



Microbial Nano-Bioremediation for Wastewater Treatment: Advances in Heavy-Metal and Pharmaceutical Effluent Removal

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Abstract

The introduction and continued enforcement of rigorous waste-discharge regulations have heightened the need for innovative waste-treatment methods. Wastewater treatment is becoming increasingly important as the global human population grows rapidly. Hence, wastewater treatment technologies are designed to maintain high densities and activity of microorganisms that meet various purification requirements. If the pharmaceutical industry's waste is not adequately managed, it can harm the environment and public health. One advanced and promising technique for removing and reducing heavy metals from contaminated soil and water is bioremediation. Microorganism-based bioremediation has significant potential for future development due to its affordability and environmental friendliness. Numerous microorganisms, such as bacteria, yeasts, fungi, and algae, can operate as physiologically active methylators and alter harmful species. Microorganisms are essential to the bioremediation of heavy metals. By creating environmentally friendly nanomaterials to reduce these pollutants, nanotechnology may reduce industry costs. As a growing field, green biotechnology benefits from the use of microorganisms for nanoparticle synthesis, thereby lowering costs and promoting sustainable production. Nanotechnology can enhance traditional technologies across various fields, including medicine, wastewater treatment, and pollution management.

1. Introduction

Water is indispensable for sustaining life on Earth, and maintaining its purity is equally vital. With rapid industrialization, the demand for water in production processes has increased considerably, leading to the generation of large quantities of industrial effluents. These wastes have become a primary environmental concern, making cost-effective and sustainable remediation methods essential for long-term ecological and industrial stability.

Various technologies have been developed to minimize the toxicity of pollutants and promote their safe management and reuse (Mandeep et al., 2019). The continuous rise in global population has intensified human activities, which, in turn, have introduced harmful substances into the environment from multiple sources. These pollutants have severely affected ecosystems, highlighting the urgent need for sustainable solutions (Ali et al., 2021; Zhao et al., 2017). Among several remediation techniques, bioremediation has emerged as a practical, eco-friendly approach to mitigate

pollution. It involves using biological organisms, such as bacteria, fungi, algae, and plants, to degrade, transform, or detoxify hazardous organic and inorganic compounds through natural metabolic processes (Zhao et al., 2017).

These biological treatments operate through oxidation or reduction reactions that occur under suitable nutrient, pH, and oxygen-availability conditions (Mahanty et al., 2020). In many cases, bioremediation is carried out in bioreactors equipped with efficient aeration and mixing systems to optimize microbial activity. However, it can also occur in natural settings such as constructed wetlands. Owing to their reliance on natural biochemical mechanisms, biodegradation methods are generally considered both economical and environmentally sustainable (Kumar et al., 2018). In the pharmaceutical industry, wastewater treatment requires specialized attention because the effluents often contain complex and hazardous substances. Before treatment, it is crucial to identify and characterize these components to ensure compliance with safety and quality standards. Pharmaceutical wastewater typically includes teratogenic, mutagenic, and carcinogenic compounds that are highly persistent and toxic. Compared to inorganic pollutants, these organic substances pose greater environmental and health risks. Therefore, wastewater treatment plants play a vital role in converting these harmful pollutants into stable or neutral forms, thereby reducing their environmental impact (Yang et al., 2019).

Recently, nanotechnology has gained prominence as an innovative approach for removing heavy metals and other toxic compounds from contaminated water. The integration of nanomaterials with existing treatment technologies has enhanced the efficiency and selectivity of pollutant removal, making it a valuable tool in achieving sustainable wastewater management.

2. Environmental need for wastewater remediation

In recent years, large volumes of wastewater containing heavy metals have been released into the environment, posing severe ecological and public health threats (Lee et al., 2012). Among the most hazardous pollutants found in aquatic systems are cadmium (Cd), copper (Cu), nickel (Ni), chromium (Cr), and lead (Pb). These metals are of great concern due to their teratogenic and carcinogenic properties, as well as their ability to bioaccumulate within the food chain, resulting in toxic effects in humans and other organisms (Ali et al., 2021). Therefore, effective removal of heavy metals

from contaminated water and soil has become an essential environmental priority. The rapid pace of industrialization and urbanization has dramatically increased wastewater production. In many regions, this wastewater is now being reused for agricultural irrigation, which, while providing economic benefits and supporting the livelihoods of low-income farming communities, also alters the chemical and physical properties of natural water bodies. Such practices contribute to the spread of contaminants into the environment, increasing ecological and health-related risks.

Simultaneously, the ongoing expansion of cities and industries has exacerbated water pollution and sanitation challenges, particularly in developing and underdeveloped countries. The consumption of unsafe, contaminated water continues to endanger human health. Moreover, as industrial and urban demands for water rise, renewable water sources are diminishing due to groundwater depletion, pollution, and inefficient water management. These problems are further intensified by climate change, which contributes to increased droughts, higher evaporation rates, and reduced precipitation, collectively disrupting the natural hydrological cycle (Chen et al., 2014). Consequently, addressing heavy metal contamination and ensuring sustainable water resource management are critical steps toward mitigating the combined impacts of pollution, industrial growth, and climate variability.

