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Innovative Enzymatic and Microbial Approaches for Wastewater Bioremediation: Advances in Enzyme Engineering, Biotechnology, and Nanotechnology for Sustainable Water Management—A Comprehensive Review

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ABSTRACT

The increasing global population and escalating levels of industrial and domestic effluents underscore the urgent need for innovative and sustainable wastewater-treatment technologies. Conventional wastewater-treatment methods—including physical, chemical, and biological processes—are widely implemented worldwide. However, these approaches often fall short in effectively mitigating the environmental and health impacts of emerging contaminants, particularly those originating from pharmaceutical and industrial sources. In recent years, microbial bioremediation has emerged as a promising and environmentally friendly alternative for the removal of heavy metals and other toxic compounds from wastewater and contaminated soils. This narrative review explores the potential of microbial bioremediation, emphasizing the roles of bacteria, fungi, algae, and yeast in transforming and detoxifying harmful pollutants. Additionally, the integration of nanotechnology with microbial processes offers a novel approach that enhances the efficiency and cost-effectiveness of bioremediation strategies. The synthesis of green nanomaterials using microbial systems holds great promise for developing advanced, eco-friendly treatment solutions. This review aims to highlight the ecological viability and economic feasibility of microbial-assisted nanobioremediation as a forward-looking solution for sustainable wastewater management.

Keywords: Bioremediation, Wastewater treatment, Microbial remediation, Nanotechnology, Heavy metal removal

Introduction

Water availability is crucial for the sustainability of life, and removal of effluents from water is equally significant. Water demand has increased as a consequence of industrialization due to its use in manufacturing processes. High production yields generate vast volumes of industrial effluents. For the long-term health of the ecosystem and industry, cost-effective and robust strategies for managing these effluents are crucial. Several remediation methods have been proposed to eliminate hazards and enable the recovery and reuse of wastewater pollutants.[1] The growth of communities worldwide has exposed our ecosystem to a large number of toxic effluents from multiple sources, while human activities and associated challenges are accelerating rapidly.[2,3] As a biologically driven process, bioremediation offers an outstanding tool to address the effects of effluents and eliminate environmental contamination by harmful substances (Figure 1).[4]

Wastewater treatment has become a reliable process for addressing water scarcity and protecting the environment from the toxic impacts of polluted water.[5] Many regions now enforce strict regulations requiring effluent treatment prior to discharge into water bodies. Conventional physical and chemical processes (e.g., precipitation and adsorption) are effective but costly, require careful conditioning, and depend on complex infrastructure. Consequently, there is a pressing need to adopt green and innovative technologies—particularly microbial wastewater treatment methods—as sustainable alternatives to conventional approaches.[6] Microbial treatment of wastewater using bacteria, microalgae, and fungi has recently attracted significant scientific attention. Nutrients such as nitrogen, carbon, and phosphorus—present in municipal effluents—support the growth of these organisms.[7] Phytoremediation is often used to polish treated effluent; however, high nutrient levels can produce plant toxicity. To enhance plant growth under municipal wastewater irrigation, Sarawaneeyaruk et al.[8] isolated plant growth-promoting bacteria (*Bacillus* spp.) from wastewater. Such eco-friendly technologies are sustainable and help maintain balance between human use and the environment. One promising strategy is the application of nanotechnology to remove harmful substances from contaminated water. This emerging field augments existing methods for heavy metal removal (Figure 2).[9]

Origins of Water Pollution

Numerous organic pollutants are detected in water bodies, including herbicides, pesticides and haloalkanes.[10] Toxic metals from acid mine drainage and inorganic pollutants such as sediments from stormwater runoff are examples of industrial waste. Various household effluents enter water sources. Herbicides used in gardens can also enter aquatic systems.[11] Cleaning products, soaps, and personal care items contain numerous contaminants that can pollute water systems making them unsuitable for use.[12] Chemicals, particularly acidic compounds from paper and steel manufacturing plants, are released into freshwater bodies.[13] Freshwater systems receive over 70% of industrial discharges, including many toxic compounds.[9] Primary agricultural discharges include fertilizers, pesticides, and other agrochemicals. Fertilizer production increases annually to enhance yield, resulting in high waste generation. Irrigation contributes

