activities related to petroleum, such as oil spills during production, transportation, and storage, as well as industrial discharges and urban runoff (Majeed et al., 2025). These pollutants, including aliphatic hydrocarbons, polycyclic aromatic hydrocarbons (PAHs), and total petroleum hydrocarbons (TPHs), penetrate soils, sediments, groundwater, and marine environments, leading to extensive environmental harm (Truskewycz et al., 2019). In marine settings, contaminants such as BTEX (benzene, toluene, ethylbenzene, xylene) and PAHs accumulate in sediments. Ecological risk assessments reveal moderate to severe threats from heavy metals and TPHs, particularly in areas like the Bohai Sea (Mekonnen et al., 2024). Land ecosystems are also compromised, with older spills producing TPH levels exceeding 8,000 mg/kg, disrupting soil health and microbial life (Elgazali et al., 2023).

The impacts of hydrocarbon pollution are multifaceted, affecting ecological, human health, and economic dimensions. Ecologically, these compounds induce toxicity, genotoxicity, and bioaccumulation in food chains, reducing microbial diversity and plant productivity by inhibiting nutrient and water access while altering soil physicochemical properties (Radhakrishnan et al., 2023). Human health risks include exposure through contaminated water and soil, potentially leading to carcinogenic effects from PAHs and other persistent compounds (Padhye et al., 2023). Economically, polluted sites hinder land reuse for agriculture or development, exacerbating global burdens in regions with frequent spills (Majeed et al., 2025). Critically, co-contaminants like heavy metals (e.g., Pb, Zn) amplify these effects by inhibiting microbial metabolism and increasing mobility under changing pH conditions (Elgazali et al., 2023).

Conventional remediation methods, such as physical (e.g., soil excavation, vapor extraction), chemical (e.g., oxidation, solvent extraction), and thermal treatments (e.g., incineration, pyrolysis), face significant limitations (Mekonnen et al., 2024). These approaches are often costly, energy-intensive, and site-specific, with risks of secondary pollution from chemical by-products and incomplete removal of recalcitrant hydrocarbons like high-molecular-weight PAHs (Radhakrishnan et al., 2023). For example, natural attenuation achieves only 31% TPH reduction in nutrient-limited soils, while nutrient amendments alone yield 43-45% efficiency but fail to address bioavailability issues in clay-rich or aged contaminations (Truskewycz et al., 2019). Moreover, these methods can mobilize heavy metals to groundwater, posing long-term eco-toxicological risks and requiring extensive monitoring (Padhye et al., 2023). Overall, conventional strategies lack sustainability, underscoring the need for biologically driven alternatives.

Emergence of Genetically Engineered Microbes (GEMs) as a Paradigm Shift in Bioremediation

The limitations of traditional remediation have spurred the development of GEMs, which represent a transformative approach in bioremediation by enhancing microbial capabilities for pollutant degradation (French et al., 2020). GEMs are microorganisms modified using techniques such as CRISPR-Cas9, synthetic biology, and metabolic pathway engineering to optimize enzymes, including dehalogenases, oxygenases, and dioxygenases, for breaking down recalcitrant compounds such as hydrocarbons and PAHs (Shams et al., 2024). This shift from



reliance on indigenous microbes to engineered strains enables broader substrate specificity, higher efficiency, and resilience in harsh environments, achieving up to 90% TPH removal in integrated systems (Mekonnen et al., 2024).

Historically, bioremediation relied on wild-type microbes, but GEMs expand degradation ranges for xenobiotics such as toluene, xylene, and persistent organics, outperforming natural consortia at laboratory and pilot scales (Tomar et al., 2024). For instance, engineered *Escherichia coli* overexpressing genes such as *almA*, *xylE*, and *p450cam* facilitate hydrocarbon degradation, and horizontal gene transfer (HGT) enables dissemination to indigenous communities for sustained remediation (French et al., 2020). Advances in omics (metagenomics, metaproteomics) and AI further refine GEM design, predicting efficiencies and mitigating risks such as gene escape through kill-switches (Olawade et al., 2025). Critically, while GEMs offer cost-effective, eco-friendly solutions, regulatory frameworks in the U.S. (e.g., EPA under TSCA) emphasize risk assessments for environmental releases, underscoring the need for balanced innovation and biosafety (Shams et al., 2024). This paradigm shift positions GEMs as a sustainable alternative, particularly for complex contaminations where conventional methods falter.

Scope and Objectives of the Review: Bridging Global Innovations with African Contexts

This review comprehensively examines advances in GEMs for hydrocarbon bioremediation, integrating global case studies with tailored applications in oil-polluted African ecosystems, including the Niger Delta (Ehis-Eriakha et al., 2020). The scope encompasses molecular tools, metabolic optimizations, ecological risks, real-world implementations, and future prospects, with an emphasis on interdisciplinary approaches such as AI and metagenomics (Mekonnen et al., 2024). Objectives include: (1) evaluating GEM efficacy relative to native consortia; (2) analyzing risks such as HGT and biodiversity loss; (3) drawing lessons from North American, European, and Asian trials; and (4) proposing feasibility roadmaps for African hotspots, accounting for tropical climates, soil heterogeneity, and socioeconomic factors (Okafor et al., 2021).

Africa faces acute hydrocarbon burdens in regions such as Nigeria's Niger Delta, where aged spills yield high TPH (8,695 mg/kg) and PAH (989 mg/kg) levels, necessitating adapted GEMs with functional genes (e.g., *alkB*, *C230*) from indigenous degraders such as *Pseudomonas* and *Bacillus* (Ehis-Eriakha et al., 2020). Bridging global innovations requires capacity building, technology transfer, and alignment with Sustainable Development Goals (SDGs), addressing challenges such as regulatory gaps and community involvement (Radhakrishnan et al., 2023). Critically, this review advocates for equitable partnerships to foster context-specific solutions, highlighting GEM potential in consortia that achieve 25% TPH reduction in crude oil-polluted African soils (Okafor et al., 2021).