3. Microbial interventions in wastewater management

3.1. Single-Strain microbial systems in bioremediation

Microorganisms, particularly bacteria, possess strong potential for treating organic waste due to their rapid growth, adaptability, and metabolic versatility. Studies have shown their ability to decolorize azo dyes under both anaerobic and aerobic conditions, where enzymes such as azoreductase play a vital role in breaking azo bonds (Delli et al., 2013). Using microbial consortia instead of pure cultures offers advantages, as different strains can target various compounds and utilize degradation byproducts produced by others, enhancing efficiency. Research on industrial textile wastewater (ITW) has demonstrated effective removal of organics using aerobic and thermophilic bacteria, with *Bacillus subtilis* WB600 employing CotA-laccase showing high dye decolorization (Cervantes-Paz et al., 2017). Mixed bacterial-fungal cultures have also achieved higher COD reductions and color removal. Fungal species, especially ligninolytic fungi, remove dyes through biodegradation and

biosorption by secreting oxidative enzymes such as manganese peroxidase, laccase, and lignin peroxidase. These enzymes are non-specific and capable of breaking down diverse xenobiotics (Ramadhan et al., 2021). Due to their mycelial structure, fungi exhibit stronger interactions with the environment, and immobilized fungal systems further improve stability and color removal efficiency.

Microalgae also contribute to ITW treatment, serving both as a low-cost wastewater treatment option and a bioenergy feedstock. They remove dyes and nutrients through bioadsorption and bioconversion, functioning under both autotrophic and heterotrophic conditions. Recent studies have introduced microalgal biocathodes in microbial fuel cells for simultaneous dye degradation and power generation, highlighting algae's potential for sustainable wastewater remediation, especially in sun-rich regions (Egede et al., 2016).

3.2. Role of activated sludge in bioremediation

Activated sludge consists of a complex microbial community of bacteria and protozoa that form flocs capable of sedimentation during wastewater treatment. These microorganisms operate under anaerobic, anoxic, and aerobic conditions, enabling efficient removal of diverse pollutants. Various dyes, such as basic, direct, vat, and disperse dyes, can

be removed by adsorption or centrifugation (Dai et al., 2014). Typically, azo dye degradation occurs in two stages: an initial anaerobic phase for color removal, followed by an aerobic phase for organic compound degradation. To enhance microbial performance, co-substrates such as glucose, tapioca, and peptone are added to increase carbon availability (Nawaz et al., 2014). Incorporating adsorbents into aerobic activated sludge improves dye sorption and converts conventional sludge into a more robust biofilm system. Combining membrane processes such as microfiltration and ultrafiltration in membrane bioreactors (MBRs) increases biomass retention, biodiversity and effluent clarity (Figure 1).

Sequential systems such as up-flow anaerobic sludge blanket (UASB) reactors followed by aerobic continuously stirred tank reactors (CSTRs) have shown higher efficiency in degrading textile dyes and reducing chemical oxygen demand (COD) (Baele et al., 2012). The integration of fungal enzymes further enhances biodegradation, providing superior resilience to shock loading and improved organic removal. Overall, activated sludge systems remain a key biological treatment method for industrial and textile wastewater due to their adaptability and effectiveness.