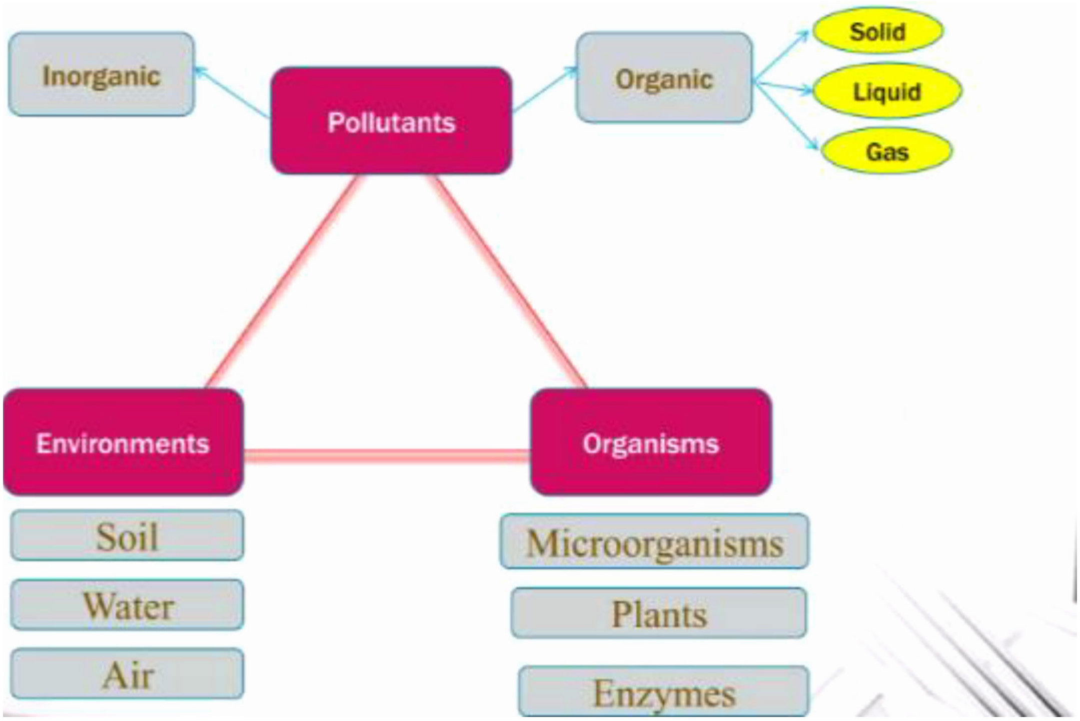

Fig 1 | Bioremediation as a triple corner process

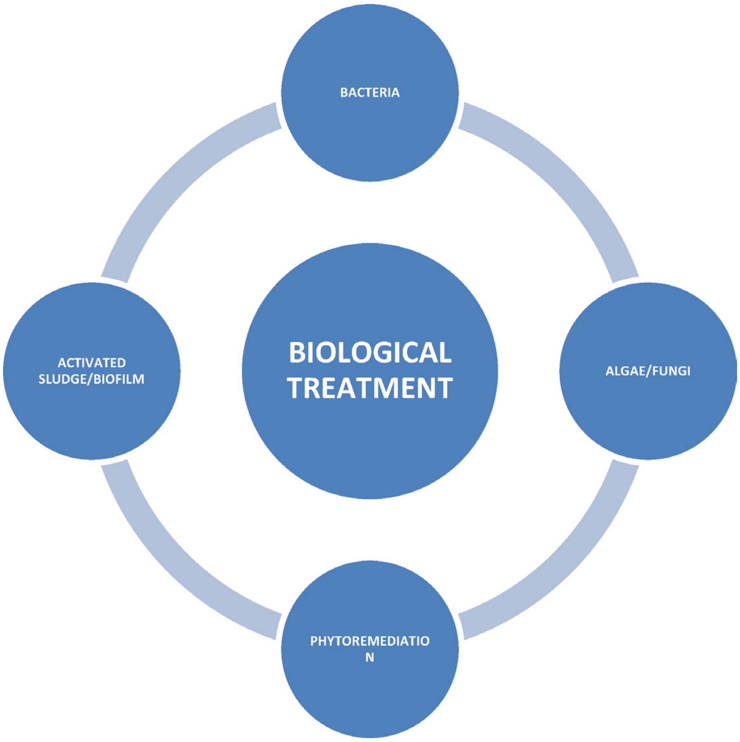

Fig 2 | Biological treatment processes

significantly to surface water contamination in China and to nitrogen contamination of groundwater in the United States.[10] Toxic compounds can accumulate in humans and ultimately reach dangerous thresholds, disrupting the food chain. Another significant source of water contamination is runoff (Table 1).

Need for Wastewater Treatment

Sewage wastewater containing toxic metals has been released into the environment on a large scale in recent decades.[14] Hazardous pollutants such as zinc (Zn), copper (Cu), sulfur (S), cadmium (Cd), chromium (Cr), nickel (Ni), and lead (Pb) are present in drinking water. The carcinogenic properties of these metals pose major health concerns as they bioaccumulate in humans via the food chain.[15] Consequently, treatment of heavy metals in polluted wastewater is essential. Rapid urban sprawl and industrial expansion have led to the discharge of large volumes of wastewater, which is often reused for agricultural irrigation. This practice provides cost-effective water resources, particularly for resource-limited farmers, but it alters water quality and poses ecological risks.[16] Water contamination is increasing daily due to population growth and industrial activities, exacerbating sanitation challenges and increasing health risks in both developing and developed regions. Consequently, water demand is projected to rise significantly, while available water bodies diminish due to depletion of nonrenewable sources, pollution, and climate change. This leads to longer droughts, increased evaporation, reduced precipitation, and altered hydrological cycles.[17,18]

Biological Methods for Industrial Wastewater Treatment

Biological methods employ microorganisms or biological processes to degrade pollutants in wastewater. As a green technology tool, microbes are essential to wastewater treatment and environmental protection. Various microbial groups—including bacteria, fungi, yeast, and microalgae—are utilized in biological treatments. Compared to physical and chemical methods,

Table 1 | Types of aquatic pollutants and their possible effects

Pollutant	Origin	Effects on Environment
Sediments	Excavation and soil erosion	Suspended sediments reduce light penetration and can suffocate aquatic organisms
Organic compounds	Organic matter	Oxygen depletion leads to aquatic life mortality
Heavy metals / pesticides	Sewage, agricultural, industrial discharge	Severe health effects including carcinogenicity
Nutrients	Overland flow	Nutrient enrichment causes algal blooms
Oil spillage	Oil spills	Form surface films that block oxygen transfer
Bio-pollutants	Raw sewage discharge	Waterborne viral diseases
Protozoa	Human waste discharge	Protozoan infections

Table 2 | Biological wastewater-treatment techniques

Biological Method	Advantages	Characteristics
Microbial consortia	Use of multiple species; effectively removes biodegradable organic matter and toxic metals; reduces color	Mixed cultures of bacteria, fungi, algae
Bioreactors	Simple to operate; scalable	Use of pure or mixed microbial cultures
Enzymatic degradation	Reduces biochemical oxygen demand (BOD) and suspended solids; high specificity	Free or immobilized enzymes
Activated sludge	Well established; cost-effective; widely implemented	Aerobic microbial flocs; sedimentation of biomass

biological treatments are more cost-effective. Table 2[19] summarizes the most commonly employed biological wastewater treatment techniques.