Advances in Genetic Engineering for Hydrocarbon-Degrading Microbes

Recent advances in genetic engineering have significantly enhanced microbial capabilities for hydrocarbon bioremediation, enabling precise modifications that improve degradation efficiency, substrate range, and environmental adaptability. This section examines key molecular tools, metabolic optimizations, resilience enhancements, and gene delivery systems that underpin these advances.



Molecular Tools and Techniques: CRISPR-Cas9, Synthetic Biology, and Multi-Omics Integration

The advent of advanced molecular tools has revolutionized the engineering of hydrocarbon-degrading microbes. CRISPR-Cas9, a precise genome-editing technology, enables targeted insertions, deletions, or modifications of catabolic genes to enhance degradation pathways (Jaiswal et al., 2019). For instance, CRISPR-Cas9 has been used to optimize *Pseudomonas putida* strains for improved breakdown of aromatic hydrocarbons and xenobiotics by disrupting competing metabolic pathways or overexpressing key oxygenases (Shams et al., 2024). This tool's efficiency in non-model organisms, combined with base-editing variants, enables rapid prototyping of degraders resilient to polluted sites.

Synthetic biology complements CRISPR by enabling the design of novel pathways and regulatory circuits. Bottom-up assembly of microbial consortia incorporates hydrocarbon catabolic modules from diverse sources into chassis organisms such as *Escherichia coli* or *Pseudomonas* spp., broadening substrate specificity for aliphatic and aromatic compounds (French et al., 2020; Wang et al., 2023). Modular genetic circuits, including quorum-sensing elements, coordinate degradation within consortia, enhancing efficiency in complex contaminations.

Multi-omics integration—encompassing metagenomics, transcriptomics, proteomics, and metabolomics—guides engineering by uncovering functional genes and regulatory networks in native degraders. Metagenomic analyses of oil-contaminated sites reveal diverse alkane monooxygenases (e.g., *alkB*) and dioxygenases, informing gene mining for synthetic constructs (Mekonnen et al., 2024). Integrated omics with machine learning predicts pathway bottlenecks, accelerating strain optimization for TPH and PAH degradation (Wang et al., 2023). Together, these tools shift GEM design from trial-and-error to data-driven precision.

Metabolic Pathway Optimization: Engineering Catabolic Enzymes for Broad-Spectrum Hydrocarbon Breakdown

Optimizing catabolic pathways targets rate-limiting enzymes to broaden substrate range and improve kinetics. Key enzymes, including alkane hydroxylases (*AlmA*, *AlkB*), cytochrome P450 monooxygenases, and ring-hydroxylating dioxygenases, are engineered via directed evolution or rational mutagenesis to degrade recalcitrant high-molecular-weight PAHs and long-chain alkanes (Wang et al., 2023). For example, site-directed mutations in *P450cam* increase affinity for PAHs, resulting in threefold higher degradation rates in engineered *Pseudomonas* strains (Radhakrishnan et al., 2023).

Pathway reconstruction integrates peripheral routes (e.g., from salicylate to catechol) into central metabolism, funneling diverse hydrocarbons into TCA cycle intermediates. Overexpression of genes such as *xylE* (catechol 2,3-dioxygenase) and *ndo* (naphthalene dioxygenase) in GEMs broadens specificity for BTEX and PAHs (French et al., 2020). Co-expression of efflux pumps mitigates intermediate toxicity, while redox cofactor engineering (e.g., NADH regeneration) sustains oxygenase activity in hypoxic, polluted environments. Metabolic flux redirection eliminates competing pathways, channeling carbon toward degradation. In *Pseudomonas putida*, knockout of glucose catabolism prioritizes hydrocarbon



utilization, resulting in up to 90% TPH removal (Mekonnen et al., 2024). These optimizations enable GEMs to outperform native consortia in broad-spectrum degradation.

Enhancing Microbial Resilience: Genetic Modifications for Abiotic Stress Tolerance in Polluted Environments

Hydrocarbon-polluted sites impose abiotic stresses such as salinity, pH extremes, heavy-metal co-contamination, and oxidative stress from reactive intermediates. Genetic modifications bolster tolerance, ensuring GEM survival and activity. Overexpression of stress-response genes, including heat shock proteins (e.g., GroEL/ES chaperones) and osmoprotectant synthases (proline, trehalose), enhances resilience to temperature and osmotic fluctuations common in oil spills (Truskewycz et al., 2019). Efflux pumps (e.g., MexAB-OprM in *Pseudomonas*) are upregulated to expel toxic hydrocarbons and metals, thereby reducing intracellular accumulation (Padhye et al., 2023).

Antioxidant systems counteract ROS generated by oxygenase activity; superoxide dismutase and catalase overexpression mitigates oxidative damage (Elgazali et al., 2023). Biofilm-promoting genes (e.g., *pel* and *psl* in *Pseudomonas*) enhance adhesion and protection in soil matrices. Multi-stress tolerance is achieved through global regulators such as sigma factors (RpoS) or two-component systems that sense environmental cues. Adaptive laboratory evolution, combined with engineering, selects robust variants; for instance, evolved *Pseudomonas* strains tolerate high TPH while degrading efficiently (Majeed et al., 2025). These modifications extend GEM efficacy across heterogeneous African ecosystems characterized by tropical heat and variable soils.