3.3. Role of biofilm in bioremediation

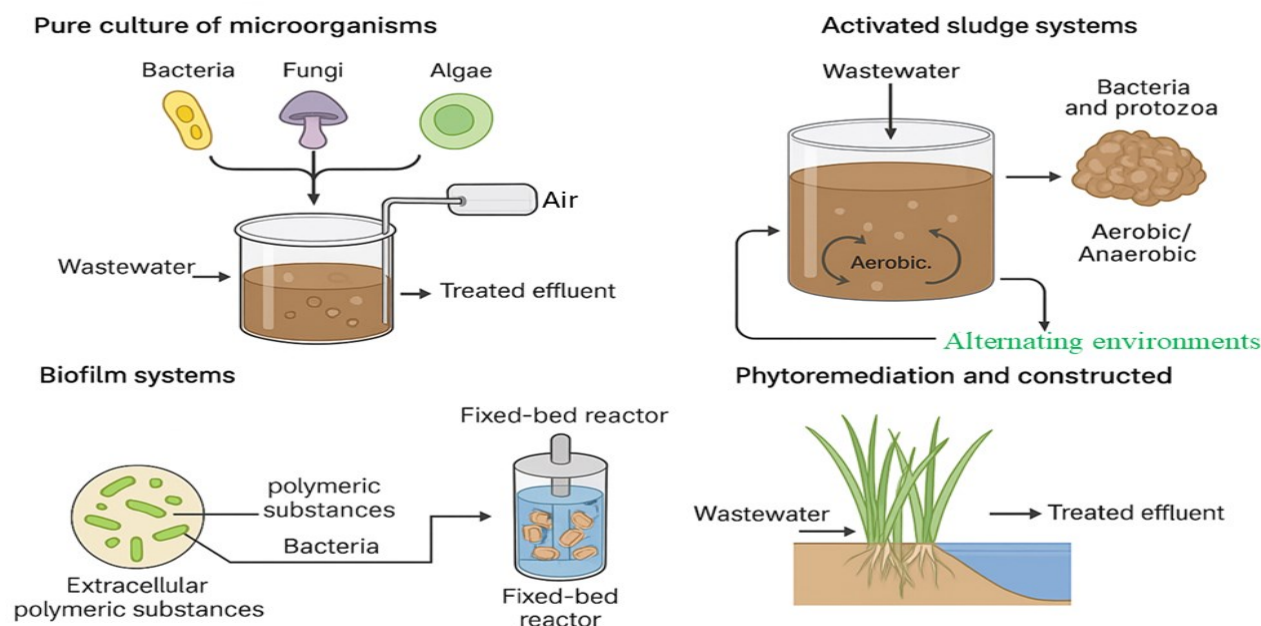


Figure 1. Microbial and plant-based systems clean wastewater effectively

A biofilm consists of microbial cells attached to a solid surface and embedded within a matrix of extracellular polymers, inorganic, and organic materials. Biofilms can develop on natural or artificial supports, which may be fixed (as in fixed-bed bioreactors) or movable (as in fluidized or moving bed systems). Their structural thickness and activity depend on operational conditions and the type of support used. Dense biofilms create gradients of substrates and oxygen, facilitating simultaneous processes such as dye decolorization, nitrification, and denitrification (Gong et al., 2016). Biofilms support diverse microbial communities and yield higher concentrations of active biomass, making them more stable and less sensitive to fluctuations in temperature, pH, or toxic compounds. Compared to activated sludge, they allow faster treatment and greater pollutant degradation. The use of moving support media in biofilm reactors further enhances performance by preventing clogging, eliminating the need for sludge recycling, improving mass transfer, and providing stable conditions for biomass growth (Kornaros et al., 2006).

3.4. Phytoremediation and constructed wetlands

Anaerobic reactors performed decolorization more effectively and served as a prior approach, similar to active sludge systems. Another method was supplementation with either glucose or acetic acid. The oxidation of nitrogen and ammonia, and the elimination of total nitrogen (Vijayalakshmi et al., 2013), were achieved using fixed-bed reactors with aeration. Anoxic conditions in dense biofilm layers allowed for denitrification. Examples of aerobic reactors with moving biofilm include MBBR ("Moving Bed Biofilm Reactors"), AFIBR ("Aerobic Fluidized Bed Bioreactors"), and SBBR ("Sequence Biofilm Bioreactors"). It combines the advantages of a high-capacity granular SBR, ensuring there are not too many suspended solids. Biomass in the SBBGR is contained within a single zone rather than suspended, as in SBRs. In different places, precipitation or adhesion, sedimentation, and the ensuing enzyme-enhanced breakdown are the primary methods used to extract textile dyes from plants.

Plants exposed to xenobiotics can activate enzymes that use aromatic compounds as substrates, completely degrading them into harmless metabolites. Additionally, plants provide essential support within an extensive system that promotes aeration by serving as homes for both aerobic and anaerobic microorganisms. Using solar energy, phytoremediation

offers long-term benefits and is inexpensive, making it easy to implement. One of the most prevalent kinds of phytoremediation systems is constructed wetlands. Several researchers used constructed wetlands as tertiary treatment. Nonetheless, unadulterated IW was used in most of the research. With documented COD removal rates of 40% and 91%, phytoremediation proved very successful. The fundamental drawback of these systems, however, is their long hydraulic retention times (HRTs), which must be at least 72 hours. This, together with the wetlands' low elevation, necessitates the construction of sizable wastewater treatment facilities. To speed up this process, it was investigated to add microorganisms such as *Ochrobactrum* sp. (Roy et al., 2014), *Bacillus pumilus* with *Microbacterium arborescens*, single *Bacillus pumilus*, and *Nostoc* spp. (algae) (Álvarez-Dávila et al., 2017).

4. Functional mechanism of microbial bioremediation

In natural ecosystems, microorganisms are abundant and often thrive in environments contaminated with heavy metals, where they play a vital role in transforming toxic metals into less harmful or non-toxic forms through diverse biochemical processes. During bioremediation, these microbes metabolize organic pollutants, converting them into stable end products such as water (H₂O) and carbon dioxide (CO₂), as well as other metabolites that support cellular growth and energy generation. Microbes exhibit two primary defense mechanisms against toxicity: the production of enzymes that degrade specific contaminants and the development of resistance mechanisms to withstand heavy metals. Using various methods such as adsorption, immobilization, oxidation-reduction reactions, biotransformation, and volatilization, microorganisms detoxify and restore polluted environments (Ahemad, 2014). Understanding the mechanisms that regulate microbial growth, metabolism, and responses to environmental stress is essential to enhance bioremediation efficiency (Table 1). Several bioremediation processes, including microbial-metal interactions, biosorption, biomineralization, bioleaching, bioaccumulation, and biotransformation, contribute to reducing heavy metal concentrations in soil and water. Some microorganisms can also oxidize and reduce transition metals, facilitating their stabilization or removal. Although exposure to organic solvents can damage microbial cell membranes, particular species have evolved protective adaptations such as solvent efflux systems and hydrophobic

barrier pumps. Additionally, many bacteria possess energy-dependent or plasmid-encoded metal efflux systems, where

