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# MICROBIAL BIODIVERSITY AND BIO-MITIGATION ASSESSMENT – RECENT ADVANCES AND STRATEGIES

## ABSTRACT

The ever-increasing population growth and industrialization have stemmed the genesis of several environmentally related contaminants of emerging concern. Numerous of them influence the living ecosystem, including pharmaceutically bioactive residues, antibiotics, pesticide-based polluting agents, industrial-based synthetic dyes, etc. Indeed, several approaches, e.g., chemical and physical treatments and their possible combinations, have been proposed, established, and executed to treat and remove environmental pollutants from numerous matrices. However, the existing approaches are becoming inefficient due to the growing quantity and complex nature of environmental contaminants. Therefore, more effective and suitable measures are needed to sustain the green environment by remediating and mitigating the hazards of emerging concerns associated with environmentally related pollutants. Among other recent approaches, microbial biodiversity, bio-mitigation assessment, and removal of current pollution load using robust materials are of supreme interest with evident merits. In addition, microorganisms have an enormous capacity to adapt and survive in divergent environments and, once established, produce unique biocatalytic molecules that break down and transform the contaminating agents, thus making it possible to revive the polluted matrices. Likewise, nanozymes are nanostructured materials with enzyme-like activities that effectively catalyze the breakdown of contaminating agents and, thus, are other suitable candidates to upgrade modern bioremediation practices. This review thoroughly examines the most recent developments in novel bio-mitigation and nanozyme-based approaches. Finally, we highlight their current challenges to provide a perspective on potential future research directions.

**KEYWORDS:** Bioremediation; Hazardous contaminants; Water matrices; Mitigation strategies

## INTRODUCTION

Environmental pollutants, e.g., antibiotics and pesticides, can directly or indirectly have harmful influences on humans and the entire living environment. These effects include respiratory problems, developmental issues, and cancer (Bilal et al., 2019; Saeed et al., 2022). In addition, pollution can cause damage to ecosystems, including the loss of biodiversity and changes in the behavior and survival rates of different animal and plant species.

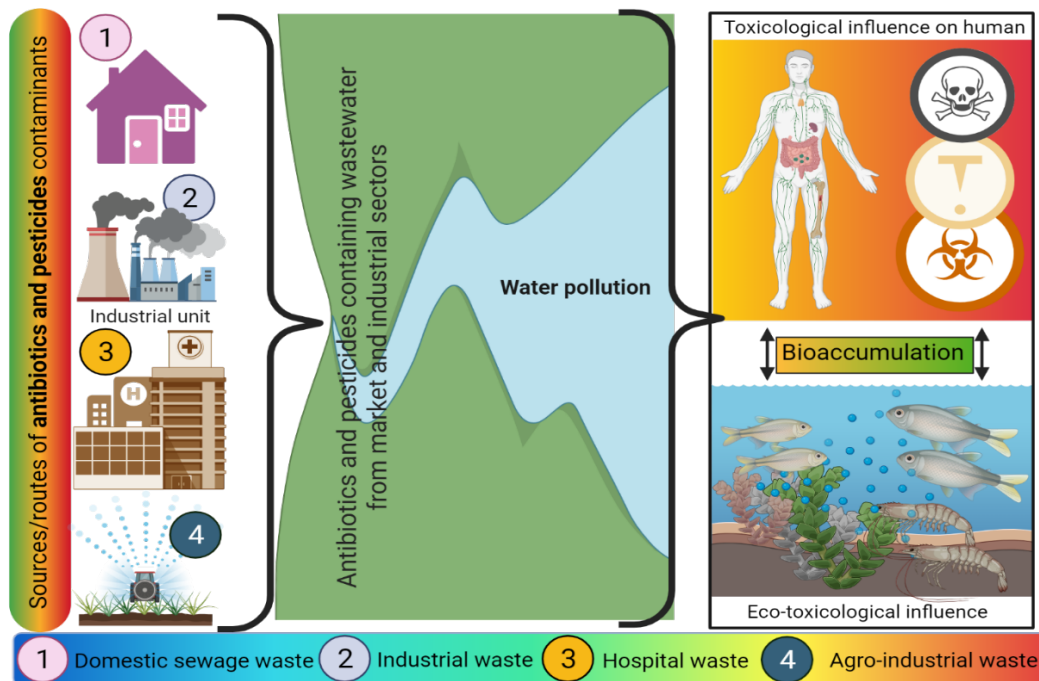
Hence, it is essential to eliminate and take preventive measures to reduce pollution to shield the living environment. There are many examples of environmental polluting agents, such as carbon dioxide emissions from cars and factories, oil spills in the ocean, chemical waste dumped into rivers, plastic pollution in the sea, and deforestation (Cárdenas-Alcaide et al., 2022). Other examples include hazardous and pharmaceutically active micropollutants, which can harm people and the environment (Bilal et al., 2022).

