Sharma et al.
Biotechnology for the Environment (2024) 1:8
https://doi.org/10.1186/s44314-024-00008-z

Biotechnology for the Environment

REVIEW

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Microbial remediation of polluted environment by using recombinant *E. coli*: a review

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Abstract

An increased amount of toxins has collected in the environment (air, water, and soil), and traditional methods for managing these pollutants have failed miserably. Advancement in modern remediation techniques could be one option to improve bioremediation and waste removal from the environment. The increased pollution in the environment prompted the development of genetically modified microorganisms (GEMs) for pollution abatement via bioremediation. The current microbial technique focuses on achieving successful bioremediation with engineered microorganisms. In the present study, recombination in *E. coli* will be introduced by either insertion or deletion to enhance the bioremediation properties of the microbe. Bioremediation of domestic and industrial waste performed using recombinant microbes is expensive but effectively removes all the waste from the environment. When compared to other physicochemical approaches, using microbial metabolic ability to degrade or remove environmental toxins is a cost-effective and safe option. These synthetic microorganisms are more effective than natural strains, having stronger degradative capacities and the ability to quickly adapt to varied contaminants as substrates or co-metabolites. This review highlights the recent developments in the use of recombinant *E. coli* in the biodegradation of a highly contaminated environment with synthetic chemicals, petroleum hydrocarbons, heavy metals, etc. It also highlights the mechanism of bioremediation in different pollution sources and the way in which this genetically altered microbe carries out its function. Additionally, addressed the benefits and drawbacks of genetically engineered microbes.

Keywords Genetic engineering, Bioremediation, *E. coli*, Bioaccumulation, Bioaugmentation

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Introduction

The advent of global industrialization has brought about critical environmental challenges with pollution being a significant concern. While industrialization has contributed significantly to economic development, technological advancements, and improved living standards for human being, but simultaneously, it has also led to adverse environmental impacts particularly in the context of pollution of the environment [1]. Environmental pollution refers to the degradation of the natural environment because of the introduction of pollutants. There are several types of environmental pollution including air pollution, water pollution, and soil pollution [2–5]. Pollutants encompass substances that, while sometimes naturally occurring, are deemed contaminants when surpassing natural levels. Pollutants can be categorized into biodegradable and nonbiodegradable types. Biodegradable pollutants, like phosphates and organic waste, can be broken down by living organisms. In contrast, nonbiodegradable pollutants, such as plastics, metals, pesticides, glass, and radioactive isotopes, resist decomposition by living organisms, persisting in the ecosphere for extended periods [6].

Environmental pollution, a ubiquitous and pressing issue, casts a looming shadow over the planet, threatening the delicate balance of ecosystems and endangering the health of both flora and fauna, including humans. From air and water pollution to soil contamination, the consequences of human activities on the environment are manifold and far-reaching [7]. The call for India to prioritize environmental protection amid its rich biodiversity and stark socio-economic disparities has never been more urgent. Joutey et al. and Rabani et al. [8, 9] underscore the critical need for India to balance economic development with environmental conservation. The government of India has taken major steps to prevent pollution in our country (Table 1). Addressing environmental pollution requires a combination of regulations, technological advancements, public awareness, and sustainable practices to minimize and mitigate the

Table 1 Govt. programs/initiatives to reduced pollution-causing agents in India

Pollutants	Programs	Year	Steps taken
Air	National Air Quality Monitoring Programme (NAMP)	2018	Regular monitoring of mainly four air pollutants, viz., SO_2, NO_2, suspended particulate matter (PM10), and fine particulate matter (PM2.5), in 307 cities/towns in 29 states and 6 union territories of the country
	National Ambient Air Quality Standards (NAAQS)	2009	To check the quality of the outdoor air (industrial, residential, rural, and other areas) Sulfur dioxide (SO_2), nitrogen dioxide (NO_2), particulate matter, ozone, lead, carbon monoxide, ammonia, etc
	National Air Quality Index (AQI)	2014	Monitoring eight pollutants (PM10, PM2.5, NO_2, SO_2, CO, O_3, NH_3, and Lead (Pb)), 71 cities in 17 states of country
	Forty-Two Action Points	-	To counter air pollution, which include control and mitigation measures related to vehicular emissions, resuspension of road dust and other fugitive emissions, biomass/municipal solid waste (MSW) burning, industrial pollution, construction and demolition (C&D) activities, mainly in Delhi and NCR area
	Environment Pollution (Prevention and Control) Authority (EPCA)	1998	Proper control and observation of environmental pollution in the NCR region
	Graded Response Action Plan (GRAP)	-	Action plan for Delhi and the NCR region
	Advanced Vehicle Emission and Fuel Quality Standards — BSIV	2017	Reduction and observation of vehicle emission pollutants
	Advanced Vehicle Emission and Fuel Quality Standards — BS-VI	2020	
	National Electric Mobility Mission Plan	2020	
Soil	E-waste (management)	2018	Soil waste management
	Biomedical Waste Management Rules Plastic Waste Management Rules Solid Waste Management Rules Construction and Demolition Waste Management Rules	2016	
	Hazardous and Other Waste (Management and Transboundary Movement) Rules 2019	2019	
	National Green Tribunal Act	2010	
Water	Water (Prevention & Control of Pollution) Act	1974	Amended deals with natural water bodies issues
	Water Cess Act	1977	Impose a cess on water abstracted from natural resources by industries and local authorities
	Coastal regulation zone	2019	-
Regulatory authorities	The key regulatory authorities are the following: • Ministry of Environment, Forests and Climate Change (MoEFCC) • Central Pollution Control Board (CPCB) • State Pollution Control Board (SPCBs) • District Level Authorities		

impact of pollutants on the planet. Numerous laws have been enacted to tackle the escalating pollution levels and set emission standards [10]. These legislative measures represent crucial milestones in India's environmental stewardship journey.

Dealing with pollution is a complex challenge, and various methods, both physical and chemical, have been employed to address the pervasive environmental issues (Table 2). However, their effectiveness and cost often limit their widespread use. Natural solutions, while safe and effective, face challenges due to the rapid accumulation of pollution from industrialization and the presence of nonbiodegradable synthetic materials [11, 12]. Physical methods, such as filtration and soil excavation, can be time-consuming and costly. Chemical alternatives, on the other hand, may pose inherent dangers to the environment and human health. Moving forward, it is imperative for India to adopt a holistic approach to environmental conservation—one that integrates environmental considerations into all aspects of policymaking and development planning. This approach should prioritize the protection of ecosystems, biodiversity, and public health while fostering sustainable economic growth and social equity. Advances in science and technology play a crucial role in pollution mitigation.

In response to these limitations, bioremediation has emerged as a promising and environmentally friendly approach. Bioremediation involves the use of microorganisms to assimilate, digest, or transform hazardous substances into less harmful or nontoxic forms [13–15]. Microorganisms exhibit remarkable capabilities in degrading, detoxifying, and even accumulating toxic organic and inorganic substances [16, 17]. The use of genetically modified organisms (GMOs), such as the genetically modified *Escherichia coli*, has become a powerful tool in the field of bioremediation. These engineered microorganisms are designed to efficiently remove toxins that indigenous bacteria may struggle to break down, offering a targeted and effective approach to environmental cleanup [18]. In contemporary bioremediation methods, genetically modified organisms play a pivotal role in addressing environmental pollution, particularly in situations where natural bacterial populations are insufficient to handle specific pollutants. The introduction of a foreign gene into bacteria transforms them into unique strains with enhanced capabilities for rapidly breaking down pollutants, such as hydrocarbons, in the environment [19]. The use of genetically modified *E. coli* in bioremediation has several advantages such as precision, efficiency, and versatility. However, it is essential to consider potential ethical and ecological concerns associated with the release of genetically modified organisms into the environment. Robust containment measures and thorough risk assessments are crucial to prevent unintended consequences. Therefore, in this article, the use of genetically modified *E. coli* in bioremediation is discussed which exemplifies the intersection of biotechnology and environmental science, offering innovative solutions to address pollution challenges. As technology continues to advance, the application of genetic engineering in bioremediation holds significant promise for developing tailored and efficient approaches to environmental cleanup.