Table 1. Overview of bioremediation techniques and their mechanism

Technique	Mechanism	Suitable Microorganisms	Pollutants Targeted	Key Advantages	Limitations	References
Biosorption	Passive adsorption of metals onto microbial cell walls	<i>Aspergillus niger</i> , <i>Bacillus subtilis</i> , <i>Penicillium</i> sp., <i>Chlorella vulgaris</i>	Pb, Cd, Cr, Ni, Cu	Simple, low-cost, effective with dead cells	Limited reusability	(Gadd, 2009; Wang & Chen, 2009)
Bioaccumulation	Active uptake and intracellular storage of metals	<i>Saccharomyces cerevisiae</i> , <i>Pseudomonas putida</i>	Cu, Ni, Zn, Hg	High specificity and efficiency	Requires live cells and nutrients	(Gadd, 2010)
Biodegradation	Enzymatic breakdown of organic pollutants	<i>Pseudomonas fluorescens</i> , <i>Acinetobacter</i> sp., <i>Rhodococcus</i> sp.	Hydrocarbons, dyes, phenols	Converts toxins to harmless compounds	May produce intermediate toxins	(Vidali, 2001; Varjani, 2017; Robinson et al., 2001)
Bioleaching	Microbial solubilization of metals from solids	<i>Thiobacillus ferrooxidans</i> , <i>Leptospirillum ferrooxidans</i>	Fe, Cu, Zn, Co	Efficient recovery of metals	Produces acidic effluents	(Rawlings, 2002; Rohwerder et al., 2003)
Bio-mineralization	Microbial precipitation of metals as insoluble compounds	<i>Bacillus sphaericus</i> , <i>Serratia</i> sp.	Uranium, Arsenic	Permanent removal of metals	Slow kinetics	(Newsome et al., 2014; Oremland & Stolz, 2003; Gadd, 2010)
Biotransformation	Chemical modification of pollutants by enzymes	<i>Alcaligenes</i> sp., <i>Clostridium</i> sp.	Nitroaromatics, pesticides	Converts pollutants into less toxic forms	May need co-substrates	(Ju & Parales, 2010; Singh & Walker, 2006)
Phytoremediation (Plant-microbe system)	Plants + microbes degrade or absorb contaminants	<i>Rhizobium</i> sp., <i>Azospirillum</i> sp.	Nitrates, dyes, metals	Eco-friendly and solar-powered	Long retention time	(Glick, 2010; Pilon-Smits, 2005; Vacheron et al., 2013)
Bio-augmentation	Addition of efficient microbial consortia	<i>Bacillus licheniformis</i> , <i>Nitrosomonas</i> sp.	COD, TSS, sulfates	Improves degradation rates	Requires acclimatization	(El Fantroussi & Agathos, 2005; National Research Council, 2000)
Bio-stimulation	Enhancement of native microbial activity using nutrients	<i>Pseudomonas</i> sp., <i>Micrococcus</i> sp.	Organic contaminants	Accelerates natural degradation	Over-nutrition may inhibit microbes	(National Research Council, 2000; Atlas & Bartha, 1993; Vidali, 2001)
Nano-bioremediation	Microbial synthesis of nanoparticles for pollutant removal	<i>Aspergillus tubingensis</i> , <i>Chlorella vulgaris</i> , <i>Escherichia</i> sp.	Heavy metals, dyes	Combines bio- and nanotechnology	Still under development	(Kamyab et al., 2025)

ATPases and chemiosmotic proton or ion pumps play key roles in conferring resistance to heavy metals such as chromium (Cr), cadmium (Cd), and arsenic (As) (Naz et al., 2016).

4.1. Role of microbes in heavy metal bioremediation

Without the use of chemicals, several bacteria can degrade metals on an inadequate large scale. Resistance to heavy metals and microbial stress is influenced by sanitary efficiency (Wasilkowski et al., 2012). Genetically modified microbes may then provide a solution to this issue (Figure 2). A crucial step in altering these bacteria's metabolic pathways is genetic engineering. Limiting harmful activities is another benefit of heavy metal activity. Microorganisms were transformed from inorganic to organic form by employing techniques such as oxidation-reduction. Through genetic engineering, microorganisms' properties can be altered to produce desired outcomes, such as resilience to harsh conditions, lower culture costs, faster growth, and pH stability. Additionally, it enhances water filtration by allowing microbes to build metabolic structure while permitting heavy metal deposition or reducing the harm caused by these metals (Anjum et al., 2020).