Innovative Delivery Systems: Plasmid-Based Gene Drives and Horizontal Gene Transfer Mechanisms

Sustained in situ activity requires stable gene maintenance and dissemination. Plasmid-based systems, often conjugative, encode catabolic operons (e.g., TOL for toluene and NAH for naphthalene) and transfer via conjugation, thereby amplifying degradation in indigenous communities (French et al., 2020). Horizontal gene transfer (HGT) mechanisms, including "gene drives" on plasmids, flood native populations with degradative genes without persistent GEM release, thereby addressing biosafety concerns (French et al., 2020). Engineered broad-host-range plasmids carrying *alkB*, *almA*, or *xylE* transfer to soil bacteria, enabling consortium-level remediation of crude oil and PAHs. Kill-switches and containment strategies (e.g., toxin-antitoxin systems) prevent uncontrolled spread. Natural HGT in polluted sites disseminates catabolic plasmids, and bioaugmentation with donor strains further enhances this dissemination (Tomar et al., 2024). These systems promote self-sustaining bioremediation, particularly suited to remote African hotspots.

Applications of GEMs in Hydrocarbon Bioremediation

Genetically engineered microbes (GEMs) have evolved from conceptual tools to practical applications in hydrocarbon bioremediation, demonstrating enhanced degradation across scales. This section details laboratory demonstrations, pilot trials, integrations with complementary technologies, and quantitative comparisons with native consortia, highlighting efficiencies, challenges, and critical insights for real-world deployment.



Laboratory-Scale Demonstrations: In Vitro Degradation Kinetics and Substrate Specificity

Laboratory-scale studies provide foundational evidence for GEM efficacy, focusing on controlled in vitro environments to quantify degradation kinetics and substrate specificity. These demonstrations often use chassis organisms such as *Escherichia coli* or *Pseudomonas* spp., modified with catabolic plasmids (e.g., TOL, NAH, OCT) or synthetic pathways to target aliphatic and aromatic hydrocarbons (Jiménez-Díaz et al., 2022). For instance, an engineered *E. coli* strain incorporating phenol hydroxylase and catechol-degrading genes degraded 5 mM phenol in crude-contaminated wastewater within 7 hours, using it as a sole carbon source, a feat native *E. coli* cannot achieve (Jiménez-Díaz et al., 2022). Kinetics revealed rapid metabolic flux, with half-lives shortened from days in wild-type strains to hours, underscoring the impact of synthetic modules on rate-limiting steps.

Substrate specificity is engineered through gene insertions and optimizations, enabling broad-spectrum degradation. In one study, *Burkholderia thailandensis* MSMB121 was modified with an orthologous alkane monooxygenase (*ladA*) gene. Homology modeling and mutations improved binding energy across various alkane chains, enhancing specificity for long-chain hydrocarbons (Jiménez-Díaz et al., 2022). A synthetic consortium of *E. coli* HY1 and *Pseudomonas aeruginosa* PH2 degraded 71% of phenanthrene in 9 days, with HY1 performing initial oxidation to dihydroxy intermediates and PH2 completing ring cleavage to TCA cycle entry. GC-MS confirmed metabolite profiles, showing specificity for polycyclic aromatic hydrocarbons (PAHs) (Jiménez-Díaz et al., 2022). Additionally, overexpression of *almA*, *xylE*, and *p450cam* in *E. coli* DH5 α achieved 60-99% degradation of crude oil, dodecane, and benzo(a)pyrene, with substrate promiscuity for complex mixtures (French et al., 2020).

Critically, these in vitro kinetics highlight GEM advantages in controlled settings, including 77-fold pathway efficiency gains via protein scaffolds that mimic substrate tunneling (Jiménez-Díaz et al., 2022). However, limitations include potential overestimation of field performance due to the absence of environmental stressors; horizontal gene transfer (HGT) risks, while mitigated by kill-switches, require rigorous testing to prevent unintended ecological shifts (Shams et al., 2024). These studies emphasize data-driven design using omics to predict kinetics, but scalability requires addressing metabolic bottlenecks in hypoxic or nutrient-limited conditions (Wang et al., 2023).

Pilot and Semi-Field Trials: Bioaugmentation Strategies in Simulated Contaminated Matrices

Pilot and semi-field trials translate laboratory insights into practical applications by employing bioaugmentation to introduce GEMs into simulated contaminated soils, sediments, or water matrices. These strategies often use conjugative plasmids or gene drives for in situ gene dissemination, enhancing degradation without persistent GEM survival (French et al., 2020). In semi-field simulations of oil spills, GEMs achieved 85% TPH reduction in 21 days in seawater and sediment matrices contaminated with crude oil or diesel, compared with 62% by native bacteria; bioaugmentation used oxygen-tolerant strains that maintained stable dissolved oxygen levels (Al-Ghamdi, 2025). Another pilot used *Pseudomonas putida* KT2440 engineered with dehalogenase variants to target recalcitrant pollutants (analogous to hydrocarbons) in



packed-bed bioreactors treating contaminated groundwater, achieving 95-97% removal at hydraulic retention times of 116-133 hours (Modin et al., 2020).

Bioaugmentation strategies incorporate HGT mechanisms, such as plasmid conjugation or nanotube-mediated transfer, with frequencies of 19-84% within 48 hours, resulting in >90% dissemination in indigenous communities (French et al., 2020). In polluted sediment trials, inoculation with pSF-OXB15-p450cam vectors led to a 46% TPH reduction over 60 days, as genes transferred to diverse native bacteria (e.g., *Pseudomonas*, *Flavobacteria*) after donor cell death (French et al., 2020). Semi-field setups mimicking coastal spills integrated GEMs with biosurfactants, improving hydrocarbon dispersion and achieving 78% TPH reduction when combined with plants such as *Spartina alterniflora* (Al-Ghamdi, 2025).

Critically, these trials reveal GEM superiority in hostile environments (e.g., low oxygen, high salinity) but also highlight biosafety concerns. Regulatory frameworks require containment (e.g., flagella gene deletions for biofilms) and monitoring for gene persistence (Modin et al., 2020; Shams et al., 2024). Efficiencies vary with matrix heterogeneity, and semi-field results often fall below lab results due to dilution effects, underscoring the need for adaptive strategies, such as pre-mated indigenous hosts, to minimize ecological risks while maximizing scalability (Wang et al., 2023).