Modern mass production attempts have signified environmental contamination issues. By considering the adverse hazards of environmentally related polluting agents, suitable measures are needed to sustain the green environment by remediating and mitigating the dangers associated with environmentally related pollutants of emerging concerns (Liu et al., 2019). The emerging concerns related to environmental impacts and toxic effects of contaminants on humans are documented in the literature. For example, the improper disposal and misuse of antibiotics are related to antimicrobial resistance (González-González et al., 2022c; WHO, 2014). Endocrine-disrupting chemicals (EDCs) can cause adverse health effects as well – even at very low concentrations – due to their capacity to alter the endocrine system and bioaccumulate (González-González et al., 2022b). The global concern caused by these pollutants and others like dyes and pesticides is maximized due to their persistence, bioaccumulation, and frequent occurrence in environmental matrices. Examples of sources/routes of antibiotics and pesticide-based contaminants of high concern and their toxicological impacts are shown in Figure 1.

Many techniques have been proposed and categorized into three main groups: physical, chemical, and biological. Furthermore, novel approaches involving integrated systems involving more than one technique have demonstrated enhanced efficiencies (Al-Maqdi et al., 2021). The chemical and physical methods have shown good efficiencies and large-scale application, but limitations remain, like high energy demand, expensive operation, and formation of toxic by-products (Al-Maqdi et al., 2021; Bilal et al., 2019). In these circumstances, biological methods are greener, safer, less expensive, and more eco-friendly alternatives.

Microbial bioremediation is a process where microorganisms are used to clean up contaminated environments. This could include breaking down and removing harmful chemicals or pollutants from soil, water, and even air (Saeed et al., 2022). Microorganisms like bacteria, fungi, and algae can break down the pollutants into less harmful substances or even convert them into harmless forms that can be safely disposed of. This process is often used in environmental cleanup efforts or industries producing many waste products. It is a natural and eco-friendly alternative to traditional hazardous waste removal methods.

Traditionally, enzymes have been considered highly efficient biocatalysts with excellent selectivity. Thus, enzyme-assisted methods allow the enzymatic conversion of pollutants into smaller and less toxic molecules. However, enzymatic treatments for water decontamination have several drawbacks, such as reduced efficiency due to harsh environmental conditions and limited enzyme reusability (Al-Maqdi et al., 2021). This review examines the mechanism of action of microbial bioremediation by analyzing recent advances in the treatment of water contaminated by emerging contaminants such as antibiotics and pesticides. An in-depth discussion is also given regarding the function of nanomaterials as potential artificial enzymes for the degradation of dyes and phenolic compounds.



**Figure 1:** Sources/routes of antibiotics and pesticide-based contaminants and their toxicological impacts on humans and the environment. Figure was created with BioRender.com and extracted under premium membership.