Table 2 Measurements of air pollutants (https://metnet.imd.gov.in, https://www.env.go.jp)

Pollutants	Method	Instrument
SO_2	*Ultraviolet fluorescence method* (analyzed sample is exposed to UV-lamp irradiation with energetic excitation of SO_2 molecule)	Fluorescence analyzer
NOx (NO, NO_2, and NOx)	*Chemiluminescence method* (nitrogen molecule excitation by ozone)	Chemiluminescence analyzer
NH3	*Chemiluminescence method* (same principles as a NOx analyzer, but with an ammonia converter)	Chemiluminescence analyzer
CO	*Gas-filter correlation* (GFC) *spectroscopy* (absorption of infrared radiation by the CO molecule)	NDIR gas-filter correlation (GFC) spectroscopy or gas chromatography-flame ionization
Suspended particulates matter (SPM) Mineral dust, organic dust, metallic compounds	*Air sampler* (SPM is measured by sucking air through a filter and determining the weight of dust collected) Absorption spectrophotometry fluorescence analysis	High-volume air sampler Absorption spectrophotometer High-performance liquid chromatography
BTEX	*Gas chromatograph* (detector is specific to volatile organic compounds — benzene, toluene, ethylbenzene, and xylene's)	BTEX analyzer
Ozone	Total ozone measurement by *Dobson spectrophotometer* (measuring the relative intensities of selected pairs of ultraviolet wavelengths)	Dobson spectrophotometer
Organic gas (vapor)	**Vessel method, adsorption method, coolant condensation method**	Infrared spectrophotometry, gas chromatography, high-performance liquid chromatography

Bioremediation

To deal with pollution, a variety of methods (physical and chemical) are available. Due to their high cost and low effectiveness, most of them are of limited use. Physical methods are time-consuming and expensive, whereas chemical alternatives are inherently dangerous. Natural solutions are safe and effective, although they are sluggish and becoming less effective as a result of industrialization's rapid pollution buildup and non-biodegradable synthetic materials [11, 12]. Bioremediation is gradually becoming the standard method for restoring contaminated with heavy metals because it is efficient and cost-effective technology for the transformation of contaminants [13–15]. Biodegradation is a series of chemical reactions that occur in the presence of living organisms such as bacteria, fungi, yeast, algae, and insects in an environment with optimal light, temperature, and oxygen [20]. Microbes mitigate heavy metals and improve soil fertility, and plant development makes them more preferable source for bioremediation. The molecular nature, gene and enzyme induction, metabolite production, growth efficiency, and survival rate all influence individual bacteria' potential to act as bioremediation agents [21]. At higher moisture rate, anaerobic condition persists which slow down the degradation rate. In cold condition, microbial degradation of heavy metal is slow, as metabolic activities are inhibited as the microbial transport routes are frozen by the subzero water [22, 23]. Similarly, at higher temperature, the rate of heavy metal solubility increases, which increases their availability and the rate of microbial biodegradation [24]. The rate of microbial biodegradation is determined by the metal or pollutant's chemical structure, bioavailability, concentration, toxicity, and stability. The degradation of the n-alkanes is more effortless in comparison to the branched alkanes, aromatics with low molecular weight, hydrocarbons with high molecular weight, and the asphaltenes [25]. Molecular mechanisms play a crucial role in deciphering the microbial metabolism, genes, characteristics, variety, and fluctuations of microorganisms engaged in microbial remediation. Metabolic and protein analysis, sequencing, and the utilization of sophisticated bioinformatics tools are specifically employed to decipher the various categories of microorganisms and the factors influencing them in the bioremediation process [23].

Microorganisms currently employed in bioremediation have the potential to be genetically engineered in order to augment their enzymatic production, thereby amplifying their capacity for biodegradation. These organisms' genetic architecture makes them useful for biodegradation, biotransformation, biosorption, and bioaccumulation [26] (Fig. 1). The use of recombinant DNA allows an organism to develop the ability to digest a xenobiotic via degradative genes. Recombinant microorganisms and genetically modified microbes have been used as an effective technique for pollution breakdown [27]. In the current bioremediation technique, genetically modified organisms are employed to efficiently eliminate pollutants that native bacteria are unable to decompose [18]. There are varieties of bacteria reported to be capable of feeding on hydrocarbons under anaerobic and aerobic conditions [28]. Toxic substances may be converted to nontoxic ones by the bioremediation process by a variety of bacteria species such as *Achromobacter*, *Pseudomonas*, *Dehalococcoides*, *Rhodococcus*, *Comamonas*, *Burkholderia*, *Alcaligenes*, *Bacillus subtilis*, *Aspergillus niger*, *Deinococcus radioduran*, *Acidithiobacillus ferrooxidans*, *Mesorhizobium huakuii*, *Pseudomonas* K-62, *Ralstonia*, *Rhodopseudomonas palustris*, and *Sphingomonas* [29]. Similarly, nitrate-reducing bacterial strains, *Brevibacillus* sp. and *Pseudomonas* sp., were identified in petroleum-contaminated soil. *Bacillus*, *Corynebacterium*, *Staphylococcus*, *Streptococcus*, *Shigella*, *Alcaligenes*, *Acinetobacter*, *Escherichia*, *Klebsiella*, and *Enterobacter* were the best hydrocarbon-degrading bacteria [30].

Microbe's genetic sequences have been manipulated keeping specific goal in mind [31]. The term "genetically engineered organisms" (GEMs) refers to microorganisms (bacteria, fungi, and yeast, among others) that have been altered by humans utilizing molecular biology in vitro procedures [32]. There has been an explosion in the expansion of genetic engineering and recombinant DNA in breeding microorganisms, resulting in a huge number of bacteria with effective engineering that boosted pollutant-degrading abilities [18, 27, 33]. Bioremediation research is awaiting the introduction of gene editing technologies that produce knock-in and knockout. According to recent articles, researchers have mostly used the CRISPR-Cas system with model organisms such as *Pseudomonas* or *E. coli* [34]. Even non-model organisms like *Achromobacter* sp. HZ01 and *Comamonas testosteroni* may be employed for bioremediation due to new insights into CRISPR tools and the synthesis of gRNA to express function-specific genes pertinent to remediation [35, 36]. In experiments involving organophosphate and pyrethroid bioremediation, genetically altered *Pseudomonas putida* KT2440 was employed [37]. White rot fungus produces enzymes that break down polycyclic aromatic hydrocarbons (PAHs), TNT (2,4,6-trinitrotoluene), and polycyclic aromatic hydrocarbons (PCBs). When the enzyme esterase D combines with the insecticide endosulfan (an organochlorine), it produces simpler molecules. LiP-encoded hemoproteins in *Phanerochaete chrysosporium* degrade PAHs [38].