Methodological Approaches in Microbial and Nanotechnology-Assisted Treatment of Wastewater

The remediation of industrial textile wastewater (ITW) has been approached through diverse biological, physicochemical, and hybrid techniques. Recent advances focus on microbial bioremediation, enzymatic degradation, and nanotechnology-assisted systems. This section synthesizes the methodologies commonly reported in the literature, highlighting their mechanisms, operational parameters, and comparative efficiencies.

Microbial Cultures in Bioremediation

Bacterial Strains

Numerous studies utilize bacterial species such as *Pseudomonas*, *Bacillus*, *Acinetobacter*, and *Sphingomonas* for their notable dye-degrading and heavy-metal-accumulating abilities. These strains are typically cultivated in nutrient-rich media such as Luria-Bertani broth under aerobic or facultative anaerobic conditions. Their capacity for decolorization and detoxification is enhanced by optimizing incubation parameters such as pH (6.0–8.0), temperature (30–37°C), and agitation.

Fungal Cultures

Filamentous fungi—including *Aspergillus*, *Trametes*, and *Penicillium* spp.—are frequently used for their production of ligninolytic enzymes. Solid-state and submerged fermentations are common cultivation methods. Their extracellular enzymatic systems facilitate the breakdown of complex dye structures and recalcitrant organics.

Algal Systems

Microalgae such as *Chlorella vulgaris* and *Spirulina platensis* offer dual benefits of nutrient uptake and biomass generation. Cultivation typically occurs in Bold's Basal Medium under controlled light-dark cycles and CO_2 supplementation. Algal systems are often integrated into photobioreactors for enhanced treatment efficiency and biomass recovery.

Bioreactor Configurations

A variety of bioreactors have been designed to leverage microbial consortia for textile wastewater treatment:

- **Batch Reactors and Sequencing Batch Reactors** allow controlled degradation cycles, suitable for variable effluent loads.
- **Continuous-flow systems**, including activated sludge processes, offer operational stability but require constant monitoring of hydraulic retention time and organic loading rates.
- **Fixed- and moving-bed biofilm reactors** provide increased surface area for microbial attachment, enhancing treatment resilience and degradation efficiency.

Studies often report significant reductions in BOD, chemical oxygen demand (COD), and color in systems optimized for microbial synergy and biofilm stability.

Enzymatic Treatment Approaches

Enzyme-mediated degradation is a key component of fungal and bacterial wastewater-treatment strategies. Enzymes such as laccase, manganese peroxidase (MnP), lignin peroxidase (LiP), and azoreductase have been widely investigated.

- **Production:** Enzymes are typically induced under nutrient-limited conditions in submerged fermentation.
- **Activity assays:** Substrates like 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) and guaiacol are used to quantify enzyme activity spectrophotometrically.

- **Applications:** Enzyme immobilization on supports such as alginate beads or nanomaterials has been explored to enhance stability and reusability in continuous operations.

Biofilm-Based Systems

Biofilms offer structural and metabolic stability for long-term bioremediation. Supports such as ceramic rings, polyurethane foam, and activated carbon are commonly used for biofilm formation.

- **Advantages**: Biofilms confer resistance to toxic shocks, enable multi-species integration, and improve pollutant contact time.
- **Characterization**: Confocal laser scanning microscopy and scanning electron microscopy are employed to assess biofilm development and morphology.

These systems are especially valuable in treating dye-laden effluents, where shock loading and high toxicity challenge conventional treatments.

Phytoremediation in Constructed Wetlands

Constructed wetlands using macrophytes like *Typha latifolia*, *Phragmites australis*, and *Eichhornia crassipes* have shown potential in polishing pretreated wastewater.

- **Mechanism**: Plant roots provide surfaces for microbial colonization and facilitate uptake of nutrients and certain heavy metals.
- **Effectiveness**: Literature indicates moderate reductions in COD, ammonia, and specific metals over treatment cycles of 48–96 hours.

Integration of phytoremediation with microbial treatment enhances overall system sustainability and biodiversity.

Heavy Metal Bioremediation

Biological mechanisms such as biosorption, bioaccumulation, and enzymatic reduction have been employed for heavy metal removal.

- **Microbial resistance**: Tolerant strains are selected through minimum inhibitory concentration testing.
- **Quantification**: Metal uptake is typically assessed using atomic absorption spectroscopy or inductively coupled plasma mass spectrometry.
- **Biosorbents**: Algal biomass, bacterial extracellular polymeric substances, and fungal mycelium serve as effective biosorbents.

Nanotechnology-Enhanced Treatment Techniques

Photocatalysis

Metal oxide nanoparticles such as TiO_2 and ZnO have been widely studied for their photocatalytic degradation of dyes under UV and visible light, mineralizing complex organics into simpler, nontoxic compounds.

Adsorption

Nanostructured materials including carbon nanotubes, graphene oxide, and metal-organic frameworks offer high surface area and functional group versatility for the adsorption of dyes and heavy metals.