Integration with Complementary Technologies: Synergies with Biostimulation, Nanomaterials, and Phytoremediation

Integrating GEMs with biostimulation, nanomaterials, and phytoremediation creates synergistic systems that amplify degradation by addressing bioavailability, nutrient limitations, and microbial resilience. Biostimulation supplies NPK fertilizers or compost to sustain GEM activity, achieving up to 93.14% TPH degradation in 42 days in diesel-contaminated soil, outperforming bioaugmentation alone (84.7% in 60 days) (Chen et al., 2025). At crude oil sites, GEMs with NPK achieved 91% removal in 60 days, enhancing enzyme activities and rhizosphere microbiomes (Chen et al., 2025).

Nanomaterials such as rhamnolipid-modified biochar provide surfaces for GEM attachment, catalyzing degradation and slowly releasing nutrients; combinations achieved 80.9% efficiency for aliphatics in 50 days (Chen et al., 2025). Sludge biochar with rhamnolipid sustained 28% removal, improving oxygen diffusion and reducing toxicity (Chen et al., 2025). Phytoremediation synergizes through root exudates that stimulate GEMs; compost with plants such as tall fescue and biochar reached 68% removal, promoting *alkB* gene expression (Chen et al., 2025). Legumes (*Medicago sativa*) with manure yielded 94.8% in 90 days, restoring soil nutrients (Chen et al., 2025).

Multi-component integrations (e.g., biochar + compost + GEMs + plants) achieved 62% TPH in 90 days, with field examples in Nigeria's Ogoniland exceeding 70% in 90 days (Chen et al., 2025). Critically, synergies yield a mean 61.9% removal (SD 21.3%) and 3-5-fold increases in gene abundance, but these effects vary with C:N:P ratios (optimal 100:10:1). Challenges include nutrient leaching and site heterogeneity, which risk eutrophication or incomplete degradation of heavy fractions (Chen et al., 2025; Mekonnen et al., 2024). Future directions include AI-optimized monitoring for sustainable, context-specific applications (Wang et al., 2023).



Quantitative Performance Metrics: Efficiency Gains Over Native Microbial Consortia

Quantitative metrics underscore GEM advantages over native consortia, with engineered strains achieving faster, more complete degradation. In lab trials, GEMs degraded 71% of phenanthrene in 9 days, compared with 45% by native *Pseudomonas*, with 77-fold pathway efficiency via scaffolds (Jiménez-Díaz et al., 2022). Engineered *E. coli* outperformed native *Pseudomonas putida* on dodecane (99% in 10 days) but underperformed on crude oil (60% vs. 79%), highlighting substrate-specific gains (French et al., 2020). Pilot bioreactors with GEMs removed 95-97% recalcitrant pollutants at 116-133 h retention, compared with <50% in native systems, with doubling times ~20 h on sole carbon sources (Modin et al., 2020).

Efficiency gains include 85% TPH in 21 days vs. 62% native in semi-field trials, with GEMs adapting to low oxygen (Al-Ghamdi, 2025). Integrated systems achieve 91% in 60 days, outperforming slower native attenuation (Chen et al., 2025). Critically, GEMs mineralize completely (stoichiometric chloride release), avoiding toxic intermediates from native cometabolism; however, high initial concentrations (>1 mg/L) are required for growth, limiting low-level applications (Modin et al., 2020). Costs (~0.26 €/m³) favor GEMs at concentrated sites, but regulatory barriers and ecological risks (e.g., gene escape) necessitate balanced assessments against native resilience across diverse microbiomes (Shams et al., 2024; Radhakrishnan et al., 2023).

Ecological, Health, and Regulatory Risks of GEM Deployment

The deployment of genetically engineered microbes (GEMs) for hydrocarbon bioremediation offers substantial benefits but raises significant concerns regarding unintended ecological consequences, biosafety, human health, and regulatory oversight. This section critically evaluates these risks, drawing on empirical evidence and expert assessments to inform balanced implementation, particularly in vulnerable ecosystems.

Potential for Unintended Ecological Disruptions: Microbial Community Shifts and Biodiversity Loss

GEM introduction into contaminated sites can profoundly alter indigenous microbial communities, potentially leading to biodiversity loss and ecosystem dysfunction. Engineered strains, often designed for superior degradative capabilities, may outcompete native microbes for resources, causing shifts in community structure and function (Wu et al., 2021). For instance, multi-functional GEMs (MFGEMs) targeting co-contaminated soils with heavy metals and PAHs exhibit enhanced adaptation but pose risks of disrupting native consortia, reducing diversity, and altering nutrient cycling (Wu et al., 2021).

Metagenomic studies reveal that GEMs can induce selective pressures, favoring resistant taxa while suppressing others, potentially leading to loss of functional redundancy essential for ecosystem resilience (Rafeeq et al., 2023). In soil microcosms, engineered *Pseudomonas* strains persisted and altered bacterial composition, with unknown long-term impacts on non-target organisms (French et al., 2020). Critically, while GEMs accelerate hydrocarbon degradation, their proliferation outside target sites risks invasive behavior, exacerbating biodiversity erosion in already stressed environments like oil-polluted soils (Shams et al., 2024).

Evidence from contained trials indicates transient shifts, but open-release scenarios amplify uncertainties, including secondary metabolite production or altered trophic interactions (Modin



et al., 2020). Mitigation via biocontainment (e.g., kill-switches) is promising, yet incomplete; stratified risk assessments are essential to prevent irreversible disruptions, especially in biodiverse tropical ecosystems (Wang et al., 2023).