5. Mechanism of heavy metal removal by microorganisms

5.1. Microbial approaches to metal remediation

Specific yeasts, such as *S. cerevisiae* and *Aspergillus*, and fungi, such as *Penicillium*, have been shown to extract pollutants, particularly heavy metals, from various bodily fluids. Microorganisms like *Saccharomyces boulardii*, *Bacillus subtilis*, and *E. coli* have also been used to remove heavy metals from wastewater. Bio-driven technology for heavy metal remediation is a simple, inexpensive, and environmentally friendly process that employs biomass to extract heavy metals from effluents. The efficacy of a bioremediation process is affected by the technique used to bind a particular site in the biomass. Microorganisms use both active and passive mechanisms to extract the metal solution. These methods can handle variables such as experimental conditions and target pollutants. Algae and other organisms with organic activity are used in their natural forms throughout the bioremediation process. A more practical and popular solar-powered technique is bioremediation. It eliminates contaminants from secondary air and soil water and is effective against hydrophobic and organic compounds.

Mechanism of action of Microbial Bioremediation

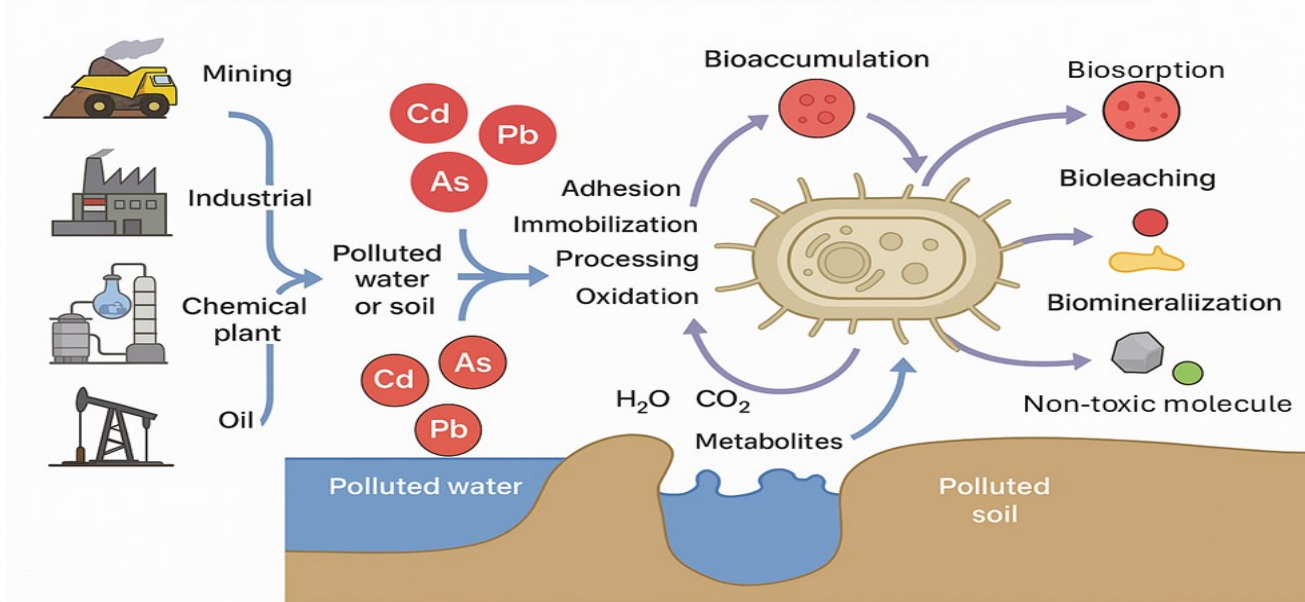


Figure 2. Microbes remove toxic metals from polluted water and soil

5.2. Biosorption mechanism

One way to describe biosorption mechanisms is quick, reversible, passive adsorption processes. Metals are maintained by their interactions with functional groups on the cell surface, including adsorption and crystallization. Several variables, including the ion's strength, temperature, presence in the solution, biomass, particle size, and pH, influence biosorption (Table 2). The extracellular surface of bacteria contains anionic groups that bind cationic heavy metals at pH 7. Because biosorption is not cell-based, it can be used with both living and dead biomass. The study of organisms with prospective biosorption capabilities may be enabled by advances in genomic research technologies, which could lead to several future applications in bioremediation. The bioaccumulation process is determined by both external and intracellular pathways, where passive absorption is restricted. The intricate buildup process varies according to the direction of metabolism and the amount of metal in the body. Microorganisms can enhance bioaccumulation. The key genes involved in bioaccumulation were revealed by a transcriptomics study (Anjum et al., 2020).