### Microbial-assisted degradation of antibiotics and pesticides

In natural environmental matrices, several microbial species thrive to naturally remediate the environment polluted by numerous hazardous polluting agents, e.g., antibiotics and pesticides based on active residues. Microbes transform any/many of these toxic residues into non-toxic forms through their natural and super-active enzyme system. In most cases, the end-products of microbial remediation include  $H_2O$ ,  $CO_2$ , and other small metabolites, which microbes use as principal growth substrates throughout the reaction process (Saeed et al., 2022). Antibiotics belong to the important class of drugs used to fight bacterial infections in living beings. Regardless of the undeniable benefits of antibiotic drugs, any/many of them can also threaten the environment once disposed of in sewage systems. Once they enter the environment through wastewater discharges and agricultural runoff, antibiotics can bioaccumulate and thus pose harmful effects on living aquatic organisms (Bilal et al., 2019, 2020; Cruz-Cruz et al., 2022). Besides the free discharge of antibiotic residues in sewage matrices, the abuse and mismanagement of antibiotics in humans and animals have led to antibiotic-resistant bacteria. Thus, it is imperative to reduce the amount of antibiotics released into the environment by properly disposing of expired medications and investing in research to develop new antibiotics and alternative treatments.

In microbial-based bioremediation treatment of antibiotics, microorganisms such as bacteria and fungi act as the agents of degradation and perform an appropriate breakdown of the target antibiotic molecules effectively. These organisms produce novel enzymes and use them to break down antibiotics into more simple substances that are no longer harmful or at least less toxic to the environment. However, microbial degradation can prevent this problem by breaking down the antibiotic before it can cause harm (Singh Aditi and Saluja, 2021). Another antibiotic that is subject to microbial degradation is chloramphenicol. It can cause

environmental problems if it accumulates in the soil or water. Microbial degradation can prevent this by breaking down the drug into safer, simpler substances. In conclusion, microbial degradation is crucial for reducing the environmental impact of antibiotics such as tetracycline and chloramphenicol. It is essential to focus on strategies to promote this process, such as optimizing environmental conditions and finding new microorganisms that can effectively degrade antibiotics. By doing so, the harmful effects of drugs can be minimized in the environment and safeguard a healthier planet for future generations.

Antibiotics can be removed by three primary mechanisms, including biodegradation, bioadsorption, and bioaccumulation, with biodegradation being the most common mechanism (Eheneden et al., 2023). An ample range of antibiotics have been removed by microbial approaches; for example, Gao et al. (2023) reported the removal of sulfadiazine by employing *Chlorella* sp. G-9 in a membrane photobioreactor. They reported the effective removal in synthetic wastewater, between 54 – 94 %. Moreover, they achieved simultaneous antibiotic removal and enhanced lipid production since sulfadiazine stress increased the lipid content of microalgae in the membrane photobioreactor (Gao et al., 2023). Microbial degradation has been employed for the removal of different antibiotics, such as sulfanilamide, cephalosporins, oxytetracycline, and ofloxacin, among others (Guo et al., 2016; Zhang et al., 2023; Zhang et al., 2022). Furthermore, antibiotics can be effectively removed by symbiotic systems such as microalgae-bacteria consortia, which have shown additional benefits like minimal operational costs and reduced carbon emissions (Wang et al., 2022).

There are many types of pesticides, including insecticides, herbicides, fungicides, and rodenticides. Regardless of the beneficial use of pesticides as pest-controlling agents, they also negatively influence environmental health and soil and adversely impact human health. Microbes can degrade many different types of pesticides through various metabolic processes. Microbes such as bacteria and fungi have developed the ability to break down these chemicals and effectively remove them from the environment. However, the removal effectiveness varies based on the type of pesticide and the environmental conditions in which it is found. In some cases, additional treatments may be required to remediate contaminated areas fully (Liu et al., 2019). *Stenotrophomonas* sp. G1 was evaluated for the degradation of eight organophosphorus pesticides. Among them, methyl parathion, methyl paraoxon, diazinon, and phoxim were completely degraded in 24h. Parathion also reached a good degradation efficiency of 95% within the same period. Authors reported that methyl parathion hydrolase, an intracellular enzyme presented in Strain G1 cells, is responsible for pesticide degradation (Deng et al., 2015). In another study, soil microbes sampled from different regions were tested for the degradation of imidacloprid.