Horizontal Gene Transfer and Antibiotic Resistance: Long-Term Biosafety Concerns

Horizontal gene transfer (HGT) represents a primary biosafety risk in GEM deployment, enabling dissemination of engineered catabolic genes or selectable markers (e.g., antibiotic resistance) to indigenous microbes. Conjugative plasmids carrying degradative operons facilitate high-frequency transfer (>90% in some consortia), potentially creating super-degraders or spreading resistance (French et al., 2020).

Antibiotic resistance genes (ARGs), often used as markers in GEM construction, amplify concerns; HGT in biofilms or stressed environments accelerates ARG proliferation, contributing to global resistance crises (Brown et al., 2024). In hydrocarbon-contaminated sites, selective pressures from pollutants and residuals may enhance transfer rates, with engineered pathways persisting post-GEM death via nanotube-mediated or plasmid-based mechanisms (French et al., 2020).

Critically, while HGT occurs naturally, engineered constructs bypass barriers, risking unintended enhancement of pathogenic or environmental bacteria (Shams et al., 2024). Long-term monitoring reveals gene stability in recipients, underscoring needs for marker-free designs and containment (e.g., toxin-antitoxin systems) (Modin et al., 2020). In African contexts with limited surveillance, such transfers could exacerbate resistance burdens, necessitating precautionary approaches (Mekonnen et al., 2024).

Human Health Implications: Toxicity Pathways and Exposure Risks in Contaminated Sites

Human exposure to GEMs during bioremediation poses potential health risks via direct contact, inhalation, or food chain accumulation. Engineered strains may produce toxic intermediates during hydrocarbon breakdown or elicit immune responses, particularly in occupational settings (Meressa & Tseha, 2024). Toxicity from GEM cells or metabolites could trigger inflammation or allergic reactions, with lateral gene transfer to gut microbiota potentially disrupting homeostasis and contributing to dysbiosis-linked diseases (Meressa & Tseha, 2024). In contaminated sites, aerosolized GEMs or spores risk inhalation, while dermal exposure during application raises pathogenicity concerns, especially if strains acquire virulence factors via HGT (Shams et al., 2024). Critically, while most GEMs (e.g., *Pseudomonas*, *E. coli* derivatives) are non-pathogenic, unintended mutations or environmental adaptations could enhance toxicity (Modin et al., 2020). Vulnerable populations near spill sites, such as in the Niger Delta, face amplified risks from incomplete containment (Okafor et al., 2021). Evidence remains largely hypothetical, derived from lab models, but underscores needs for exposure assessments and non-toxic chassis selection (Wang et al., 2023). Balanced against remediation benefits, risks demand robust monitoring to prevent adverse outcomes.



Global Regulatory Landscapes: Biosafety Protocols, Risk Assessment Frameworks, and Ethical Considerations

Regulatory frameworks for GEMs vary globally, emphasizing case-by-case risk assessments under precautionary principles. In the United States, the EPA regulates intergeneric GEMs under TSCA, requiring pre-manufacture notices (MCANs) or experimental release applications (TERAs) for bioremediation agents, focusing on persistence, transfer, and toxicity (Shams et al., 2024). FIFRA oversees pesticidal GEMs, while exemptions apply to intragenetic modifications.

Internationally, the Cartagena Protocol on Biosafety governs transboundary LMO movements, mandating advance informed agreement for environmental releases, including bioremediation microbes (Secretariat of the Convention on Biological Diversity, 2000). Many countries align with this, incorporating stratified assessments. Critically, gaps persist: limited field data hinders approvals, ethical concerns involve equity in technology access, and potential dual-use for bioweapons (Shams et al., 2024). In developing regions like Africa, capacity constraints delay adoption, raising justice issues (Mekonnen et al., 2024). Future frameworks should integrate adaptive monitoring, public engagement, and equitable benefit-sharing to reconcile innovation with safety.

Global Case Studies: Lessons from Real-World and Contained Applications

Global applications of genetically engineered microbes (GEMs) for hydrocarbon bioremediation remain limited because of stringent regulatory hurdles, biosafety concerns, and the predominance of laboratory-scale demonstrations over field deployments. Nevertheless, select pilots and experiments provide high-impact insights into GEM efficacy, ecological integration, and scalability challenges. This section examines region-specific case studies, highlighting successes in degradation kinetics, failures in persistence or unintended effects, and critical lessons for adapting GEMs to diverse geographies, including oil-polluted African ecosystems.

North American Pilots: Engineered *Pseudomonas* in Petroleum Refinery Sediments (e.g., California Trials)

North American pilots, particularly in the United States, represent some of the earliest and most extensively documented field applications of GEMs, driven by regulatory frameworks such as the EPA's TSCA and historical petroleum refinery contamination. A landmark case is the 1996 EPA-approved outdoor field trial of *Pseudomonas fluorescens* HK44 at the Department of Energy's Y-12 site in Oak Ridge, Tennessee, targeting polycyclic aromatic hydrocarbons (PAHs) in refinery-like sediments (Ripp et al., 2000). This engineered strain, modified with a recombinant plasmid (pUTK21) that fuses *nah* and *sal* degradative pathways with *luxCDABE* bioluminescence genes, served dual purposes: degrading PAHs (e.g., naphthalene, anthracene, phenanthrene) and acting as a biosensor for real-time monitoring via light emission proportional to contaminant bioavailability.

In the trial, 10^{14} cells were sprayed onto lysimeters (enclosed soil columns) spiked with 1,000 mg/kg naphthalene and 100 mg/kg other PAHs, mimicking refinery sediment pollution. Outcomes included effective PAH degradation, bacterial populations peaking at 10^7 CFU/g before declining as contaminants diminished, and bioluminescence correlating with



naphthalene levels down to low ppm (Ripp et al., 2000). Successes included containment (viable cells limited to within 4 m of the site) and proof-of-concept for integrated biodegradation-biosensing, reducing monitoring costs compared to traditional GC-MS methods. However, failures highlighted plasmid instability under field stresses and potential horizontal gene transfer (HGT) of antibiotic resistance markers (tetracycline), necessitating hypochlorite sanitization and two-year monitoring.

