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Review

Enzyme sources in wastewater treatment: Their influence on enzymatic bioremediation and large-scale applications

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ABSTRACT

Enzymes have recently garnered increased attention in wastewater treatment, and enzyme sources are critical for enzymatic applications, influencing enzyme types, abilities, production strategies, costs, large-scale applications, and other factors. However, existing studies on enzyme sources for wastewater treatment are fragmented, often concentrating on specific enzyme sources or their applications rather than offering a comprehensive overview. Additionally, research on scaling up enzymatic wastewater treatment remains limited. This study addresses these gaps by providing a comprehensive analysis of enzyme sources and their feasibility in wastewater treatment. Enzymatic wastewater treatment primarily relies on enzymes derived from animals, plants, microorganisms, organic waste, and synthetic sources, each offering distinct advantages and limitations. Commonly used enzymes in sewage bioremediation include lipases, proteases, laccases, peroxidases, glucose oxidases, and nanozymes, each with specific functional mechanisms, applications, and origins. While commercial enzymes are available from various sources, high costs and stability issues remain significant barriers to large-scale application. To address these challenges, this study establishes key criteria for scale-up and evaluates the feasibility of different enzyme sources in large-scale applications. Finally, perspectives on the challenges of enzymes in wastewater treatment regarding enzyme sources and future research directions are proposed to facilitate the industrial applications of enzymatic wastewater treatment.

1. Introduction

The earth experienced a sharp population increase during recent decades, leading to a subsequent increase in wastewater generation. Consequently, wastewater treatment has been one of the top priority missions to protect our surviving environment. However, activated sludge, the most prevalent wastewater treatment method worldwide, is not considered a green and energy-effective strategy since it is an energy-intensive process and produces a large amount of waste sludge that adversely impacts the surroundings. To overcome these issues, various strategies have been developed for effective wastewater treatment, such as membrane bioreactor [1,2], microalgae [3–5], reverse osmosis [6,7], photocatalysis [8], and enzyme-based wastewater treatment [9,10].

Enzymes are biocatalysts that can effectively biodegrade organic pollutants in wastewater, such as proteins, oils, greases, pesticides, pharmaceuticals, personal care products, and phenolic compounds. Since the diverse nature of enzymes and their distinct optimal working conditions, current research focuses on optimizing the conditions to enhance enzymatic performance on pollutant removal [11,12]. Moreover, the enzymatic systems are crucial to the wastewater treatment efficiency. Free enzyme systems, immobilized enzymes, and enzyme mediators-related enzyme systems are the most common enzymatic systems in wastewater treatment [9]. However, enzymes are vulnerable and might be biodegraded by microorganisms easily in free enzyme systems, while immobilized enzyme systems could overcome this issue.

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Immobilization reuses enzymes, extends the lifespan of enzymes, reduces loss of enzyme activity, and enhances resistance to pH and temperature [9]. Therefore, recent researchers are focusing on effectively immobilizing enzyme and maintaining their activity, stability, and reusability by exploring new matrices and suitable enzyme immobilization methods [13–15]. Furthermore, enzymes with high abilities on a specific substrate/reaction have been explored, such as artificial enzymes [16,17] and engineered enzymes [18]. Besides being used as catalysts for biodegrading organic pollutants, enzymes also have applications in bacterial disinfection [19–21] and as biosensors for monitoring specific pollutants in wastewater [22–24].