Interestingly, the degradation efficiency varied according to the geographic location where the sample was recovered due to the different soil microbiota composition. It was reported that *Achromobacter* sp. could remove 100% of the pesticide in 20 days; However, its degradation activity was enhanced when combined with *Paracoccus* sp., reaching complete removal within 15 days (Gao et al., 2021). Different microorganisms, such as *Pseudomonas* and *Bacillus*, have demonstrated excellent degradation activities for pesticides like chlorpyrifos and pyraclostrobin through metabolic pathways involving enzymes (Chen et al., 2018; Liu et al., 2023).

## Emerging contaminants and traditional enzymatic techniques

Pharmaceutical products and their metabolites are a class of organic chemical compounds with a high presence in aquatic environments. Those pollutants can reach the environment by different pathways. The primary sources of these pollutants include hospital and domestic effluents with loads of pharmaceutical compounds excreted by feces and urine, inadequate disposal of expired/unused drug products, agricultural effluents with pharmaceutical content due to feces and urine of animals, and direct discharges from manufacturing plants in the pharmaceutical industry (González-González et al., 2022c). The processes currently implemented to mitigate pharmaceutical products in wastewater treatment plants (Stadlmair et al., 2018).

EDCs are compounds of different origins that have gained great relevance due to their potential to interfere with the endocrine system and generate various health problems in humans and animals (Rodríguez-Hernández et al., 2022). The occurrence of these pollutants is highly problematic since their removal is difficult by current technologies in wastewater treatment plants. In addition, the high persistence of EDCs, their high bioaccumulation in living organisms, and the environmental conditions exacerbate the problem. The biological approaches involving microorganisms or enzymes outweigh the large diversity of methods due to their many advantages and capacity to degrade various emerging contaminants. Biological processes are biotechnologies considered green catalysis due to their lower cost, lower energy expenditure, high efficiency in the degradation of contaminants, even presented at low concentrations, and eco-friendly nature (Feng et al., 2021; Morsi et al., 2021).

A recent strategy to further increase the efficiency of biocatalysts is the immobilization of enzymes in nanostructured supports. Compared to free enzymes, the immobilized-enzyme approach results in systems with greater thermal stability, easier separation, increased reusability, and catalytic activity in broader reaction conditions (Wong et al., 2019). The genetic engineering strategy is a different approach, allowing "building" modified enzemergering contaminants effectively and at a lower cost. Thus, this strategy is suitable for improving biocatalytic performance and stability in different adverse media, such as wastewater (Bhatt et al., 2021; Feng et al., 2021). Finally, enzyme-mimicking nanomaterials or nanozymes have recently emerged with superior features to natural enzymes. For instance, they possess high catalytic activity and stability, functionalization ease, and cost-effective manufacturing processes (Lopez-Cantu, et al., 2022a, 2022b). In this manner, multiple applications can be achieved using novel nanozymes, which include the efficient degradation of pollutants.

## Nanomaterials as potential artificial enzymes

Enzymatic remediation techniques have demonstrated their great potential, ample diversity, and capacity to degrade emerging contaminants. However, such biological approaches have significant challenges like enzyme reusability and enzyme stability (Al-Maqdi et al., 2021). Different alternatives have evolved, with nanozyme-based techniques emerging as innovative remediation approaches. Although nanomaterial-based artificial enzymes present significant structural differences from natural enzymes, they can imitate their catalytic activity and general principles. These alternative catalytic materials encompass a great diversity of nanostructured materials. Metal-based nanomaterials have been presented as potential candidates for mimicking the activity of several natural enzymes. Some examples are platinum-based, palladium-based,



silver-based, and gold-based nanomaterials. Metal-based nanozymes are characterized by easy synthesis procedures and the requirement of few reagents. Gold nanoparticles have been extensively studied for their glucose oxidase activity, while platinum nanomaterials have shown peroxidase and catalase. The study by Zhang et al. (2019) is a representative example of using gold nanoparticles as nanozymes. The authors tested gold nanoparticles and five other common nanozymes for estradiol degradation in their study. The results demonstrate that gold nanoparticles can convert estradiol into estrone by dehydrogenase-mimicking activity. In addition, it was found that the particle size had a significant effect on the catalytic activity since smaller nanoparticles presented higher efficiency (Zhang et al., 2019).