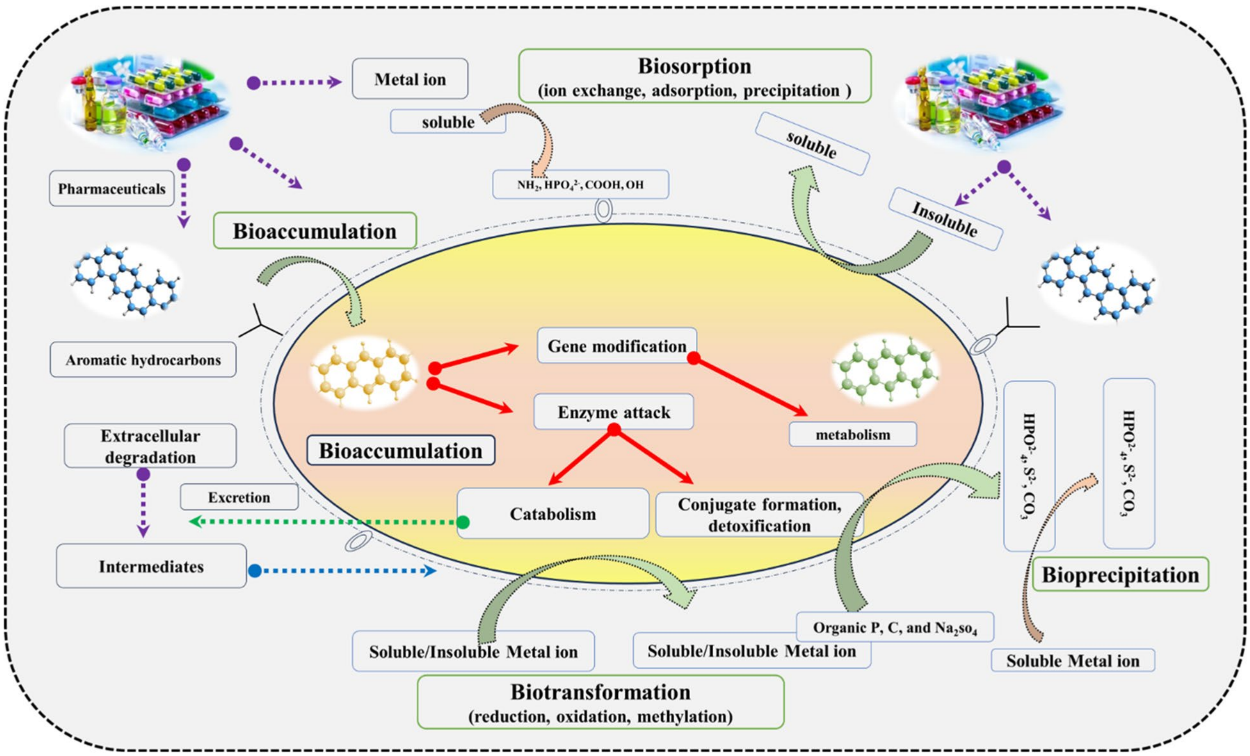

Fig. 1 Different approaches adapted by the microbes for the degradation of toxic compounds

Recombinant *E. coli* in bioremediation

E. coli is a rod-shaped facultative coliform bacterium belonging to the genus *Escherichia* that measures only about 1 µm long by 0.35 µm wide. It is one of the model organisms used in bioremediation (Fig. 2). *E. coli* is generally known as the "work horse" of molecular biology for its fast growth rate in chemically defined media and the various tools available for its genetic modifications. *E. coli* harbors a genome with features like an organized structure, a remnant of many phages, insertion sequences (IS), and high transport capacity towards the cytoplasm [39]. *E. coli* is a preferred host for gene cloning due to the ease with which DNA molecules may be introduced into the cells. Protein production in *E. coli* is expected due to the strain's rapid growth and high protein expression levels [40]. Various studies show that enteric bacterium like *E. coli* form phenol and p-cresol when grown on natural media (peptone and casein media) as well as in chemically defined media, i.e., L-tyrosine and p-hydroxybenzoic acid media [41]. According to a study conducted by Burlingame and Chapman [42] in 1983, it was found that *E. coli* has the capability to mineralize several aromatic acids, such as PA (phenylacetic acid), HPA (hydroxyphenylacetic acid), PP (phenyl propionic acid), 3HPP (hydroxyl phenyl propionic acid), and 3HCl. These research findings emphasized the ability of *E. coli* to break down and utilize a diverse range of aromatic acids [22]. An *E. coli* bacterium that has been genetically modified is employed as a highly effective agent in the process of bioremediation. The incorporation of a gene into bacteria results in the conversion of the bacteria into a distinct strain that possesses the ability to efficiently eliminate hydrocarbon pollutants from the surrounding environment (Fig. 3) [19]. There exist multiple methods for manipulating microbial genetics through genome editing, each of which is quite efficient and has been used in *E. coli* genome editing, making it capable of degrading pollutants and converting them to less harmful molecules [43]. The curli of an *E. coli* cell was genetically modified to produce BIND-PETase [40]. The *E. coli* SE5000 strain underwent genetic modification by introducing the nixA gene, which enables the expression of a nickel transporting system. This system has the ability to degrade nickel from aqueous system [44, 45]. The *E. coli* FACU strain possesses a significant capacity to reduce Cr (IV) to Cr (III) exhibiting great potential as a viable agent in the bioremediation of hazardous chromium species in aerobic environmental conditions [46].

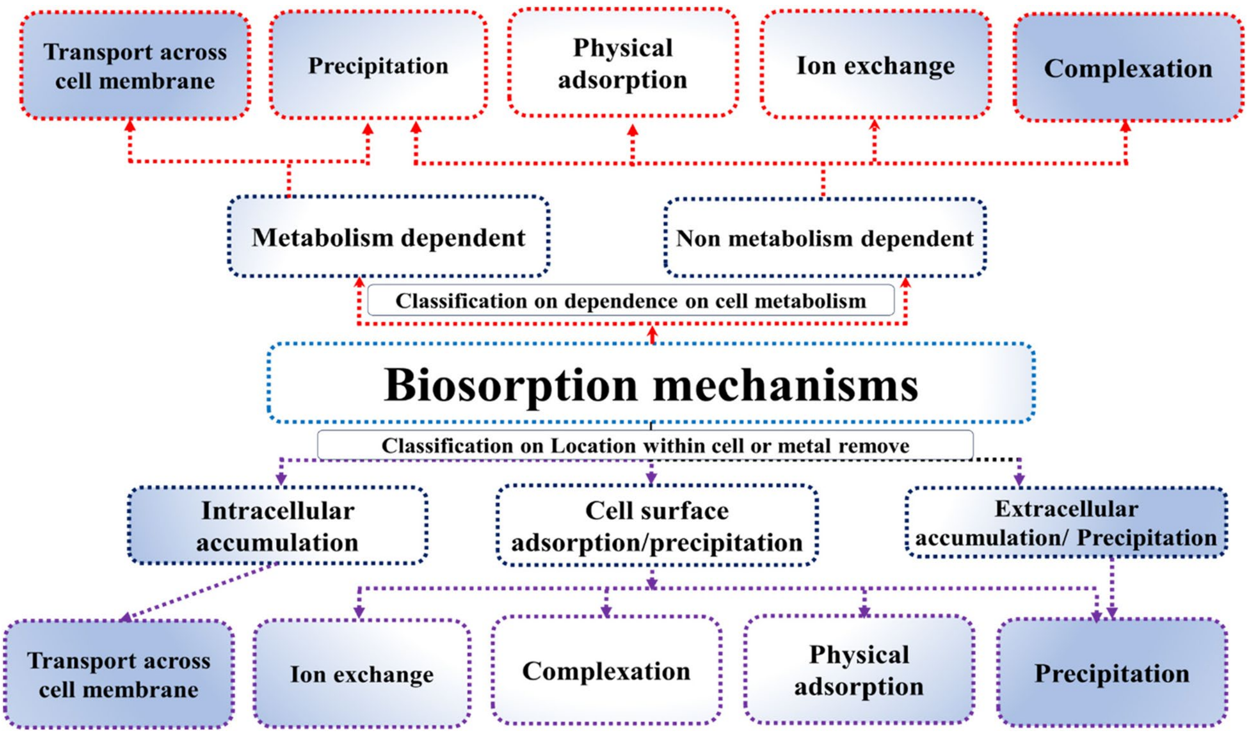

Fig. 2 Mechanism of biosorption on the basis of cell metabolism and its location within cell or metal removable