Critically, this Tennessee pilot underscores regulatory foresight; EPA's consent order imposed site-specific restrictions and risk assessments, yet revealed scalability barriers: GEM persistence was transient, and toxic metabolites (e.g., salicylate analogs) posed unresolved risks for broader refinery applications (Shams et al., 2024). These cases highlight North America's leadership in contained pilots but also critique overreliance on intergeneric scrutiny, which delays commercialization and underscores the need for adaptive, low-risk designs in high-salinity refinery environments.

European Field Experiments: GEMs for PAH Remediation in Industrial Brownfields

European field experiments with GEMs for PAH remediation in industrial brownfields are scarce, constrained by the EU's precautionary biosafety directives (e.g., Directive 2001/18/EC) and an emphasis on natural attenuation or non-GM bioremediation (Shams et al., 2024; Brewer et al., 2024). Most applications remain at semi-field or contained scales. Lab-to-pilot transitions have demonstrated enhanced dioxygenase activity in engineered *Pseudomonas* strains, but approvals for open releases have been denied due to biosafety and ethical concerns (Wang et al., 2023; Rafeeq et al., 2023).

Outcomes included improved bioavailability through biosurfactant co-expression, but failures stemmed from microbial community shifts that threatened functional redundancy (Rafeeq et al., 2023). Critically, these pilots reveal Europe's risk-averse approach: while the Cartagena Protocol guides assessments, national variations (e.g., stricter in Germany) limit field data, with only semi-enclosed trials approved under controlled conditions. High-impact insights highlight synergies with sustainable practices: combining GEMs with biochar in brownfield trials enhanced retention and degradation, yet scalability is hampered by public opposition and regulatory fragmentation (Chen et al., 2025). Critically, Europe's focus on eco-toxicological modeling predicts biodiversity impacts but underestimates adaptive GEM benefits, underscoring the need for harmonized frameworks to bridge lab efficacy and field application, particularly for PAH-laden industrial legacies.

Asian and Middle Eastern Examples: Adaptations for Crude Oil Spills in Arid and Marine Ecosystems

Asian and Middle Eastern GEM applications adapt to arid and marine ecosystems affected by crude oil spills, leveraging indigenous strains for resilience in high-salinity, hypoxic conditions. In China, a recent lab-to-pilot study engineered *Vibrio natriegens* VCOD-15 with five gene clusters to degrade biphenyl, phenol, naphthalene, dibenzofuran, and toluene in saline marine sediments and industrial wastewater (Su et al., 2025). Trials in bioreactors and pot arrays reduced pollutant levels to 2% of initial concentrations, converting them into benign intermediates for further microbial metabolism, with success in handling complex mixtures from Bohai Sea spills.



In Kazakhstan (Central Asia), microbial enhanced oil recovery (MEOR) pilots using GEMs such as *Pseudomonas* spp. with inserted alkane hydroxylases targeted arid reservoir spills, achieving 15-25% additional oil recovery and 60% TPH degradation in field tests (Yernazarova et al., 2024). Outcomes included adaptation to extreme aridity, but failures included biofilm clogging and incomplete breakdown of the heavy fraction.

Critically, these cases highlight regional innovations, e.g., salinity-tolerant chassis in arid zones, yet expose vulnerabilities: HGT in dense microbial communities risks the spread of resistance, and marine dispersal amplifies ecological risks (Al-Ghamdi, 2025). Scalability insights reveal cost-effectiveness in oil-rich nations but also underscore understudied long-term impacts on fragile arid/marine biodiversity, advocating for contained systems aligned with the SDGs.

Comparative Analysis: Success Factors, Failures, and Scalability Insights from Diverse Geographies

North American pilots excel in regulatory maturity and contained efficacy (e.g., 95% PAH reduction in HK44 trials), driven by EPA frameworks, but falter in open scalability due to HGT concerns (Shams et al., 2024). European experiments prioritize precaution, achieving high lab efficiencies but limited field data, with public acceptance failures hindering brownfield redevelopment (Chen et al., 2025). Asian/Middle Eastern adaptations shine in arid/marine resilience (60-90% degradation), fueled by oil economies, yet suffer from incomplete mineralization and biosafety gaps (Wang et al., 2023).

Success factors include indigenous chassis selection, pathway optimization, and integration (e.g., biosensors), yielding 2-3x gains over native approaches. Failures stem from persistence issues, community disruptions, and regulatory silos, with scalability hindered by data scarcity (Brewer et al., 2024). Critically, these geographies inform African prospects: North American containment models suit Niger Delta pilots, European ethics guide equity, and Asian adaptations address tropical/arid challenges. Global partnerships are essential to overcome knowledge asymmetries for sustainable, high-impact deployment (Mekonnen et al., 2024).

As global adoption of genetically modified organisms (GMOs) and GEMs accelerates, as evidenced by expanding pilots in Asia and regulatory developments in North America and African nations, particularly Nigeria (with its chronic hydrocarbon burdens in the Niger Delta), Angola, Algeria, and Libya must proactively prepare to embrace this transformative technology. Building local capacity, harmonizing biosafety regulations, and fostering international collaborations will enable equitable access, ensuring that GEMs contribute to environmental restoration and sustainable development without exacerbating existing vulnerabilities.

Prospects for GEMs in Oil-Polluted African Ecosystems

Africa's hydrocarbon-rich landscapes offer unique opportunities for genetically engineered microbes (GEMs) in bioremediation, yet implementation faces substantial barriers. This section explores tailored prospects, emphasizing mapping of pollution hotspots, environmental adaptations, socio-economic alignments, and strategic roadmaps, while critically assessing feasibility amid regulatory, ecological, and equity challenges.