Moreover, the enzyme-mimicking activity of bimetallic nanomaterials has also been explored. Interestingly, bimetallic nanozymes have shown significant improvement compared to monometallic nanomaterials due to a synergistic effect associated with their unique physicochemical properties. In this respect, Naveen Prasad et al. (2022) evaluated the performance of Cu nanoparticles combined with different metal ions such as silver, palladium, gold, and platinum. The Cu-Pt nanozyme exhibited the highest peroxidase-mimicking catalytic activity for detecting urine glucose (Naveen Prasad et al., 2022). Similarly, metal oxide-based nanomaterials such as ferric oxide, nanoceria, vanadium pentoxide, and ruthenium oxide nanoparticles, among others, have presented enzyme-like activity. In this respect, nanoceria is one of the most widely studied metal oxide nanozymes due to its excellent multienzyme-mimicking activity. Moreover, recent research has aimed to propose different strategies to regulate and enhance the catalytic activity of nanoceria nanozymes. For instance, Yue et al. (2021) developed a coordination chemistry approach to regulate the peroxidase-like activity of ceria nanorods. The authors observed a synergistic effect that significantly enhanced the catalytic activity of nanoceria nanozyme by chelation of metal ions (Yue et al., 2021). Iron oxide, cobalt oxide, copper oxide, manganese dioxide, and vanadium pentoxide are additional examples of metal oxide-based nanomaterials with enzyme-mimicking potential (Šálek et al., 2020; Wei and Wang, 2013).

Most of the reported nanozymes are composed of metallic elements; however, in recent studies, several carbon-based nanostructures have exhibited enzyme-like activity with reduced toxicity, increased biocompatibility, and eco-friendly properties. In this category, different carbon-based nanomaterials such as fullerene-like structures, carbon nanotubes, graphene, and carbon dots have present catalytic activity (Li et al., 2013; Lopez-Cantu et al., 2022a; Sun et al., 2013). Some research groups have further studied the mechanism involved to propose effective approaches to enhance their enzyme-like activities (Duan et al., 2019; Sun et al., 2013). In addition, metal-organic frameworks (MOFs) have been studied for their potential to imitate the enzymes' activities (Wang et al., 2020). Pores within MOFs provide a unique confined environment in which new physical properties and chemical reactions can be developed (Zhou and Kitagawa, 2014). These materials contain metal nodes, which might provide possible active sites for catalytic reactions (Niu et al., 2020). MOFs-based nanozymes present multi-enzyme activities under certain conditions (Wang et al., 2020). Overall, nanozymes offer multiple advantages over natural enzymes. For example, feasibility for low-cost and large-scale manufacturing, robustness to harsh conditions, which can be presented when environmental samples are involved, long-term storage, high stability, and tunable activity are among other advantageous features. Due to these advantages, a vast diversity of nanozymes have been established and employed to deplete emerging pollutants.