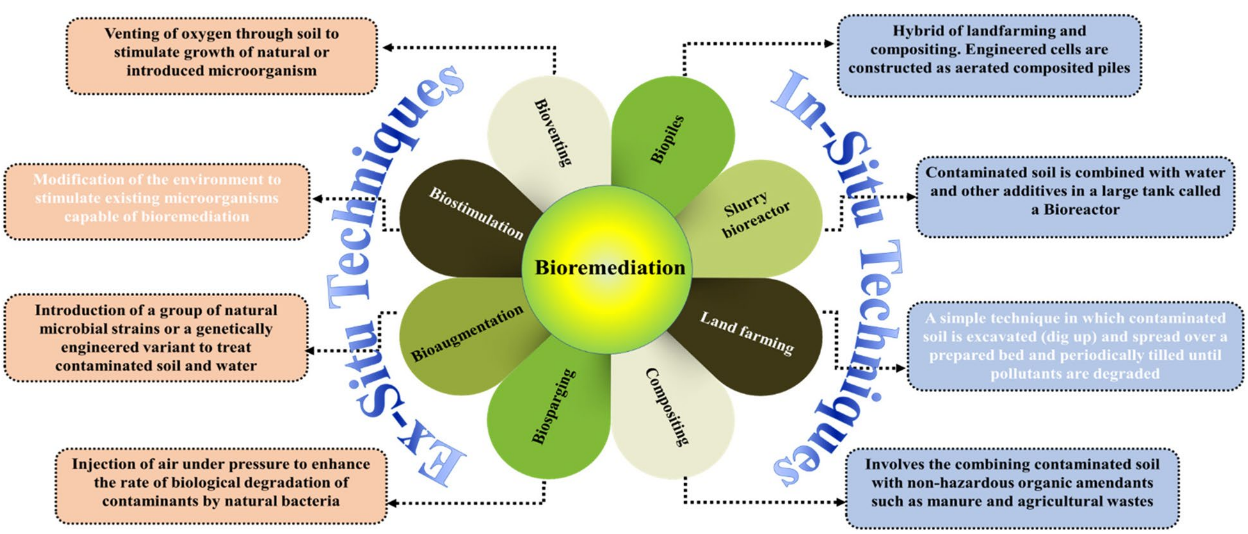

Fig. 3 Overview of bioremediation methods

General mechanism of degradation of pollutant by recombinant microbe

Genetic manipulation possesses the ability to create or mend microorganisms, leading to the development of biological detection systems that exhibit enhanced internal robustness, specificity, and resilience in various environments. Genetically engineered microorganisms (GEM) refer to microorganisms that have undergone genetic modifications using techniques of genetic engineering (inspired by the natural genetic exchange observed between microorganisms) [47, 48]. GEMs (genetically engineered microbes) have shown promise in the bioremediation of soil, groundwater, and activated sludge, with improved degrading capabilities for a variety of chemical contaminants [28]. Microbes possess inherent biological mechanisms that enable them to withstand intense metal stress or eradicate metals from their surroundings. Microbial bioremediation employs the following mechanisms [49]:

(1) Cell wall components or intracellular metal-binding proteins and peptides, such as metallothioneins (MT) and phytochelatins, play a crucial role in sequestering toxic metals. Additionally, substances like bacterial siderophores, which are mainly catecholates, are also involved in this process. It is worth noting that fungi produce hydroxamate siderophores [50].
(2) Altering metabolic processes directly blocks metal uptake.
(3) Enzymes are used to convert metals into harmless forms.
(4) Efflux mechanisms have the potential to decrease metal levels within the intracellular milieu.

Environmental contaminants such as chlorobenzene acids, toluene, and other halogenated insecticides and toxic wastes are broken down into less harmful forms by using important genes. A different plasmid is required for each chemical [51, 52]. Plasmids are classified into four groups [53].

1) OCT plasmid (degrades octane, hexane, and decane).
2) XYL plasmid (degrades xylene and toluenes).
3) CAM plasmid (degrades camphor).
4) NAH plasmid (degrades naphthalene).

The appearance and dissemination of genes that break down pesticides can yield a beneficial impact on the elimination of hazardous waste from the surroundings. The potency of *E. coli* in the degradation of various pollutants has been shown in Table 3. The genetically engineered strain of *E. coli* is able to express the Hg^{2+} and metallothionein transport systems. Excessive exposure to *Saccharomyces cerevisiae* glutathione S-transferase fusion protein and pea metallothionein significantly increased Hg^{2+} expression delivered by MerP and MerT, which protect cells from Hg^{2+} [54, 55]. Similarly, horizontal gene transfer (HGT) methods have been employed for incorporating petrol-contaminated organisms with *E. coli* carrying the vector pSF-OXB15-p450 cam fusion, which showed that *E. coli* bacteria are useful for the degradation of heavy metals [56]. Recombinant *E. coli* that expresses the metallothionein gene (*Neurospora crassa*) for Cd uptake was created using plasmid-encoded biochemical information and genetic engineering techniques, yielding a significantly faster Cd uptake than the donor microbe [57].

Different types of pollution and their bioremediation using recombinant *E. coli*

Soil contamination

Soil is an essential ecosystem consisting of both living and nonliving elements. The entirety of the natural world relies on soil in various ways. It serves as a connection between the biosphere, atmosphere, and hydrosphere, thereby playing a crucial role in maintaining the ecological equilibrium [67]. There exists a disagreement in the definition of "soil contamination." According to certain viewpoints, soil is deemed contaminated when the concentration of chemicals exceeds its typical range. While some individuals express concerns regarding the establishment of the standard range for pollutants. Hence, it can be asserted that "soil which is unsuitable for utilization and incapable of fulfilling its purpose is deemed as contaminated" [68]. The quality of soil and its role in ecological balance are affected by the addition of

Table 3 Genetically modified *E. coli* strains for bioremediation

Pollution treated	Bioremediation process	References
Water pollution	Bioremediation potential of cadmium by recombinant *E. coli* surface expressing metallothionein MTT5 from *Tetrahymena thermophila*	[58]
	E. coli strains (XL10 Gold, DH5α, and six types of BL21 (DE3)) showed bioremediation capacity against copper	[59]
	E. coli SE5000 strain showed bioremediation capacity against Ni^{2+}	[60]
	E. coli expressing metallothionein protein affecting against cadmium	[61]
Soil pollution	*E. coli* SE5000 strain express nickel transporting system: nixA which degrade nickel from aqueous system	[62]
	Engineered *E. coli* with atzA gene was proven to be successful in remediating soil polluted by atrazine	[63]
	E. coli JM109 expressed *merT–merP* protein and *metallothionein* (MT) which degraded mercury	[64]
	Engineered *E. coli* with linA and mpd genes was responsible for organochlorine and organophosphate degradation	[65]
	E. coli DH5α containing alkB, almA, xylE, ndo, and p450cam is responsible for petroleum hydrocarbon degradation	[56]
	E. coli BL21 containing PbrR, PbrR691, and PbrD genes is help in lead adsorption	[66]

soil contaminants due to natural processes and human activities like industrial wastes, the use of fertilizers in agricultural activities, and domestic and commercial construction [69]. Broadly, two types of contaminants contribute to soil pollution: inorganic and organic. In the category of inorganic pollutants, heavy metals are placed at the top of the list and are present in most of the contaminated sites. The most common toxic heavy metal contaminants found in soil include mercury (Hg), arsenic (As), copper (Cu), cadmium (Cd), chromium (Cr), Zinc (Zn), lead (Pb), and nickel (Ni) [70]. These soil contaminants sink into the soil through bad agricultural practices, inefficient industrial effluent disposal techniques, unauthorized waste dumping, etc. The persistence of heavy metals in natural environments presents a more formidable obstacle in comparison to organic contaminants, as they exhibit resistance to both microbial and chemical degradation. As a result, the elimination of heavy metals becomes a long-lasting challenge once they are introduced [71]. Organic contaminants encompass carbon-containing substances, regardless of the presence or absence of functional groups within their structures. The list of organic contaminants that contribute to soil pollution includes insecticides (e.g., captan, benomyl, endosulfan, heptachlor), herbicides (atrazine, alachlor, acetochlor, etc.), oil hydrocarbons (e.g., alkanes, alkenes), chlorinated compounds (e.g., polychlorinated biphenyls (PCB), polychlorinated dibenzodioxins (PCDD), polychlorinated dibenzofurans (PCDF)), aromatic hydrocarbons (e.g., BTEX, i.e., benzene, toluene, ethylbenzene, xylene), biocides (benzalkonium chloride), and polycyclic dibenzo-p-dioxins (e.g., benzopyrene, chrysene, fluoranthene) [72]. Persistent organic pollutants (POPs) are classified as organic contaminants, which are regarded as the most prioritized category of organic contaminants due to their high toxicity, carcinogenic properties, and ability to bioaccumulate in the environment [73]. Taking this into consideration, numerous nations have implemented limitations or outright prohibited the utilization and production of persistent organic pollutants (POPs). The POP compounds encompass substances such as DDT, endrin, hexachlorobenzene, PCBs, PCDD, PCDF, and others [68, 74] (Fig. 4).