Nanomembranes

Nanocomposite membranes with embedded antimicrobial or catalytic nanoparticles are increasingly applied for ultrafiltration, nanofiltration, and forward osmosis processes. Their performance is evaluated in terms of flux rate, fouling resistance, and pollutant rejection.

Antimicrobial Action

Certain nanomaterials exhibit intrinsic antimicrobial properties, disrupting cell membranes or generating reactive oxygen species. These are useful in reducing microbial contamination in treated effluents.

Analytical and Statistical Techniques

Physicochemical Monitoring

Parameters such as pH, conductivity, turbidity, BOD, COD, total dissolved solids, and heavy metal concentrations are standard metrics for evaluating treatment efficiency, following American psychological association (APHA) and International organization for standardization (ISO) protocols.

Advanced Instrumentation

Techniques such as gas chromatography-mass spectrometry, high-performance liquid chromatography, and UV-Vis spectrophotometry are employed to identify and quantify pollutants and degradation products.

Culture of Microorganisms (Fungi, Bacteria, and Microalgae)

Bacteria and other microorganisms are highly adapted for biological processes, easy to cultivate, and proliferate more quickly than other microbes, making them promising for removing organic contaminants. Numerous investigations have examined the ability of microorganisms to decolorize azo dyes in anaerobic environments. However, due to the action of redox mediators, the azoreductase enzyme cleaves azo bonds.[20] Conversely, several bacterial species degrade wastewater contaminants under aerobic conditions.[21] The use of microbial consortia rather than pure cultures offers several advantages. Different strains can target various dye components, and metabolic byproducts from one strain's activity may serve as substrates for others.[22]

The primary goal of research on treating ITW using bacterial cultures is the decolorization of wastewater contaminants. One study examined how aerobic, thermophilic bacteria remove organics from wool effluent.[23]

Fungi can eliminate dyes through two processes: biodegradation and biosorption.[24] Various ligninolytic fungi produce high levels of oxidative, extracellular enzymes—such as laccase, manganese peroxidase, and lignin peroxidase—that facilitate the solubilization of recalcitrant substrates (Figure 3).[25]

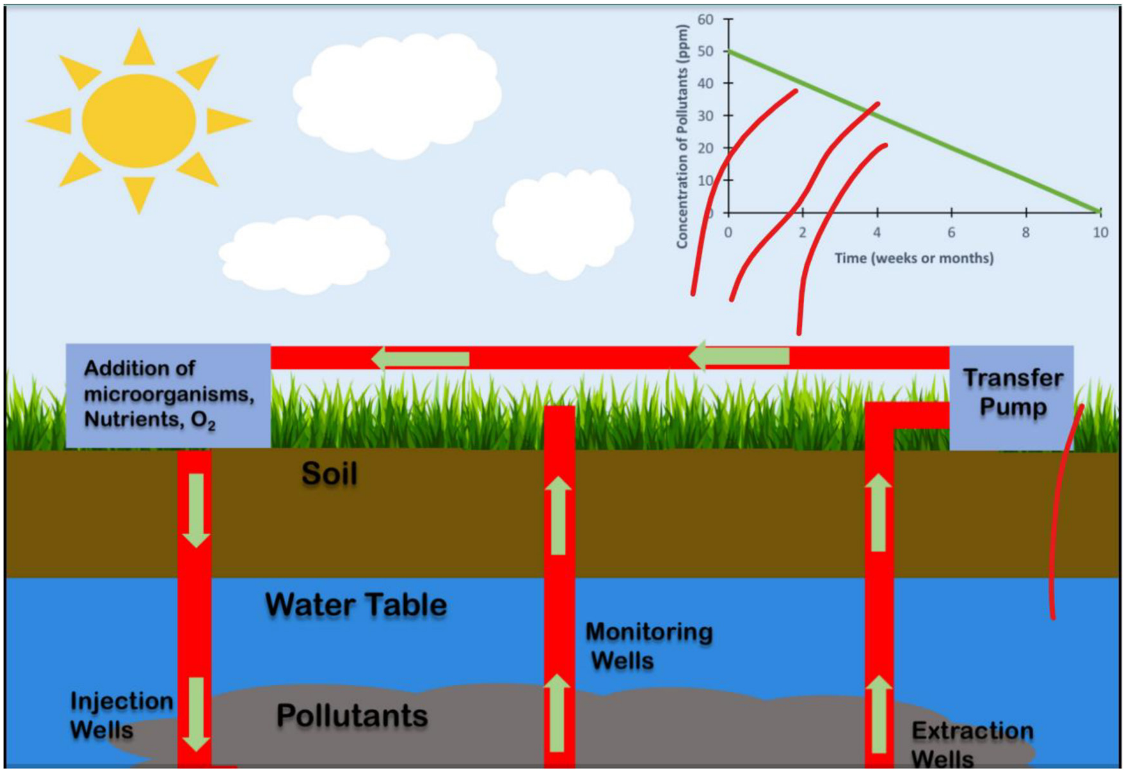

Fig 3 | In-situ microbial bioremediation

Removal of color is the main focus of most studies on fungal remediation of ITW. Effectiveness varies widely depending on enzyme specificity and fungal morphology, making immobilization of fungal biomass advantageous.[26]

Two applications for microalgae in ITW treatment are "low-cost wastewater treatment" and "bioenergy feedstock supply".[27] Dye and nutrient removal can be achieved via bioadsorption and bioconversion using live or dead biomass.[28] Microalgae can grow mixotrophically, consuming organic compounds in addition to photosynthesis. The first paper on microalgal treatment of ITW was published in 2010.