## Dyes-based pollutants in the environment

Dyes have been used by many industries like textile, pharmaceutical, food, cosmetics, plastics, photographic, and paper (Al-Tohamy et al., 2022). An extensive list of dyes employed in industrial activities includes malachite green, reactive blue 19, congo red, methyl orange, rhodamine B, and methylene blue, among many others. Currently, the estimated production of dyes is around 800,000 tons/year; a significant percentage ends as waste discharged without further processing (Hassaan et al., 2017). Most of the production belongs to the textile industry, which has a significant impact mainly in developing countries such as India, Bangladesh, and Pakistan; however, many of their factories are equipped with inefficient wastewater systems (Gita et al., 2017). Effluents derived from dye production contain many contaminants, including surfactants, salts, heavy metals, oxidizing agents, and reducing agents (Madhav et al., 2018). An untreated effluent could cause hazardous consequences on organisms, showing diverse adverse effects, and many studies have reported that textile dyes may also cause respiratory affections in humans after exposure, such as asthma (Tang et al., 2018). Furthermore, dyes can affect water bodies and disturb the aquatic environment, causing turbidity, changes in pH, color, and temperature, and decreasing the dissolved oxygen (Varjani et al., 2021). Therefore, the relevance of finding an effective solution to these ecotoxicological threats has increased, leading to enzyme-based and nanozyme-based approaches. Some of their limitations are the excessive costs, low efficiency, and unsustainable nature of some conventional wastewater treatments (Selvaraj et al., 2021). Concerning biological therapies, there is enough evidence in the literature to prove the effectiveness of enzymes for degrading dyes. Despite the benefits of these natural tools, many disadvantages remain, such as operational stability, recovery, production cost, etc. A nanozyme-based strategy has been proposed as an innovative solution for degrading dyes, offered by the synergistic interaction between nanotechnology and biotechnology. The degradation of dyes through this approach consists of catalytic reactions that occur by employing artificial nanostructured enzymes (Al-Tohamy et al., 2022). Peroxidase is the most useful enzyme employed for degrading many emerging pollutants like dyes. This enzyme has the potential for bioremediation of wastewater, bioleaching in the paper industry, and textile dye degradation (Bansal and Kanwar, 2013). Another example is reported by Terres et al. (2015), who analyzed the peroxidase activity in the degradation of Indigo carmine dye (IC); their results showed the disappearance of coloration (Terres et al., 2014). Another study used Co-doped magnetite nanoparticles surrounded by the carboxymethylcellulose polymer shell; the nanozyme was termed a “Co-MIONzyme” and exhibited peroxidase-like activity. The Co-MIONzyme could efficiently degrade methylene blue (MB) with an efficiency of 95%. This result was obtained at pH 3, 40°C, within 10h of the reaction, and a dye dose of 22.5 µg/mL (Mansur et al., 2022). Laccase is another enzyme utilized to degrade dyes; it has been immobilized into pine needle biochar to degrade malachite green dye (MG). The study reported a dye removal efficiency of 85%, and only the 53% efficiency could be maintained after some cycles (Pandey et al., 2022). On the other hand, Ge et al. (2021) constructed hybrid composites of copper ions and tannic acid (Cu-TA) with laccase-like activity. In addition, after several cycles, the efficiency of degrading MG was 90%, with good reusability. The storage stability is another characteristic to highlight, which was superior to natural laccase (Ge et al., 2021). Similarly, other nanozymes have exhibited excellent results in removing a great diversity of dyes, as presented in Table 1. New methods for the degradation of dyes are of vital importance in the present days; nanozymes might become one feasible and cost-effective solution. This is because nanozymes exhibit many advantageous attributes compared to their

natural counterparts; nanozymes can easily be adapted to current industries and environmental necessities. The published reports in the literature give insight into their incredible potential; however, research efforts are still required to design new nanomaterials through sustainable and optimized protocols to enhance their yield and large-scale performance. However, numerous dyes with combinations must be tested with the proposed nanozymes for further advancements, providing more data to compare results. In addition, toxicity tests need to be conducted in more studies.

### **Phenolic compounds degradation**

Phenolic compounds derived from the petrochemical, pharmaceutical, agricultural, and food industries play a relevant role in human life due to the negative impacts caused by their release and distribution in the environment. The environmental balance and human health might be seriously affected by problems such as endocrine disruption and carcinogenesis, among many other adverse effects ( Wang et al., 2019). Certain phenolic compounds such as 4-nitrophenol and 4-chlorophenol are considered high-priority contaminants by the Environmental Protection Agency (EPA) of the United States.