Traditionally, various techniques are used to remove the soil contaminants, including extraction and separation techniques, thermal methods, chemical methods, microbial treatment methods, solid waste treatments, and phytoremediation (Table 4). The existing treatments for soil pollution, as discussed above, are not very successful in the removal of contaminants; sometimes, they bring down the concentration of contaminants at the cost of soil quality. Some of the techniques are also

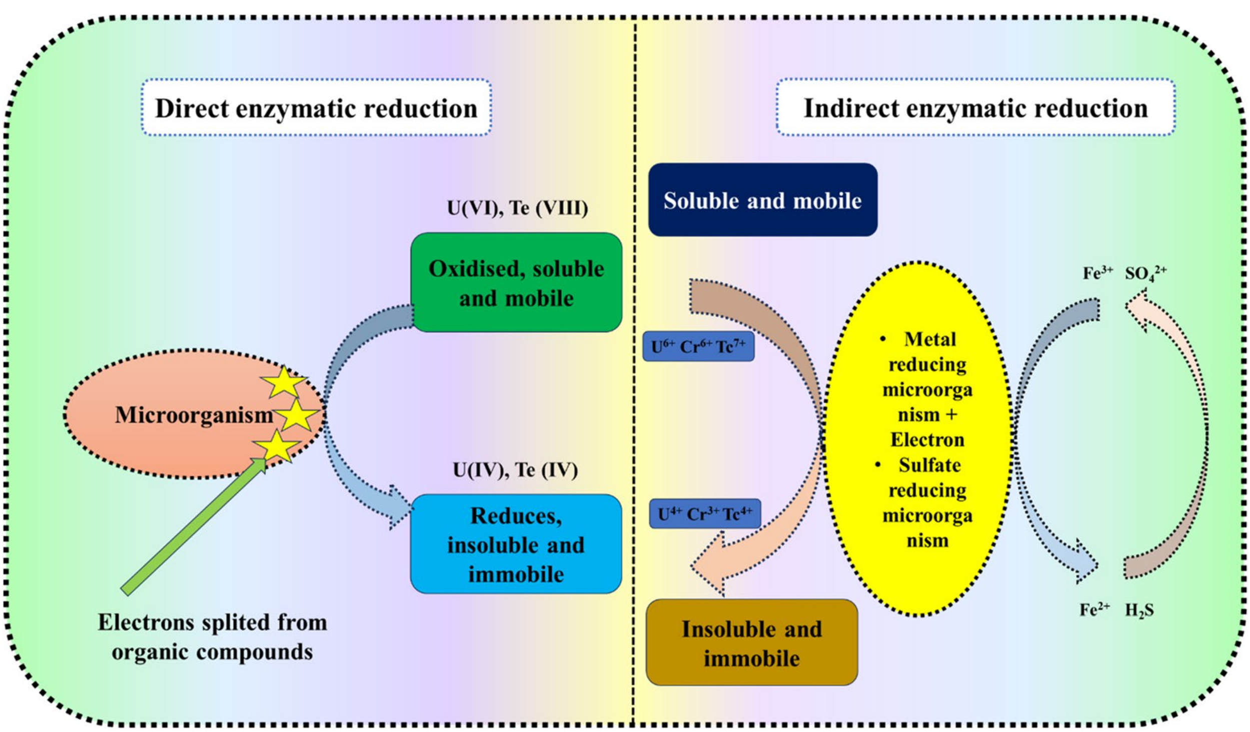

Fig. 4 Direct enzymatic and indirect mobilization of radionuclides by metal-reducing microorganisms via capturing of electrons derived by organic compounds (lactate and acetate)

Table 4 Various techniques used for treatment of soil pollution

Sr. no	Technique	Basic approach	Application	References
1	Extraction and separation technique	Removal by the use of extracting agents usually organic solvents	Removal of heavy metals, hydrocarbons, and halogenated hydrocarbons	[79]
2	Thermal treatment	Two ways of heat treatment: a) Removal of contaminants using the evaporation method b) Destruction of contaminants at an appropriate temperature	Used for the removal of volatile contaminants like toluene and trichloroethylene	[79]
3	Chemical method	Use of strong inorganic acids, weak organic acid, and chelating agent, i.e., HCl, HNO_3, H_2SO_4, H_3PO_4, NTA, EDTA	Removal of heavy metals, PTE, etc	[26]
4	Solid waste treatment	Chemical, physical, and biological treatment of waste by landfilling, excavation, incineration	Mainly for industrial waste treatment	[79]

less cost-effective [75, 76]. The main objective of soil remediation is not just the elimination of contaminants but also to restore the quality of the soil. So, we need to shift towards a new approach that gives better and more desirable results in terms of the elimination of pollutants and the restoration of soil quality [77]. Bioremediation is one of those approaches on which we can rely. In recent years, bioremediation has emerged as a great alternative to existing treatments as it is economical and does not compromise the health of the soil [78].

Table 5 Various genetically engineered bacteria used for the removal of heavy metal contamination in soil

S. no	Genetically engineered bacteria species	Targeted heavy metals	References
1	*Ralstonia eutropha* CH34	Cd	[44]
2	*Mesorhizobium huakuii* B3	Cd	[85]
3	*Pseudomonas putida* 06909	Cd	[86]
4	*Pseudomonas fluorescens* OS8	Cd	[87]
5	*B. subtilis* BR151 (pTOO24)	Cd	[88]
6	*Sphingomonas desiccabilis*	As	[89]
7	*Moraxella*	Hg	[90]
8	*Pseudomonas* K-62	Hg	[91]
9	*Pseudomonas fluorescens* OS8	Pb	[87]
10	*Pseudomonas fluorescens* OS8	Zn	[87]

Recombinant E. coli strain used for bioremediation of soil

The various studies done by Almaguer-Cantú et al. [80] on the removal of heavy metal contaminants from soil reported that genetically modified *E. coli* cells with overexpression of pea metallothionein MT improve the biosorption of Ni^{2+} and efficiently remove Ni^{2+} contamination from the affected sites. The elimination of hazardous metals from a polluted area through the utilization of biosorbent cell surface components of microorganisms is known as biosorption [81]. These biosorbent cell surface moieties are present on the outer surfaces of fungi, algae, and bacteria. Bacteria are widely regarded as the superior biosorbent when compared to other microorganisms [82]. This is primarily attributed to their possession of chemosorption sites such as teichoic acid, as well as their remarkable surface-to-volume ratio. These characteristics greatly enhance their biosorption capabilities [83]. In a study done in the United States for the removal of atrazine contamination from the contaminated fields by using recombinant *E. coli* encapsulating AtzA, which is responsible for the degradation of atrazine, they observed that after 8 weeks of inoculation, atrazine levels decreased by 52% and 77% (Table 5) in plots containing killed recombinant *E. coli* cells and combinations of phosphate, respectively [63, 84].

The successful elimination of oil contaminants in soil caused by oil spills can be achieved through the introduction of genetically modified *E. coli* cells containing catabolic genes [92]. The overexpression of three enzymes, namely almA, xylE, and p450cam, results in the degradation of petroleum hydrocarbon. According to their research, this genetically modified *E. coli* was able to decrease the level of petroleum hydrocarbon concentration by as much as 46% after a period of 60 days following inoculation [56]. Mercury (Hg) is a hazardous heavy metal and a significant inorganic pollutant found in soil, which can have harmful consequences on the organisms inhabiting contaminated areas. When it infiltrates the human body through the food chain, it gives rise to serious ailments such as neural disorders and respiratory disorders, occasionally leading to fatality. Genetically modified *E. coli* JM109 cells can assist in eliminating the Hg^{2+} contamination present in the soil [93]. This strain of *E. coli* has been genetically modified to produce the merT-merP protein and metallothionein, which are responsible for the accumulation of Hg^{2+} in the organism [64]. *E. coli* SE5000, a genetically modified strain, possesses the GSM-MT and nixA genes. The nixA gene is accountable for the activation of the Ni^{2+} transport system, enabling it to effectively eliminate Ni^{2+} contamination. On the other hand, GSM-MT is responsible for the increased production of metallothionein in the form of a glutathione S-transferase fusion protein [94].