Because bioaccumulation must be maintained in the presence of living cells, whereas bioaccumulation may be

produced by industrial waste, biosorption is less expensive than bioaccumulation. One of the main costs of biosorption is the transportation and production of biosorbents. Another crucial component that affects both processes is pH. While bioaccumulation is limited to the designated pH range, biosorption can operate across a wider pH range. Selectivity is enhanced in bioaccumulation, but because processes in bioaccumulation are faster than the intercellular accumulation required for clearance, the clearance rate is higher (Figure 3). Biosorbents are reusable and regenerable. Reuse of bioaccumulation does not occur. Bioaccumulation requires more energy than biosorption because living cells consume more energy. When biomass bioaccumulates, it cannot be utilized for other uses. Among the many benefits of microbial wastewater treatment are cost and energy savings. This method enhances air quality, eliminates odors, and kills germs. Different bacteria evolve distinct pathways to survive and interact with inorganic metals, including heavy metals. Although biological wastewater treatment is widely accepted, it has several serious drawbacks, including slow processing, limitations caused by nonbiodegradable pollutants, and the potential for microbial toxicity (Pedone et al., 2017).

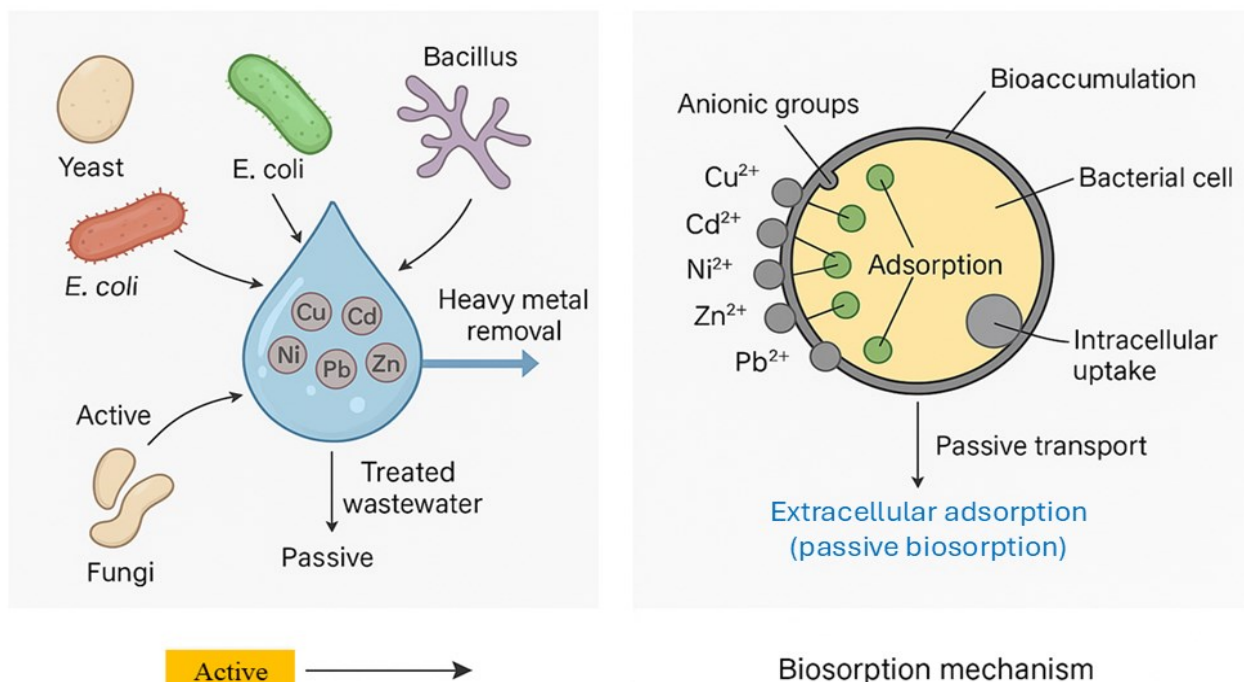


Figure 3. Microbes absorb and remove heavy metals from wastewater

Table 2. Common microorganisms used in wastewater bioremediation and their roles

Microorganism	Type	Major Application	Pollutants Removed	Mechanism / Enzyme System	References
<i>Bacillus subtilis</i>	Bacterium	Textile and pharmaceutical wastewater	Dyes, heavy metals	Laccase-like oxidation; biosorption	(Li et al., 2023)
<i>Pseudomonas putida</i>	Bacterium	Industrial effluents	Hydrocarbons, COD, dyes	Oxidation–reduction reactions, biofilm formation	(Medić, 2022; Daphedar et al., 2022)
<i>Chlorella vulgaris</i>	Microalga	Nutrient and dye removal	Ammonium, phosphate, dyes	Bioaccumulation and exopolysaccharide formation	(Kim et al., 2013)
<i>Rhodococcus</i> sp.	Bacterium	Petroleum and pharmaceutical effluents	Hydrocarbons, phenols	Oxygenase-mediated biodegradation	(Larkin et al., 2005)
<i>Acinetobacter</i> sp.	Bacterium	Chemical and industrial wastewater	Phenols, surfactants	Enzymatic oxidation	(Liu et al., 2019)
<i>Saccharomyces cerevisiae</i>	Yeast	Metal-contaminated water	Copper, nickel, zinc	Active transport and intracellular accumulation	(Brady & Duncan, 1994)
<i>Nitrosomonas</i> sp.	Bacterium	Nitrification processes	Ammonium ions	Ammonia monooxygenase (AMO)-driven oxidation	(Arp & Stein, 2003)
<i>Nitrobacter</i> sp.	Bacterium	Nitrification processes	Nitrite ions	Nitrite oxidoreductase (NXR) activity	(Meincke et al., 1992)
<i>Escherichia</i> sp.	Bacterium	Dye decolorization	Congo red (reported for) malachite green	Reductase-linked dye decolorization (varies by strain/enzymes)	(Daphedar et al., 2022)
<i>Bacillus licheniformis</i>	Bacterium	Effluent treatment and bioaugmentation	COD (also improves N-removal performance in systems)	Enhances treatment via microbial regulation/consortium effects	(Li et al., 2023)
<i>Microbacterium arborescens</i>	Bacterium	Phytoremediation systems	Textile dyes	Symbiotic degradation with plant roots (reported in plant–microbe dye-removal literature)	(Daphedar et al., 2022)
<i>Thiobacillus ferrooxidans</i>	Bacterium	Metal leaching and bio-oxidation	Copper, iron, zinc	Bioleaching and oxidation of metal sulfides	(Rawlings, 2002)