The degradation of different phenolic pollutants can be performed by different processes, either physical, chemical, or biological. Typically, they present high degradation efficiency; however, disadvantages like high implementation and maintenance costs significantly hinder broad applicability. Thus, nanozymes are considered successful strategies for the degradation of phenolic compounds. Nanozymes have advantages over conventional catalysts or biocatalysts, showing superior and tunable catalytic efficiencies, great recyclability, high stability in different media, and simple and low-cost manufacturing (Liang et al., 2022). Furthermore, researchers have explored innovative strategies to enhance the performance of nanozymes, such as the design of nanomaterials with specific characteristics or the further modification of properties like size, morphology, and shape. Similarly, the obtention of higher catalytic activities and increased selectivity of nanozymes have been pursued by generating hybrid materials, adding coatings, and surface modifications (Xu et al., 2021).

A recent study reported a new strategy for the preparation of a BSA-Cu-based laccase-mimicking nanozyme using ionic liquids; it showed enhanced stability and catalytic performance under extreme conditions of temperature, salinity, and pH values for many substrates, such as dopamine, 2, 4-dichlorophenol (2, 4-DP), guaiacol, epinephrine, and guaiacol (Huang et al., 2022). Similarly, a novel and not commonly explored strategy based on a series of multivalent Ce-MOFs, Ce-UiO-66 and Ce-MOF-808, showed great stability and enhanced efficiency for the degradation of 2, 4-DP; those results were significantly superior in comparison to those exhibited by laccase enzyme (Liang et al., 2022). Some other novel nanozymes can act in a reaction cascade, showing the activity of more than one enzyme with a higher response in terms of efficiency. For example, single Fe atom nanozymes (Fe SAEs) exhibited outstanding oxidase, peroxidase, and catalase activities. The nanozyme removed phenol from aqueous solutions with an efficiency of 83% within 30 min (Zhao et al., 2019).



**Table 1.** Recent nanozyme-based developments for the removal of dyes.

Nanozyme	Enzyme-mimicking activity	Dye	Degradation efficiency (%)	Reference
CNZ	Peroxidase	MO	93	(Geng et al., 2021)
Fe <sub>3</sub> O <sub>4</sub> NPs	Peroxidase	IC	99	(Zha et al., 2022)
Co-MIONzyme	Peroxidase	MB	18	(Mansur et al., 2022)
Cu <sup>2+</sup> -HCNSs-COOH	Peroxidase	MB	80.7	(Zhu et al., 2021)
W <sub>18</sub> O <sub>49</sub> NSs	Peroxidase	MB	91	(Zhu et al., 2018)
CoSe <sub>2</sub> MS	Peroxidase	MG	99.45	(Khagar et al., 2021)
Fe <sub>3</sub> O <sub>4</sub> @Cu/GMP	Lacasse	OPD	90	(Zhang et al., 2020)
Cu/H <sub>3</sub> BTC MOF	Lacasse	AB-10B	90	(Shams et al., 2019)
Cu-TA	Lacasse	MG	90	(Ge et al., 2021)
FeBi-NC SAzyme	Oxidase	RhB	100	(Chen et al., 2022)
Au-Au/IrO <sub>2</sub> nanocomposite	Peroxidase and glucose oxidase	RhB	98.16	(Zhong et al., 2021)
		MB	92.78	(Liu et al., 2020)
		MO	97.17	(Le et al., 2022)
Co <sub>3</sub> O <sub>4</sub> -g-C <sub>3</sub> N <sub>4</sub>	Peroxidase	RhB	90.2	(Liu et al., 2020)
H-Mn-Cu NFs	Laccase	CV	90	(Le et al., 2022)
		NR		
		RhB		