Air pollution

Despite the remarkable advancements in technology, society, and the provision of various services, the Industrial Revolution had a detrimental impact on human health due to the significant release of pollutants into the air (http://www.who.int/airpollution/en/). Air pollution is the term used to describe the existence of detrimental substances in the atmosphere of our planet, which has adverse effects on both human well-being and the environment [95, 96]. The increase in economic growth has been accomplished by elevated energy consumption. The rapid urbanization in India, coupled with swift economic progress, has led to a surge in air pollution levels within megacities [97]. Particulates, greenhouse gases, and smog-forming substances such as sulfur dioxide (SO_2), ground-level ozone (O_3), nitrogen oxides (NO_2), and volatile organic compounds, are all major air pollutants (VOCs) [98, 99]. Air pollution has adverse effects not only on humans but also on the marine environment and is responsible for climate change too. The degradation of the earth's atmosphere is closely linked to the relationship between climate change and air pollution. The elevated concentrations of methane, black carbon, aerosols, and tropospheric ozone disturb the incoming solar radiation. Consequently, the temperature is on the rise, leading to the melting of icebergs, ice, and glaciers [22, 100]. The World Health Organization provides information on different categories of air pollutants, such as particle pollution, ground-level ozone, carbon monoxide, sulfur oxides, nitrogen oxides, and lead. In 2011, Delhi recorded a PM10 level of 198 μg m^{-3}, which exceeds the minimum limit by a factor of 10 [101, 102]. In May 2014, the city of New Delhi earned the unfortunate distinction of being the most polluted city in the world, according to the World Health Organization (WHO). This was primarily attributed to the high concentration of particle matter (PM) with a diameter less than 2.5 μm, which exceeded 350 μg per cubic meter of air in New Delhi. (http://www.theguardian.com/news/datablog/2015/jun/24/air-pollution-delhi-is-dirty-but-how-do-other-cities-fare) [95]. A conference titled "Impact of Disease: Air Pollution as a Leading Cause" was organized in New Delhi on February 13, 2013, by the Centre for Science and Environment (CSE) in collaboration with the Health Effects Institute, Boston, USA, and the Indian Council of Medical Research, New Delhi (http://www.cseindia.org/content/workshop-global-burden-disease-air-pollution-amongst-top-killers-india).

Air pollution can be easily dispersed and transported between different areas. This detrimental pollution leads to significant issues for both the environment and human health. Consequently, it is imperative to discover effective decontamination strategies in order to cleanse the environment. The process of decontamination must be carried out in a manner that safeguards the well-being of both animals and humans while also promoting the circulation of clean air [31] (Perera and Hemamali, 2022). Consequently, there is an increasing desire to discover efficient methods for remediating polluted areas, whether partially or entirely, in order to restore their environmental integrity [103, 104].

Degradation of air pollutant by recombinant E. coli microbe

Bacteria facilitate the breakdown of dangerous chemicals through an assimilative mechanism, wherein they acquire carbon and energy to support their growth, ultimately leading to the conversion of the compound into minerals [105, 106]. The bacteria responsible for PAH degradation include *Achromobacter* sp., *Bacillus* sp., *Mycobacterium* sp., *Burkholderia* sp., *Pseudomonas* sp., *Rhodococcus* sp., *Stenotrophomonas maltophilia*, *Sphingomonas* sp., *Xanthomonas* sp., and *Xanthomonas* sp. [107, 108]. The initial stage of hydrocarbon degradation involves the transformation of polycyclic aromatic hydrocarbon (PAH) or alkane chain into basic alcohol, subsequently converting into aldehyde and ultimately resulting in water, carbon dioxide, and biomass through oxidation. Oxidation also leads to the conversion of reduced sulfur molecules like H_2S into inorganic sulfur and thiosulfate, forming corrosive sulfuric compounds [101, 109]. H_2S advance oxidation is completed by chemolithotrophs. Sulfate is ingested through the sulfate start pathway, which is made up of three responses: adenosine 5′-phosphorylation of APS, GTP hydrolysis, and APS 3′-phosphorylation to deliver 3′-phosphoadenosine 5′-phosphosulfate (PAPS) [110, 111]. Microbes that degrade sulfate have the ability to use hydrocarbons and hydrolyze complicated chemicals in soil, according to Rennenberg [112]. Besides, a designed strain able of debasing PAHs was made in *E. coli* by communicating salicylate oxygenase, a protein encoded by bphA2cA1c from *Sphingomonas yanoikuyae* B1 [113]. The pGEX-AZR/*E. coli* JM-109 strain was genetically engineered, resulting in enhanced efficiency for decomposing various azo dyes [114].

Water pollution

The express "water contamination" is characterized in an assortment of ways by different committees, with the objective of making strides the quality of our environment. Agreeing to the head of the science committee, Washington, USA, in 1965, characterized water contamination as an alteration within the physical, compound, and organic qualities of water that will cause risky impacts on human and maritime life. Nowadays, it is not only concerned with public health but also with destroying natural beauty, resources, aesthetics, and the conservation of water [115, 116]. Numerous anthropogenic exercises are related to water contamination and have driven to water quality disintegration, like industrialization, chemical-related cultivating, broad urbanization, and populace development [117]. There are two sorts of sources that are included in water contamination, i.e., point sources and nonpoint sources. The coordinate identifiable source, or where coordinate association is appeared, is known as a point source, such as mechanical effluents, oil spills, and metropolitan and mechanical squander water effluents. In terms of nonpoint sources, diverse sources are included within the event of water contamination, primarily urban squander, runoff from rural areas, radioactive water (from atomic reprocessing plants), and contaminants that enter ground-level water [49]. Water contamination is caused by a variety of factors, the most prominent of which being urbanization (higher phosphorus concentrations in urban catchments sewage waste (massive increase in the growth of algae or plankton that facilitate huge areas of oceans, lakes, or rivers), industrial waste (wastes containing acids, alkalis, dyes, and other chemicals), agro-chemical waste (include fertilizers, pesticides which may be herbicides and insecticides), nutrient enrichment, thermal pollution (nuclear power and electric power plants, petroleum refineries, steel melting factories, coal fire power plant, boiler from industries), oil spillage (petrol, diesel, and their derivatives pollute seawater), acid rain pollution, and radioactive pollution (radioactive sediment, waters used in nuclear atomic plants, radioactive minerals exploitation, nuclear power plants) [65, 118, 119].

Water contamination is treated using a variety of physical and chemical approaches [75, 94]. Screening (radioactive sediment, waters used in nuclear atomic plants, radioactive mineral exploitation, nuclear power plants), grit chamber (remove sand and egg shells), floatation (oils, fats, grease, sediment solids), and sedimentation tank clarifier are examples of physical treatments (remove heavier sludge solids), whereas chemical treatments are as follows: neutralization (it adjusts pH for maintaining acidity of water), flocculation, coagulation (solid removal, water clarification, lime softening by chemical flocculants and coagulants), oxidation (may reduce toxicity using biochemical oxygen demand), ozonation (degradation of organic and inorganic pollutants), and chlorination [115, 120].

Drawbacks of physical and chemical methods

So many by-products are formed, chemical consumption is so high, physicochemical monitoring of effluents, capital and energy costs are so high, high sludge production and management of disposable, and techniques are expensive and toxic to the environment (Table 6).

Biological method used for water treatment

The biological method is the most common sanitizing method used for wastewater treatment, and it is also called secondary treatment, which involves the removal of organic matter from wastewater using bacteria and other microorganisms [137]. Wastewater typically contains pathogenic organisms, heavy metals, toxins, and organic matter (garbage, waste, and partially digested foods) [138]. Biological methods can be classified into two categories: (i) aerobic—takes place in the presence of oxygen and (ii) anaerobic—takes place in the absence of oxygen. Aerobic biological treatment involves many processes, i.e., the activated sludge process, trickling filters, aerated lagoons, and oxidation ponds. Due to its ease of use, rapidity, and efficiency, this process removes up to 98% of organic contaminants. Anaerobic biological treatment is used to treat high-strength wastewater (sludge degradation and stabilization). The process is slow as compared to aerobic; biogas production is one example of biodegradation of material where it overall converts up to 60% of organic solid mass (http://neoakruthi.com/blog/biological-treatment-of-wastewater.html) [139].

Some of the examples of microorganisms that are involved in the treatment of wastewater using different processes are gram-negative bacteria (proteobacteria) for the elimination of organic elements and nutrients, *Bacillus*, Bacteroidetes, Acidobacteria, Chloroflexi, *Tetrasphaera*, *Trichococcus*, *Rhodobacter*, *Pseudomonas*, *E. coli*, *Hyphomicrobium ascomycetes* fungi, *Nitrosomonas*, etc. [137, 138, 140, 141]. For specific contaminant degradation, predominantly well-defined microorganisms are used.