6. Bioremediation techniques for the pharmaceutical industries

Conventional treatment methods employed by wastewater treatment plants (WWTPs) are generally effective in reducing the concentrations of carbon and

nitrogen compounds in wastewater. However, these systems are often inefficient in eliminating pharmaceutical residues and other emerging micropollutants, which persist through the treatment process and are subsequently released into the environment. The increasing occurrence of such micropollutants in discharged effluents poses serious

environmental and public health concerns. Moreover, chemical and physicochemical treatment processes, while effective in specific contexts, present additional challenges. These include the high cost of chemical reagents and the formation of undesirable byproducts during treatment, which can themselves be environmentally harmful. Such limitations hinder the widespread adoption of these methods in industrial applications, prompting greater interest in cost-effective, sustainable alternatives.

Consequently, attention has shifted toward biological degradation and bioremediation techniques. In biological treatment systems, it is crucial to ensure that the metabolic byproducts generated during the process are less toxic than the original pollutants. The efficiency of bioremediation depends on several factors, including the chemical structure, toxicity, and concentration of the contaminants; the retention time and environmental conditions (such as pH, temperature, and oxygen levels); the presence of other interacting compounds; and the metabolic capabilities of the selected microbial strains. A comprehensive overview of the various bioremediation strategies and their operational principles is summarized in Table 1 (Das et al., 2012). These techniques continue to evolve as researchers explore more efficient microbial systems and hybrid treatment processes to address the persistent limitations of conventional wastewater management.

6.1. Bioremediation by bacteria

Microorganisms naturally thrive in environments contaminated with heavy metals and play a crucial role in converting toxic metals into less harmful forms. During bioremediation, microbes break down pollutants into stable end products, such as water and carbon dioxide, supporting their growth and energy needs. They defend themselves by producing enzymes that degrade contaminants and developing resistance to heavy metals. Through processes such as adsorption, oxidation-reduction, biosorption, and bioaccumulation, microorganisms help detoxify polluted environments. Some species also use efflux systems and ion pumps to resist metals like chromium, cadmium, and arsenic, making them valuable agents in sustainable bioremediation (Das et al., 2012).

6.2. Bioremediation by fungi

There is potential for bioremediation with strains of different fungal spores, such as *Aspergillus niveus*, *Aspergillus fumigatus*, and *Aspergillus niger*. However,

certain strains are limited by their lengthy spore-forming cycle. It is commonly recognized that Ascomycetes may remove COD from industrial wastewater, particularly distillery effluent. Effects of the WRF strain (White Rot Fungus) *Bjerkandera adusta* MUT 2295 on the production of resistant waste sample mixes made from humic and tannic acids. The results showed that while BOD removal was 75% and 89% in the solutions of humic and tannic acids, COD elimination was 61% in the tannic acid effluent. Increasing research is using the biochemical capacity of filamentous basidiomycetes to enzymatically transform various pharmaceutical products (Das et al., 2011).

7. Environmental impact of microbial bioremediation

Microbial bioremediation strategies use native microbial species to remove contaminants from the environment. Numerous factors, including the type and quantity of the pollutant, the habitat, and the composition of native microbial communities, affect the extent to which pollutants are detoxified (Hussain et al., 2020). Among the many advantages of bioremediation are the following: It is an entirely natural procedure with no adverse side effects. Input and maintenance expenses are minimal. Very little energy is used compared to incineration and landfilling. The quick turnaround time for turning soil and water into valuable resources. Because toxins are less likely to leak, liability is decreased. Most applications are finished in situ without the need for risky transportation.