Mapping Africa's Hydrocarbon Hotspots: Focus on Niger Delta, Libya, Angola, Algeria, and Emerging Producers

Africa holds approximately 7% of global proven oil reserves, concentrated in key hotspots where pollution from extraction, spills, and legacy contamination requires innovative remediation (Mekonnen et al., 2024). The Niger Delta in Nigeria, Africa's largest wetland, is a primary hotspot; chronic spills from more than 50 years of operations have contaminated soils with total petroleum hydrocarbons (TPHs) exceeding 8,000 mg/kg, affecting mangroves and livelihoods (Ehis-Eriakha et al., 2020). Libya, with vast reserves in the Sirte Basin, faces pollution from conflict-damaged infrastructure, resulting in groundwater TPH levels up to 5,000 mg/L (Elgazali et al., 2023). Angola's offshore Cabinda and onshore fields contribute to marine spills, with sediment PAHs reaching 1,500 µg/kg in the Congo River estuary (Majeed et al., 2025). Algeria's Saharan basins, including Hassi Messaoud, show arid soil contamination from blowouts, with TPH hotspots exceeding 10,000 mg/kg (Padhye et al., 2023).

Emerging producers such as Namibia (Kavango Basin discoveries), Senegal (Sangomar offshore), and Mozambique (Rovuma LNG) amplify risks; exploratory spills in Namibia's sensitive ecosystems threaten biodiversity, while Mozambique's gas fields raise concerns about methane leakage (Truskewycz et al., 2019). Critically, GIS and remote sensing mapping reveal heterogeneity: Niger Delta's tropical, waterlogged soils contrast with Libya's arid deserts, necessitating site-specific GEMs (Radhakrishnan et al., 2023). High-impact opportunities lie in deploying GEMs in these hotspots, which could restore 20-30% more land than conventional methods, but require addressing underreported pollution in emerging areas to prevent escalation (Okafor et al., 2021).

Tailoring GEMs to African Environmental Challenges: Tropical Climates, Mangrove Systems, and Soil Heterogeneity

Tailoring GEMs to Africa's diverse environments requires engineering for tropical heat (30-40°C), high humidity, mangrove salinity (up to 100 ppt), and soil variability (from clay-rich deltas to sandy deserts) (Tomar et al., 2024). In tropical climates such as the Niger Delta, GEMs must incorporate thermotolerant genes (e.g., heat shock proteins) to sustain degradation amid fluctuating pH and redox, achieving 70-90% TPH removal in hypoxic soils (Ehis-Eriakha et al., 2020). Mangrove systems, vital in West Africa, face oil smothering; GEMs with halotolerant chassis (e.g., engineered *Halomonas*) and osmoprotectants enhance resilience, degrading PAHs in saline roots where native organisms fail (French et al., 2020). Soil heterogeneity poses challenges: Libya's arid sands require drought-resistant GEMs with efflux pumps to handle metal co-contaminants, while Angola's heterogeneous clays demand biofilm enhancements to improve bioavailability (Elgazali et al., 2023). Critically, indigenous gene mining (e.g., *alkB* from African degraders) ensures adaptation but overlooks microbial interactions; consortia approaches yield 1.5-2x efficiency yet risk biodiversity shifts in fragile mangroves (Okafor et al., 2021). High-impact tailoring via CRISPR and omics predicts performance, but climate change exacerbates volatility, demanding resilient designs to avoid failures in heterogeneous terrains (Wang et al., 2023).



Socio-Economic and Policy Opportunities: Community-Driven Remediation and Alignment with Sustainable Development Goals

GEMs offer socio-economic opportunities in Africa by empowering communities in remediation and aligning with SDGs 6 (clean water), 14 (life below water), and 15 (life on land) (Radhakrishnan et al., 2023). In the Niger Delta, community-driven pilots could restore farmlands, create jobs in monitoring and bioaugmentation, and reduce poverty (SDG 1) and health risks from contamination (SDG 3) (Okafor et al., 2021). Policy frameworks such as Nigeria's NOSDRA enable GEM integration, fostering green economies and gender-inclusive training (SDG 5) (Majeed et al., 2025).

Critically, equitable access mitigates exploitation. Collaborative models in Angola align with SDG 17 (partnerships), but weak enforcement risks elite capture (Truskewycz et al., 2019). High-impact opportunities include cost savings (GEMs are 30-50% cheaper than excavation) and tourism revival in restored mangroves, yet these overlook cultural resistance. Inclusive policies ensure benefits reach marginalized groups (Padhye et al., 2023).

Feasibility Roadmaps: Proposed Pilot Frameworks, Risk Mitigation Strategies, and Collaborative Research Agendas

Feasibility roadmaps for GEMs in Africa propose phased pilots: initial lab validations with indigenous strains, followed by scaling to contained microcosms and semi-field trials in hotspots such as Libya's basins (Shams et al., 2024). Frameworks include biosafety protocols aligned with AU guidelines and kill-switches for containment (French et al., 2020). Risk mitigation emphasizes HGT monitoring via metagenomics and community consent (Modin et al., 2020). Collaborative agendas involve Pan-African research hubs partnering on AI-optimized GEM design (Tomar et al., 2024). Critically, roadmaps address funding gaps; international ties could fund pilots in Angola but overlook capacity building; integrated agendas ensure ethical, scalable solutions amid climate pressures (Brewer et al., 2024).

Overarching Challenges and Future Directions

Despite promising advances, deploying genetically engineered microbes (GEMs) for hydrocarbon bioremediation faces multifaceted challenges that must be addressed to realize their potential. This section critically examines technical barriers, knowledge gaps bridged through interdisciplinary methods, and the imperative for global-African partnerships, offering insightful pathways toward sustainable innovation.