Activated Sludge

The complex microbial community known as activated sludge comprises bacteria and protozoa. These microbes form flocs, which are removed from treated wastewater by sedimentation. Activated sludge microbes can adapt to anaerobic, anoxic, and aerobic conditions.[29] Various dye classes—including disperse, vat, basic, and direct dyes—can be removed using centrifugation or adsorption processes. Anaerobic reactions often drive color removal, while aerobic processes degrade organic compounds efficiently but typically achieve lower decolorization. Most studies have focused on removing organic matter, with less attention to nitrogen transformations.[30]

Anaerobic cleavage of azo bonds often requires electron donors that may be lacking in textile effluents. Many studies include co-substrates to enhance azo dye degradation. Glucose is the preferred carbon source, although alternatives include sago wastewater,[31] peptone,[32] dextrin,[32] and tapioca.[33]

To achieve complete biological degradation of azo dyes, sequential anaerobic-aerobic and treatment configurations—such as an upflow anaerobic sludge blanket followed by an aerobic continuously stirred tank reactor—have been investigated.[34] In 2012, Li et al. published the first study detailing hydrogen production from industrial desizing wastewater using bioreactors. An experimental bioreactor was employed to investigate this process.[35] Figure 4 shows activated sludge for ITW treatment.

The Biofilm

A biofilm consists of cells adhering to a surface, embedded in extracellular polymeric substances (EPS), along with inorganic and organic materials.[36] Biofilms can develop on artificial or natural supports, which may be fixed (e.g., fixed-bed bioreactors) or fluidizing (e.g., moving-bed bioreactors). Depending on the support medium and operational conditions, biofilms develop layers of varying thickness.[37] Dense biofilms exhibit gradients of substrates, products, and oxygen, creating distinct zones for processes such as nitrification, denitrification, and dye decolorization.[38] Complex, multi species microbial communities are ideal for biofilm based treatment. Biofilms yield higher concentrations of active biomass due to microbial attachment to support media. Immobilized cells within biofilms are more resistant to environmental fluctuations in temperature, pH, and toxic substances.[36] This leads to shorter treatment times compared to activated sludge

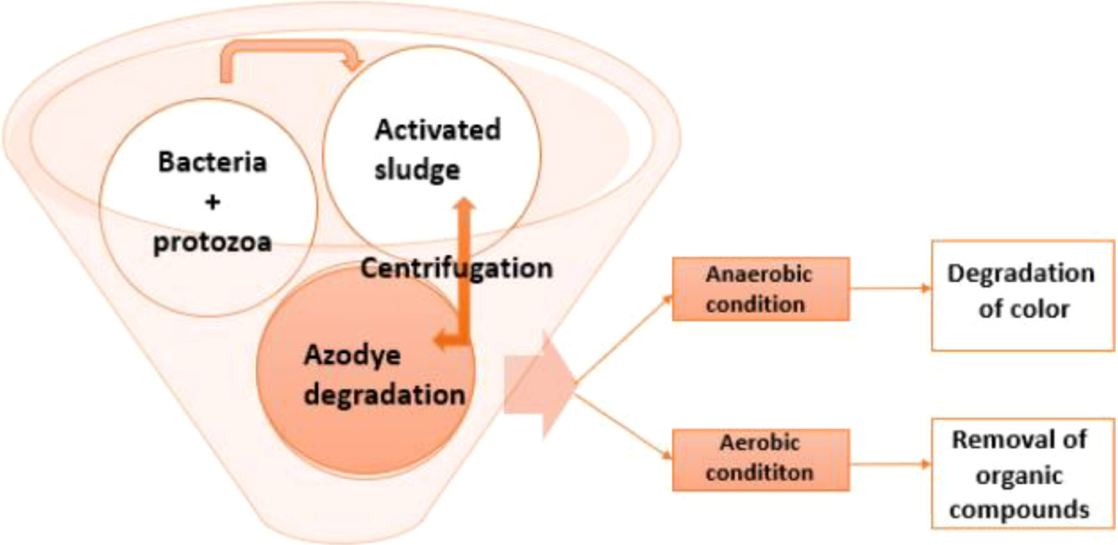

Fig 4 | Activated sludge for the treatment of textile wastewater (adopted from Elsevier; Science of the total environment)

and improved pollutant removal efficiency. Anaerobic biofilm reactors demonstrate superior decolorization performance compared to activated sludge systems and often serve as a pretreatment step.

Co-substrate supplementation with glucose[39] or acetic acid[40,41] can further enhance dye degradation.

In many regions, the primary methods for removing textile dyes from wastewater include adhesion or precipitation, sedimentation, and subsequent enzyme-enhanced degradation.[42] Additionally, plants create favorable conditions through extensive root systems that facilitate aeration and provide habitats for both aerobic and anaerobic microorganisms.

Phytoremediation, which harnesses solar energy, is a cost-effective and sustainable approach that is relatively easy to implement.[43] The most common form of phytoremediation system is the constructed wetland. Numerous studies have utilized constructed wetlands as a form of tertiary therapy or treatment.[44,45] However, the majority of the research employed unmodified ITWs.[46] Reported COD removal rates range from 40% to 91%, indicating a high level of efficacy.

The primary limitation of these systems is their lengthy hydraulic retention time, which must be at least 72 hours. Combined with the typically shallow design of wetlands, this requirement necessitates large-scale wastewater-treatment infrastructure.