7.1. Integration of nanotechnology and microbial bioremediation

Nanomaterials are ideal for wastewater treatment because of their small size. Their use in various applications is enhanced by their biological, physical, and chemical characteristics. Numerous carbon-based nanomaterials, such as nanotubes and nanocomposites, as well as metal- and oxide-based materials, have been used to remove contaminants from wastewater. Wastewater treatment uses adsorption, photocatalytic degradation, nanoparticle-based filtering, and monitoring of different pollutants and toxins (Mandeep et al., 2020). Using microorganisms and biofabricating nanoparticles makes nanotechnology more practical and environmentally beneficial. When used in aqueous solutions, chemically produced nanomaterials may exhibit several drawbacks, including self-agglomeration and the formation of chemical byproducts. Consequently, natural sources such

as bacterial and fungal enzymes and plant extracts are used to produce nanomaterials, which could serve as a viable alternative. Their function is to create metal nanoparticles and to minimize the formation of metal complexes. Coprecipitation, or the addition of bioactive substances and proteins to the nanoparticles' surfaces, increases their stability in aqueous environments.

In the Sundarbans of India, *Aspergillus tubingensis* (STSP 25) synthesizes bio-fabricated iron oxide nanoparticles from the rhizosphere of *Avicennia officinalis*, achieving over 90% removal of heavy metals such as Zn^{2+} , Ni^{2+} , Cu^{2+} , and Pb^{2+} from wastewater, with regeneration possible for up to five cycles. Similarly, *Chlorella vulgaris*-derived exopolysaccharides (EPS) were used to precipitate iron oxide nanoparticles, as confirmed by FT-IR analysis, effectively removing 91% of PO_4^{3-} and 85% of NH_4^+ . The microbial synthesis of nanoparticles is both cost-effective and eco-friendly—*Escherichia* sp. SINT7, a copper-resistant strain, produces copper nanoparticles that degrade dyes such as malachite green, direct blue-1, Congo red, and reactive black-5, with removal efficiencies above 80% at lower concentrations; additionally, *Pseudoalteromonas* sp. CF10-13 and iron-sulfur nanoparticles from Cheng et al. demonstrate efficient dye degradation through extracellular electron transfer, reducing toxic byproducts and supporting sustainable wastewater treatment (Leong et al., 2020).

8. Limitations and bottlenecks of bioremediation

Microbe-based remediation is an effective and economical approach due to its high selectivity, low maintenance cost, and firm performance in removing pollutants from pesticides, fertilizers, and wastewater. However, its success depends on selecting the right microbial strains and maintaining suitable environmental conditions. For efficient heavy metal removal, integrating microalgal biomass with chemical modification techniques or combining it with other treatment methods is often recommended (Azubike et al., 2016). Despite its potential, bioremediation has limitations, especially in pharmaceutical wastewater treatment, where nutrient deficiencies, such as insufficient nitrogen or phosphorus, can reduce microbial activity. Moreover, the process is primarily restricted to biodegradable contaminants, making it less effective for complex xenobiotics. Scaling up from laboratory or pilot studies to full-scale operations presents further challenges, including variability in microbial efficiency and potential toxicity of

intermediate products. Hybrid systems that combine biological, physical, or chemical methods, such as sequential or simultaneous bioremediation reactors, are commonly used to enhance performance and reduce the formation of resistant residues. Advances in molecular biology and bio-stimulation have improved understanding of microbial pathways and nutrient control, though excess nutrients may inhibit activity. Additionally, nanomaterials are increasingly being applied to enhance microbial efficiency by reducing activation energy and increasing surface area, offering new opportunities to improve future bioremediation systems.

9. Conclusion

Numerous sources, including industrial output and residential activities, produce wastewater, which is then treated through biological processes, microbial remediation, and nanotechnology developed from microbiology. The microorganisms involved in the treatment process must be better understood, and wastewater treatment methods must be monitored. Because of the substantial heavy metal contamination, the effectiveness of the current remedial process should be improved or, if needed, new processes implemented. The fixed ion forms of heavy metals make removal difficult. This seriously threatens the environment and public health. Furthermore, recent developments have shown that nanotechnology and the use of nanomaterials in remediation techniques have significantly improved and expanded. Traditional technologies, such as pollution management, wastewater treatment, and medicine across many disciplines, can be modified and enhanced by nanotechnology.

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12. Conflict of Interest

The authors declare no conflict of interest.

13. Authors' Contribution

Burhan Khalid, Rizwan Maqbool, and Muhammad Atiq Ashraf proposed a general idea and wrote the review. Qammar Malik and Talha Riaz contributed to the conception and design of the review. Mian Muhammad Ahmed and Muhammad Wassay reviewed all manuscript drafts and provided extensive editing. Syeda Maira Hamid, Abdullah, and Ayaz Ahmad helped draw the figures. All authors have read and approved the final version of this manuscript.

14. SDGs Addressed

This study directly contributes to SDG 6 (Clean Water and Sanitation) by advancing sustainable and efficient wastewater treatment technologies for the removal of heavy metals and pharmaceutical contaminants. It also supports SDG 12 (Responsible Consumption and Production) and SDG 14 (Life Below Water) by promoting eco-friendly bioremediation strategies that reduce toxic effluent discharge and protect aquatic ecosystems.

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