Technical Barriers: Stability, Monitoring, and Cost-Effectiveness of GEMs in Open Environments

GEM stability in open environments remains a core technical barrier, as engineered traits like enhanced catabolic pathways can degrade due to plasmid loss, mutations, or competitive pressures from native microbes (Brewer et al., 2024). In field conditions, GEMs often exhibit reduced persistence compared to lab settings, with survival rates dropping below 10% after weeks due to abiotic stresses such as pH fluctuations, temperature extremes, and nutrient scarcity, limiting degradation efficiency to 50-70% for recalcitrant hydrocarbons (Rafeeq et al., 2023). Critically, horizontal gene transfer (HGT) exacerbates instability; while beneficial



for disseminating degradative genes, uncontrolled spread risks creating unfit hybrids or antibiotic-resistant populations, undermining long-term efficacy (French et al., 2020).

Monitoring GEM performance poses another hurdle, requiring real-time tracking of gene expression, microbial dynamics, and pollutant fate in heterogeneous open systems (Shams et al., 2024). Traditional methods like qPCR or culturing are labor-intensive and biased toward culturable strains, missing 90% of microbial diversity; advanced tools like biosensors (e.g., lux-fused GEMs) offer promise but face scalability issues in vast polluted sites, with costs escalating for remote sensing integration (Modin et al., 2020). Cost-effectiveness further constrains adoption: initial engineering and deployment can exceed \$100,000 per hectare, 2-3 times higher than biostimulation, due to regulatory compliance and biosafety measures (Brewer et al., 2024). High-impact critiques highlight economic disparities; in resource-limited African contexts, GEMs must demonstrate 20-30% cost reductions through localized production to justify use over conventional approaches, yet overlook hidden expenses like post-release surveillance (Olawade et al., 2025). Future directions emphasize stable chassis (e.g., via chromosomal integration) and affordable monitoring via drones or AI, but demand rigorous field validation to balance risks and benefits.

Bridging Knowledge Gaps: Interdisciplinary Approaches Involving AI, Metagenomics, and Climate Modeling

Knowledge gaps in GEM bioremediation, such as unpredictable microbial interactions and climate impacts, necessitate interdisciplinary integration of AI, metagenomics, and climate modeling for predictive optimization (Wang et al., 2023). Metagenomics uncovers functional genes in contaminated microbiomes, enabling data-driven GEM design; for instance, sequencing oil-polluted soils reveals *alkB* and *ndo* diversity, informing pathway engineering for broader hydrocarbon spectra (Mekonnen et al., 2024). AI enhances this by machine learning models predicting degradation kinetics, reducing trial-and-error by 50-70% through simulations of enzyme-substrate interactions (Radhakrishnan et al., 2023).

Climate modeling addresses environmental variability: integrating GEM performance with projections of rising temperatures and erratic rainfall forecasts adaptation needs, such as thermotolerant strains for African hotspots (Truskewycz et al., 2019). Critically, these approaches reveal biases; metagenomics often underrepresents rare taxa, while AI models lack diverse African datasets, risking inequitable solutions that ignore local climate extremes (Padhye et al., 2023). High-impact insights underscore synergies: AI-metagenomics pipelines have optimized consortia for 80-95% TPH removal in simulations, but field gaps persist due to unmodeled HGT under climate stress (French et al., 2020). Future directions advocate open-access platforms for global data sharing, fostering resilient GEMs amid climate change, yet caution against over-reliance on models without empirical validation in dynamic open environments.

Fostering Global-African Partnerships: Capacity Building, Technology Transfer, and Equitable Innovation

Global-African partnerships are essential to overcome GEM adoption barriers in Africa, focusing on capacity building, technology transfer, and equitable innovation to address pollution inequities (Okafor et al., 2021). Capacity building through training programs empowers local scientists in CRISPR editing and omics, as seen in EU-Africa initiatives



enhancing bioremediation skills in Nigeria (Ehis-Eriakha et al., 2020). Technology transfer from North-South collaborations could adapt GEMs for Niger Delta mangroves, but often perpetuates dependencies without intellectual property sharing (Tomar et al., 2024).

Equitable innovation requires co-design models, aligning with SDGs to ensure African voices shape GEM applications, mitigating risks like biosafety lapses in under-resourced labs (Shams et al., 2024). Critically, partnerships frequently overlook power imbalances; while promising 30-50% faster remediation, they risk neo-colonial dynamics where benefits accrue globally but burdens (e.g., HGT risks) localize in Africa (Elgazali et al., 2023). High-impact strategies include Pan-African hubs for joint R&D, fostering south-south ties for context-specific GEMs, but demand enforceable frameworks for fair benefit-sharing to drive sustainable, inclusive progress (Modin et al., 2020).

Conclusion

Genetically engineered microbes (GEMs) represent a transformative frontier in hydrocarbon bioremediation, offering unparalleled potential to degrade recalcitrant pollutants with precision, efficiency, and adaptability that far surpass conventional methods. Yet, as this review has revealed, their true power lies not merely in laboratory triumphs or isolated pilots, but in the courageous navigation of profound challenges: ecological uncertainties, regulatory prudence, technical instabilities, and socio-economic inequities that demand rigorous biosafety, interdisciplinary foresight, and unwavering ethical commitment. For Africa's oil-scarred landscapes, from the ravaged Niger Delta to Libya's arid basins and Angola's mangrove fringes, GEMs offer not just remediation but restoration; a pathway to reclaim sovereignty over degraded environments, empower communities, and align innovation with sustainable development amid climate exigency. Ultimately, the exceptional promise of GEMs transcends technology; it embodies a profound moral imperative to wield human ingenuity responsibly, equitably, and decisively, transforming the scars of extraction into legacies of resilience, justice, and renewal for generations to come.

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