Microbial Bioremediation's Mode of Action

In natural environments, microbes are ubiquitous and thrive in heavy-metal-contaminated sites. Consequently, these organisms convert toxic heavy metals into less harmful forms through processes such as biotransformation, biosorption, biomineralization, bioleaching, bioaccumulation, and microbe-metal interactions. Heavy metals in soil can be sequestered by microorganisms that require minimal external nutrients.[47] Microbes also catalyze oxidation-reduction reactions of transition metals, in addition to solubilizing metal complexes. Organic solvents can damage cell membranes, but cells deploy defense mechanisms—such as efflux pumps and hydrophobic barriers—to protect their outer membranes.[48]

Many bacteria possess energy-dependent or plasmid-encoded metal efflux systems. ATPases and chemiosmotic proton/ion pumps have been implicated in chromium (Cr) and arsenic (As) resistance across various species.[49] Several heavy metal sources are depicted in Figure 5.[49]

Microbes' Roles in Heavy Metal Bioremediation

Without chemical additives, many microorganisms can remediate heavy metals, albeit on a limited scale. The resilience of these microbes to heavy-metal-induced stress depends on their intrinsic resistance mechanisms and the surrounding environmental conditions. Genetic modification of these organisms offers a promising solution by redirecting and enhancing their metabolic pathways through genetic engineering.

Controlling heavy metal bioavailability also mitigates their toxic effects. Through redox transformations, microbes convert metals from inorganic to organic species (Figure 6).[50] Genetic engineering can further endow microorganisms with desirable traits—tolerance to extreme conditions, cost-effective cultivation, accelerated growth rates, and stability across pH fluctuations (Figure 6).[9] According to Wasilkowski et al.,[51] such engineered strains improve water treatment by promoting cellular maceration processes, enhancing heavy metal accumulation, and reducing metal-associated toxicity.[51]

Environmental Bacterial Bioremediation

Microbial-bioremediation strategies utilize native microbial species to remove contaminants in situ. The type and concentration of pollutants , the composition of the indigenous microbial community, and environmental parameters all influence the extent of detoxification.[52] Advantages of environmental bacterial