Abbreviations: CNZ (cooper nanozyme); Fe<sub>3</sub>O<sub>4</sub> NPs (polymer-coated Fe<sub>3</sub>O<sub>4</sub> nanozymes); Co-MIONzyme (Co-doped iron oxide nanozymes); Cu<sup>2+</sup>-HCNSs-COOH (Cu<sup>2+</sup>-modified carboxylated hollow carbon nanospheres); W<sub>18</sub>O<sub>49</sub> NSs (W<sub>18</sub>O<sub>49</sub> nanospheres); CoSe<sub>2</sub> MS (CoSe<sub>2</sub> microsphere); Fe<sub>3</sub>O<sub>4</sub>@Cu/GMP (Fe<sub>3</sub>O<sub>4</sub>@Cu/GMP guanosine 5'-monophosphate nanozyme); Cu/H<sub>3</sub>BTC (Copper and 1,3,5-benzene tricarboxylic acid nanozyme); Cu-TA (Copper and tannic acid hybrid composites single-atom nanozymes); FeBi-NC SAzyme (Fe-Bi bimetallic MOF-derived carbon single-atom nanozymes); g-C<sub>3</sub>N<sub>4</sub> (graphite carbon nitride); H-Mn-Cu NFs (Manganese dioxide-copper phosphate hybrid nanoflowers); MO (methyl orange); IC (indigo carmine); MB (Methylene blue); OPD (o-phenylenediaminex); AB-10B (amido Black 10B); MG (malachite green); RhB (rhodamine B); CV (Crystal violet); NR (neutral red).

## Challenges and key recommendations

Regardless of current technological advancements in chemical and biological processes, microbial bioremediation is a sustainable method with excellent execution, low maintenance expenditures, exceptional process selectivity and adaptability, and no or minimal generation of byproducts. Nanotechnological implementation and functionalization or modification strategies are also recommended to improve pollution elimination. Such an approach can also facilitate microbial remediation of polluting agents or the induced production of remediating microbial enzymes (Benjamin et al., 2019; Khaliq 2023; Yagnik et al., 2023).

Regardless of the manifestation of microorganisms in a water stream, their action is repeatedly perceived as ineffective. This lack can be directly or indirectly ascribed to a deficiency of nutrients such as phosphorus or nitrogen (Azubiike et al., 2016).

Although nanozymes represent effective alternatives for monitoring and degrading several emerging pollutants, either presented in wastewater or leachates, there is still a lack of data to compare results. In this respect, an ample and diverse range of emerging pollutants should be tested to degrade; complex water samples should be used in experimentation, in which a combination of different pollutants and co-existing substances can occur. Finally, the toxicity of nanozymes has received increased research attention recently due to their broad applicability in many fields and their potential adverse effects on the environment and the population's health. The biosafety and biocompatibility of nanozymes –even carbon-based nanozymes, which are typically biocompatible– should be carefully reviewed (Lopez-Cantu et al., 2022b). Applying nanozymes to the degradation of pollutants present in the environment involves the interaction of the nanozyme with natural systems and multiple organisms. Therefore, *in vivo* and *in vitro* toxicity tests should be performed using various concentrations and applied to different biological models. A detailed understanding of nanozymes in composition, shape, and size is required to define further their effect on toxicity, biodistribution, and *in vivo* uptake; which should be considered for future studies in the field. Nanomaterials also face these concerns whether they possess enzyme-like activities or not. Thus, significant advancements in this regard are expected in the near future.

## CONCLUSIONS

In conclusion, microbial bioremediation-assisted environmental cleanup has become increasingly popular in recent years, providing a natural and sustainable alternative to traditional remediation techniques. In addition, microbial bioremediation is also considered a relatively low-cost method of environmental cleanup. Owing to the complete breakdown within the natural microbial process, it does not require removing and disposing of contaminated material, which is otherwise considered expensive and disruptive in other traditional techniques. In turn, this ultimately lowers the risk of further contamination and thus helps to promote and maintain a healthy and balanced ecosystem. Furthermore, the extensive use of harsh chemicals/reagents in chemical-based remediation measures can pose adverse side effects.

## Conflict of interests

The authors declare no conflict of interest.

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