To improve the potency of proteins to overexpress the desired character for degradation by transforming microbes using genetic engineering approaches where they are transfected with genes that encode catabolic enzymes. Nowadays, genetically engineered microorganisms (GEM) are the most feasible xenobiotic-degrading microorganisms (*E. coli* and *Pseudomonas putida*) in wastewater treatment, and with the help of GEM, we can improve the bioaugmentation process. These GEMs have been used to degrade hexane, oil spills, xylene, toluene, camphor, trichloroethylene, etc. because of their high degradative capacities for various pollutants in wastewater [131, 142]. Manipulation of the oil-degrading *Pseudomonas* bacterium with plasmids containing genes

Table 6 Advantages and limitation of physical and chemical methods in water, soil, and air pollution

Sr. no	Pollution			
1	**Soil pollution**			
a)	**Physical method**	**Advantages**	**Disadvantages**	**References**
	Soil washing	• Cost-effective and time-saving • Remove a range of contaminants, both organic and inorganic at the same time	• Require large area in the setup of system • Not work on very silty and clayey sites • Wastewater from soil washing contains chemical additives, which may need specialized treatment which is generally difficult and expensive	[121]
	Soil covering	• Cost-effective and time-saving	• Polluting ground water	[122]
	Soil replacement	• Enhance load-bearing capacity • Reduce settlement • Improve stability of soft soils	• Costly method • Require proper compaction and compatibility between the replacement material and the existing soil	[121]
	Encapsulation	• Minimum water ingress and leachate generation • Avoid the escape of gases and vapors	• Long-term program of monitoring and maintenance • Groundwater pollution	[123]
	Nano-remediation	• No groundwater is pumped out • No soil transportation • Better remediation • Very small spaces in the subsurface	• Nanoparticles remain suspended in groundwater • Nanomaterials used for remediation do not move very far from their injection point	[121]
b)	**Chemical method**			
	Vitrification	• Significant reduction of waste • Significant advantage in terms of storage and disposal • Treat various waste such as soil with buried waste, dried sludges, tailings, sediments, organic waste, chemical waste, radioactive waste, and mixture of hazardous waste	• Costly method • Maintenance of poisonous gases is difficult	[124]
	Chemical leaching	• Heavy metals can be easily recovered	• Significant amount of chelating agent is required	[121]
	Chemical fixation	• Conversion of pollutants into less toxic form and reduced their bioavailability, mobility, and ecological risk • Material used are metallic oxides, clays, or biomaterial • Cost-effective	• Influenced by environmental conditions	[125]
	Electrokinectics	• Protecting natural ecosystem • Effective in the treatment of saturated and unsaturated soils	• Consumption of electric energy • Effects of electricity on soil characteristics	[126]
	Chemical oxidation	• Effective for the removable of polycyclic aromatic hydrocarbon • Effective for the removable of various recalcitrant organic compounds • Used in combination with other remediation methods • Require less time • Can be easily modified	• By-products of chemical oxidation are of potential risk to the environment • Further research is required to achieve maximum pollutant removal efficiency	[127]
2	**Water pollution**			
a)	**Physical method**			

Table 6 (continued)

Sr. no	Pollution			
	Sewage dumping	• Labor-saving • Produce energy • Fertilizer production • Reduce public health risk • Environment friendly	• Putrid odor • Bacterial imbalance • High installation costs • More use of power • Maintenance costs • Environment footprint • Massive land utilization	[128]
	Mechanical garbage collector	• Remove suspended solids up to 2–3 mg • Available at low cost • Easy to operate • Simple construction • Potable • Work long time	• Human resources are not used • Cannot withstand higher loads • It can clear drain width up to its width only • Sound and vibration during process	[129]
	Mechanical sewage collector	• High efficiency of removable of suspended solids • Easy to opeate • Cost-effective	• Eutrophication	[130]
	Active pharmaceutical ingredient	• Removing fats and oils from wastewater • Treat wastewaters of refineries, petrochemicals and chemical plants, and other industries	• Cannot produced large varieties of products	[131]
	Corrugated plate interceptor (CPI)	• High efficiency of oil and fat removal • Low-energy consumption • Simple operation • Using corrosion-resistant plates, acid, alkalinity • Dense structure raises concerns about deformation	• Large surface area is required • Expensive oil/bottom scrapers required that are maintenance intensive • Ineffective with small oil droplets or emulsified oil • Require long retention time to achieve efficient separation	[132]
	Dissolved air flotation	• Small space requirement • Recovered solids are 3–4 times thicker than gravity sedimentation • Faster processing time	• Low initial cost but high operational cost	[133]
b)	**Chemical method**			
	Iron-enhanced sand filters	• Treat dissolved phosphate • Decrease eutrophication	• Require better analytical method for detection	[134]
	Chlorination	• Cost-effective • Remove microorganism	• Chlorine can be toxic • Cause irritation to the eyes, nasal passages, and respiratory system	[135]
	Advanced oxidation process	• Remove organic contaminants from water • Not producing large sludge • Rapid reaction rate with less retention rate • Not require large area	• High operating and maintenance cost • Complex chemistry to specific contaminants	[136]
	Photochemical degradation	• Degrade insecticides and pesticides	• Need improvement to achieve complete mineralization	[166]
	Ozonation	• Good removable rate for all pesticides	• Ozone has short life span • It should be generated on-site, which increases the cost of treatment • Free redicals causing side effects to human	[136]
	Fenton	• Remove organic contaminants	• More toxic intermediate products • Costly	[167]
	Adsorption	• Cost-effective • Fast, flexible, and simple design	• Continuous removal of entrapped sludge	[136]
3	**Air pollution**			
a)	**Physical method**			

Table 6 (continued)

Sr. no	Pollution			
	Scrubbers	• Small space requirements • No secondary dust source • Handles high temperature and humidity gas streams	• Corrosion problems • High-power requirements • Water-disposal problems	[123]
	Electrostatic precipitators	• Reduce mercury emission • Home air cleaners • Incinerators • Used in coal-burning plants	• Less effective in removing very small particles and gaseous pollutants • High setup cost	[168]
	Baghouse filter	• Gentle cleaning method extends filter bag lifespan • Low operational cost	• Require more space • Not efficient as pulse-jet system	[96]
b)	**Chemical method**			
	Flue gas desulfurization	• Simple process • No sewage and acid treatment • Low-energy consumptions • Purified flue gas does not need secondary heating	• Low sulfur removal efficiency • Large investment • Large equipment • Large floor area • High technical requirements for operation	[169]
	Carbon sequestration	• Reduces the emission of greenhouse gases	• Costly process	[120]

encoding catabolic enzymes used in the degradation of aromatic compounds [143, 144]. For biodegradation of atrazine, metal removal, and direct blue dye in waste water, a genetically modified *E. coli* strain has been used [145, 146].

Genetically modified E. coli involved in wastewater treatment

Mercury (Hg) is the most dangerous heavy metal that can be released into the environment through industrial wastewater. Mercury can be removed from contaminated water, soil, or sediment by the GE *E. coli* strain JM109 [43]. Mercury can be removed from a contaminated site using GE bacteria that possess the MerA gene [147, 148]. GE *E. coli* has been discovered to digest trichloroethylene after being transformed with a variety of phenol catabolic genes such as pheA, pheB, pheC, pheD, and pheR. Nickel (Ni) is perhaps the most tenacious toxin, and it can be extracted from water by the GE *E. coli* SE5000 strain [149]. In this way, GE microorganisms can help with the bioremediation of heavy metals from degraded sites.

Safety of using recombinant *E. coli* strain for treatment of pollutants

Artificial generation of pollutants takes place by various by-products produced by the modern human world, which leads to toxicological impacts on nature. With growing awareness about the direct and indirect impacts of environmental pollution on ecosystems, efficient, cost-effective, and environmentally safe methods are being developed for the treatment of pollutants. The rapid rise in the rate of industrialization and the manufacturing of harmful toxic products leads to a change in the homeostatic balance of ecological biodiversity. Recombinant DNA technology emerged in 1972 and became the cutting-edge technology in the modern world, leading to the mass production of human insulin, human growth hormones, interferon, and the hepatitis vaccine [150]. In medical sciences, delivery made by this technology has set mild stones to combat pollution. For this purpose, genetically modified organisms (GMO) produced by recombinant DNA technology are used as a promising option for the treatment of pollutants, and many reports have also been published in this context [151, 152]. There are a variety of pollutants that are increasing at an alarming rate in the environment and need to be monitored.