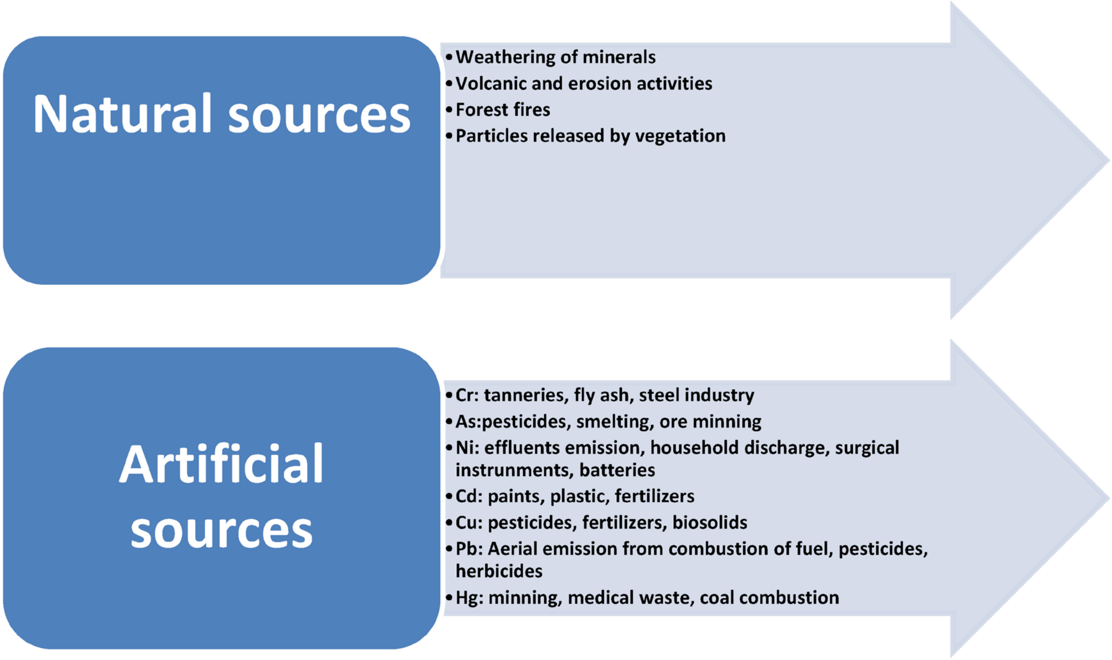

Fig 5 | Sources of heavy metals

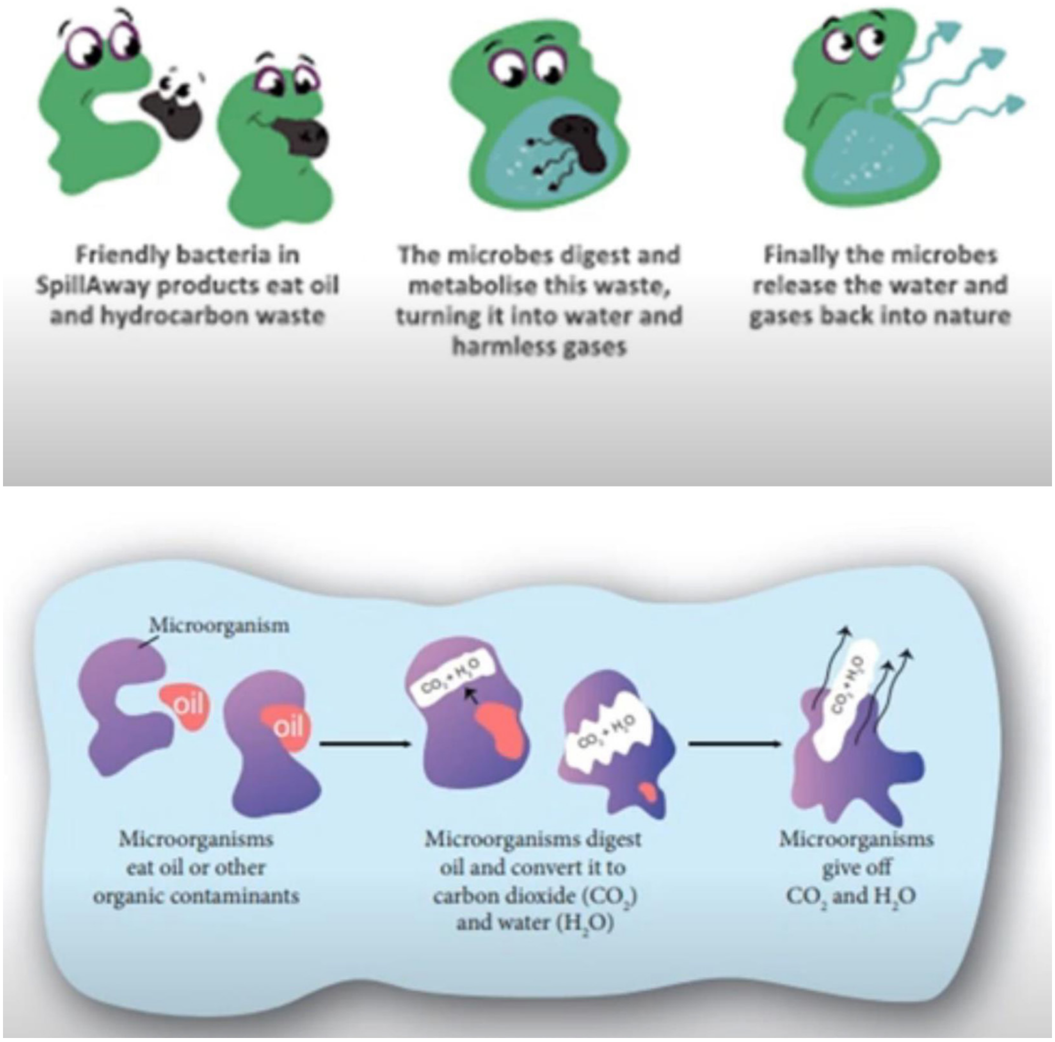

Fig 6 | Microbial functions in bioremediation

bioremediation include: 1) no adverse byproducts; the process is fully natural; 2) low input and maintenance; 3) minimal energy requirements compared to incineration or landfilling; 4) rapid conversion of contaminants into harmless end products; and 5) reduced liability due to lower risk of residual contamination. Most applications are finished in situ, eliminating the need for hazardous transport of contaminated materials.

Microorganisms-Assisted Nanotechnology for Wastewater Treatment

Nanomaterials are ideal for wastewater treatment due to their nanoscale dimensions. Their unique biological, physical, and chemical properties enhance performance across applications. Carbon-based nanomaterials (e.g., carbon nanotubes and graphene oxide) and metal-based nanomaterials (e.g., metal oxides and metal organic frameworks) are widely employed for contaminant removal. Treatment processes include adsorption, photocatalytic degradation, nanoparticle-enhanced filtration, and contaminant sensing (Table 3).[53]

Combining microorganisms with biofabricated nanoparticles enhances the practicality and environmental benefits of nanotechnology. Chemically synthesized nanomaterials in aqueous media often suffer from self-agglomeration and unwanted byproducts. As an eco-friendly alternative, researchers have turned to natural extracts—such as plant phytochemicals and bacterial or fungal enzymes—to reduce complex metal salts and form metal nanoparticles (Figure 7).

Table 3 | Different nanotechnology assisted water treatment techniques

Techniques	Target Pollutants	Pros	Cons
Photocatalysis	Organic pollutants (e.g., glycerol, dyes, pharmaceuticals, endocrine disruptors, and pesticides)	Complete pollutant mineralization; no secondary pollution; effective on nonbiodegradables	Slow reaction rates; difficult NP recovery; limited visible-light utilization
Adsorption	Heavy metals; other inorganic/ organic contaminants	High efficiency; low cost, reversible; ease operation	Poor selectivity; adsorbent saturation; regeneration challenges
Nanomembranes	Organic/inorganic contaminants; particulates; microbial pathogens	Customizable; low chemical use; high efficacy; minimal solid waste	Membrane fouling; low flux; high operating cost
Antimicrobial action	Microbial pathogens	Broad-spectrum activity; cost-effective vs. antibiotics	Nanotoxicity concerns; challenges in NP recovery post-treatment