The common pollutants that are to be taken into consideration are heavy metals, high density petroleum hydrocarbons (mercury, lead, arsenic, cadmium, etc.), polymers, chlorinated hydrocarbons, pesticides, insecticides (polycarbonates, polyethylene, polyurethane, polypropylene, etc.), explosives, detergents (GTN, TNT, and RDX), etc. [153, 154]. The combination of biotechnology and recombinant DNA technology is improving pollutant-degrading microbes through genetic modifications and strain improvement of specific metabolic and regulatory genes that are crucial in biodegradation [30]. Chakrabarty [155] established the bar by patenting petroleum oil pollution bioremediation, which was the first step towards using recombinant DNA technology for pollution mitigation. The most important and prominent tools for recombinant DNA technology are GMOs, which aid in the bioremediation of pollutants. Although they are potential deliverables, they need statutory clearance to be used in an open environment in many countries. So various regulatory guidelines are framed in different countries for their safe use.

The recombinant *E.* coli K-12 strain is extensively used for pollution control as it does not colonize the human gut and is non-pathogenic [156]. Further, it has a simple expression system as compared to other higher-level organisms and a large quantity of well-characterized genomic databases. Although certain scientific considerations are to be taken into account while assessing the environmental use of this recombinant microorganism by selecting appropriate safety measures, this may pose some negative impact on the environment [157]. The bioremediation process is monitored indirectly by measuring the polluted site's redox potential as well as temperature, pH, electron acceptor and donor concentrations, oxygen content, and concentrations of breakdown products (e.g., carbon dioxide), and petroleum-contaminated environments are analyzed by bacterial biosensors [158, 159]. In addition, microbial biosensors are increasingly being used to detect contaminants in systems based on reporter genes.

The specific metals in cellular environments are responsible for the expression of resistance genes, and this specificity of tight regulation is exploited in such biosensors [154]. The promoters and regulatory genes present in resistance operons are being used to construct metal-specific biosensors (promoter-reporter gene fusions) [160]. In addition to chemical analysis, metal-specific biosensors can be used:

1. To regulate pollutant concentration
2. Bioavailable metal concentration in the samples [110]

Thus, currently existing risk assessment and safety methods are being used to characterize the consequences of human exposure to such *E. coli* strains. Further, key difficulty lies in the assessment of interactions of the microorganism with the existing ecosystem. For example, an introduced *E. coli* strain may pass genetic material to other microbes, altering the environment and resulting in secondary impacts. Thus, two important areas of investigation related to establishment and proliferation are as follows:

(i) Fate of the recombinant *E. coli* strain and environmental transfer.
(ii) Interaction with the ecosystem.

This knowledge of recombinant *E. coli* transport and its fate (or survival) is useful for assessing potential exposures of nontarget organisms or nontarget areas and rendering it safe for remediation of pollutants [161, 162].

Policy regarding regulation of genetically modified microorganisms

The recent advancements in genetic manipulation offer vast potential and are being utilized in various innovative experiments and applications. These progressions have raised apprehensions among researchers in the biological sciences and other related fields regarding the safe conduct of research in this domain. Genetically modified organisms (GMOs) and their products are regulated in India under the "Rules for the manufacture, use, import, export & storage of hazardous microorganisms, genetically engineered organisms or cells, 1989" (referred to as Rules, 1989) notified under the Environment (Protection) Act, 1986 [163]. The Ministry of Environment, Forest, and Climate Change, the Department of Biotechnology, and state governments enforce these rules through six competent authorities. Six competent authorities and their composition have been notified under these rules that include the following: rDNA Advisory Committee (RDAC), Institutional Biosafety Committee (IBSC), Review Committee on Genetic Manipulation (RCGM), Genetic Engineering Appraisal Committee (GEAC), State Biotechnology Coordination committee (SBCC), and District Level Committee (DLC). The Recombinant DNA Advisory Committee (RDAC) has been established by the department for this specific reason. A publication outlining the Recombinant DNA Safety Guidelines has been released, based on the latest scientific knowledge, to regulate the use of this technique in research, production, and various applications. In 2014, the Department of Biotechnology (DBT) established a specialized task force focused on "Genome Engineering Technologies and their Applications."

The Coordinated Framework for Regulation of Biotechnology was issued in 1986 by the Office of Science and Technology Policy (OSTP) in United States of America (USA). The framework detailed the allocation of regulatory duties among the many authorities that deal with pesticide, food, and agricultural goods. Therefore, in compliance with the framework, the US Environmental Protection Agency (US EPA) regulates microorganisms and other genetically engineered constructs intended for pesticidal purposes and subject to the Federal Insecticide Fungicide and Rodenticide Act (FIFRA) and the Federal Food Drug and Cosmetic Act (FFDCA); USDA APHIS regulates microbes that are plant pests under the Plant Protection Act (PPA) and the National Environmental Policy Act (NEPA). Additionally, certain genetically modified microbes employed as biofertilizers, bioremediation agents, and to produce other industrial compounds including biofuels under the Toxic Substances Control Act (TSCA) are regulated by the US EPA (EPA 1999) [164].

The European Union (EU) has put in place a number of legal tools to guarantee the safety of goods made with or containing GMMs. A product must undergo a scientific risk assessment before it is allowed to be sold. A guidebook for the risk evaluation of genetically modified organisms (GMOs) in food or feed products has been released by the European Food Safety Authority's (EFSA) GMO Panel (EFSA, 2011) [165]. The evaluation is divided into two sections: the GMOs characterization and any potential impact the modification may have on the product's overall safety.

Conclusion

Genetic engineering methods have provided enough opportunities to remove pollutants and toxins from the environment. Comparing this technology with conventional technologies, it is less expensive and more ecologically friendly. It is important to consider environmental factors that may influence the bioremediation of contaminated sites. Microorganisms have an optimal environment for maximum performance as well as a limit of adaptation to certain environmental conditions. A range of biochemical, microbiological, ecological, and genetic factors influence the rate of bioprocessing and biodegradation of contaminants by genetically engineered bacteria for environmental cleanup. Scientists are continually uncovering new unique genes that can be used to generate new constructs and eventually a new strain that aids in the manufacture of derivative routes for new synthetic compounds, as well as the introduction of biodegradation capabilities in a variety of locations. Even with their great potential and encouraging results in the treatment of pollutants by recombinant host bacteria, recombinant bacteria still face a number of difficulties in the process of treating pollutants. In a complex environment with several substrates and numerous microbial interactions, only a small number of modified bacteria are involved in the treatment and removal of toxins. The greatest way to increase biodegradation variety is to use a plasmid with multiple operons rather than multi-plasmids with favorable qualities as plasmids are not only compatible but also incompatible. Protoplast fusion technique has demonstrated promising outcomes in the breeding of biodegradation-engineered bacteria, in addition to plasmids, which is a good thing. Recombinant bacterial strains were produced using the protoplast fusion process; however, the strains also contained genes that were unnecessary or harmful to breakdown. It is also important to follow the correct regulatory procedures for the safe containment and use of GMOs in bioremediation processes.

The subsequent stages of bioremediation research involve discovering and comparing gene and protein sequences that are efficient at eliminating contaminants, even though genomics, metabolomics, and proteomics in bioremediation help explore potential solutions to particular pollutants. GMOs have the ability to clean up a variety of contaminated soil and waste effluents. Utilizing bioremediation in tandem with other physical and chemical techniques can offer an all-encompassing strategy for eliminating pollutants from the surroundings and has the potential to overcome current challenges. It seems to be a long-term treatment; thus, more study in this field is required.

Acknowledgements
I acknowledge the Department of Microbiology, CSK Himachal Pradesh Agricultural University, Palampur, for providing necessary funding to carry out this work.

Authors' contributions
S.S. and S.P. wrote the main Manuscript text. S.B and R.K. prepared figures. S.B and NK prepared Tables. A.W. edit and conceptualized the whole manuscript. All authors reviewed the manuscript.

Availability of data and materials
No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

Received: 8 March 2024 Accepted: 17 May 2024
Published online: 13 August 2024

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