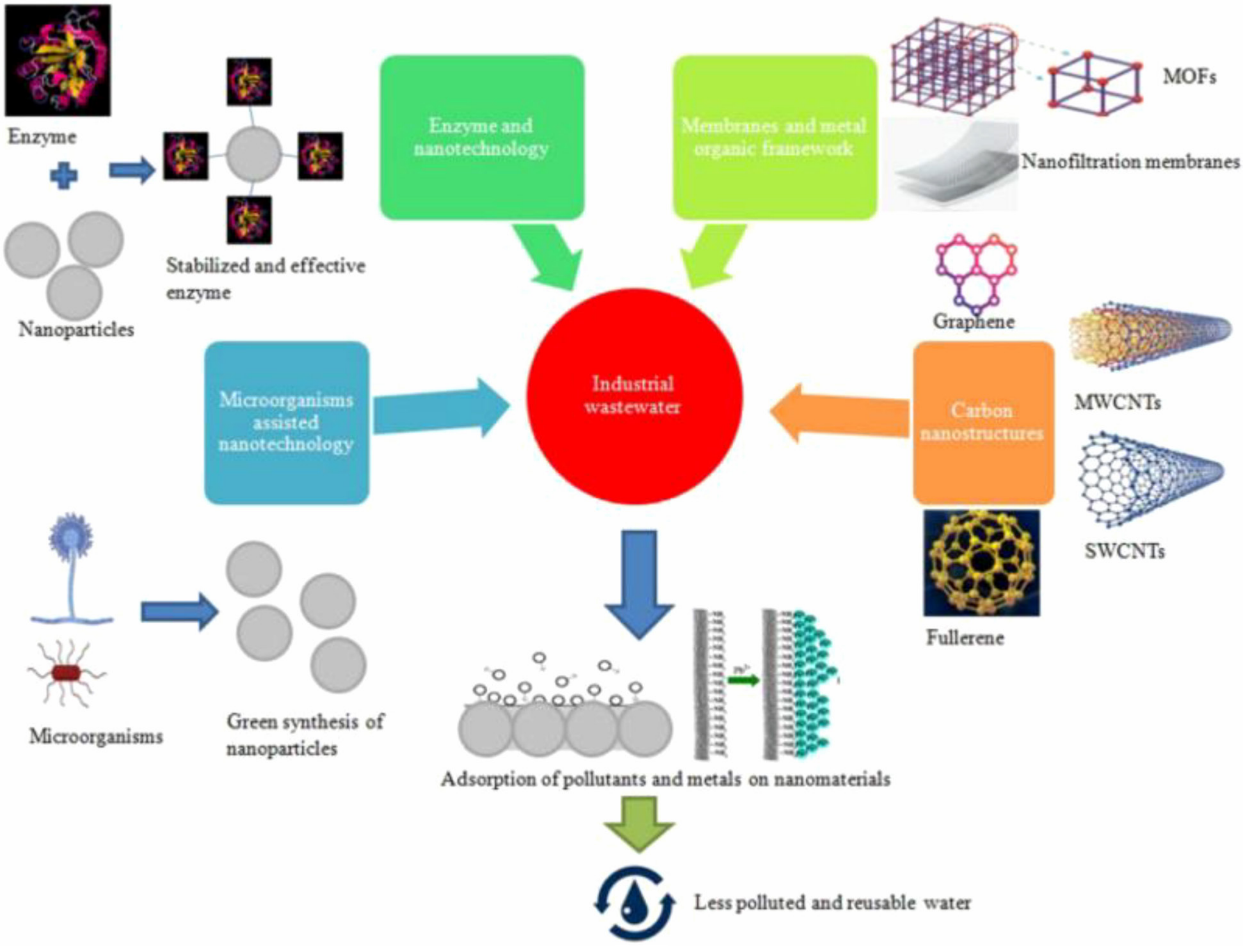

Fig 7 | Microbial assisted nanotechnologies for wastewater bioremediation; adopted from Elsevier, Source of the total environment

In aqueous environments, these particles stabilize via co-precipitation or by capping with proteins and other bioactive compounds.[54] For example, *Aspergillus tubingensis* (STSP 25), isolated from the rhizosphere of *Avicennia officinalis* in the Indian Sundarbans, has been used to biofabricate iron-oxide nanoparticles. These biosynthesized nanoparticles removed over 90% of Zn(II), Ni(II), Cu(II), and Pb(II) from wastewater and retained their efficacy through approximately five regeneration cycles.

Challenges and Drawbacks of Bioremediation Processes

However, scaling up these systems requires judicious cost–benefit analysis and the careful selection and screening of microbial strains. Notably, many bioremediation techniques validated for industrial wastewater remain unevaluated in pharmaceutical wastewater contexts. Azubuike et al. have reported that microbes introduced into contaminated streams sometimes exert minimal impact due to nutrient limitations (e.g., nitrogen or phosphorus).[55] Furthermore, bioremediation is inherently selective—most effective against readily biodegradable pollutants—making it less suitable for a broad spectrum of recalcitrant xenobiotics. To address these limitations, hybrid strategies that couple biological processes with physical or chemical treatments (e.g., simultaneous bioremediation in sequential reactors) are increasingly employed to remove persistent residues.[56] Biostimulation—supplying limiting nutrients to microbial consortia—must be carefully dosed, since excessive nutrients can inadvertently inhibit microbial activity.[57] The emerging integration of nanomaterials into these processes has shown promise by lowering activation-energy barriers and increasing reactive surface area, thereby enhancing microbial performance.[58]

Perspective

Biological processes, microbial remediation, and microbiology-derived nanotechnologies represent key strategies for treating wastewater from residential and industrial sources. To optimize treatment performance, a deeper understanding of microbial community structure and dynamics is essential. Recent advances have expanded remediation capabilities, offering enhancements to conventional methods across environmental engineering and related disciplines. Persistent heavy metal contamination—due to the stability of their ionic forms—underscores the need to improve existing technologies or develop novel solutions.

Despite the promise of bioremediation and nanotechnology, challenges remain in microbial performance, process scalability, and nanoparticle safety. Future research should focus on optimizing microbial consortia, integrating real-time monitoring systems, and developing safer, cost-effective remediation technologies.

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