Since enzymes are essential for various life activities, the functions of enzyme sources decide the enzyme types they contain. For instance, digestive organs contain hydrolases such as lipases and proteases, while microorganisms capable of biodegrading cellulose could produce cellulase. Therefore, during enzymatic wastewater treatment, researchers tend to acquire enzyme sources from the targeted substrate-polluted samples [25,26]. It is expected to observe distinct working conditions and biodegradation performance on a specific substrate for the same type of enzyme but from different sources. For instance, laccase from *Weissella viridescens* LB37 and *Coprinopsis cinerea* exhibited distinct optimal working conditions when decomposing dyes from wastewater [27,28]. The microbial lipases achieved higher animal fat hydrolysis rates than animal lipase under the same conditions [29]. Therefore, enzyme sources significantly influence both the enzymatic working conditions and the performance of enzymes. Enzyme sources also impact the cost of enzyme production as various enzyme source materials differ in price and transportation cost. Furthermore, enzyme production methods vary based on the materials used. The composition of enzyme source materials influences cell disruption methods, and impurities determine both chemicals used during enzyme extraction and techniques employed for enzyme purification. Thus, enzyme sources are crucial in shaping diverse aspects of enzyme production methods.

Current research on enzyme sources primarily focuses on identifying specific enzymes applicable to wastewater treatment, such as peroxidase and laccase, or exploring the use of particular enzyme sources for this purpose [30,31]. However, a comprehensive overview of enzyme sources suitable for wastewater treatment remains lacking. Additionally, most studies on enzymatic wastewater treatment are conducted at the laboratory scale. While some research has been extended to pilot and large-scale applications [32,33], such studies remain limited.

This review summarizes research on enzymatic wastewater bioremediation published between 2014 and 2024, primarily sourced from the ScienceDirect database. Only studies that directly employed enzymes for wastewater treatment were included. The search terms used were: “enzyme, wastewater treatment,” “enzymatic wastewater treatment,” “water, enzyme, bioremediation,” “enzyme, water purification,” “lipase, wastewater,” “laccase, wastewater,” “peroxidase, wastewater,” “garbage enzyme, wastewater treatment,” “glucose oxidase, wastewater,” “protease, wastewater,” “nanozyme, wastewater,” “large-scale enzymatic wastewater treatment,” and “pilot-scale enzymatic wastewater treatment,” among others. After applying the selection criteria, approximately 250 publications were included in this review.

This article provides a thorough view of the impacts of enzyme sources on enzymatic wastewater treatment. It introduces feasible enzyme sources for wastewater treatment, encompassing both conventional and novel sources. Moreover, commonly used enzymes in wastewater treatment and their function mechanisms, sources, and applications are reviewed. Additionally, scale-up studies on enzymatic wastewater treatment and the influence of enzyme sources are examined. Finally, the challenges associated with enzyme sources in enzymatic wastewater treatment are discussed, and future research directions are proposed.

2. Feasible enzyme sources

2.1. Traditional enzyme sources

2.1.1. Animals

Animal tissues or organs have complex functions and typically contain multiple enzymes, making animals great enzyme sources. Currently, animal enzymes are used in the food and pharmaceutical industries, among other areas. However, they have limited applications in wastewater treatment (Table 1). Lipases are the predominant animal enzymes found in wastewater, with most being derived from porcine pancreases due to their relatively low cost [34]. Compared to plants and microorganisms, animals have more extended growth periods. Currently, animal protection has attracted much attention, especially concerning endangered animals. This raises ethical concerns and reduces the available animal species that can be used for enzyme production. Due to the complexity of animal tissues, one tissue may contain various types of enzymes. The unwanted enzymes are considered impurities, leading to high purification costs. For instance, the semi-purified porcine pancreas lipase is expensive since the esterase, amylases, proteases, and other hydrolases in the porcine pancreas can act as impurities [35]. Therefore, despite the numerous types of enzymes that could be isolated from animals, they are not a suitable enzyme source for wastewater treatment.

2.1.2. Plants

Plants are one of the essential enzyme sources for wastewater treatment. Peroxidases are one of the most studied plant enzymes in wastewater bioremediation, with horseradish as the most common enzyme source. Other plants, such as soybean [44,45], *brassica rapa* [54], *Cedrela fissilis* leaves [50], cabbage leaves [49], *prosopis juliflora* [47], and tea leaves [38], have also been used as peroxidase sources for the removal of dyes, phenolic compounds pharmaceuticals, and other emerging pollutants from wastewater [38–40,42]. More examples are shown in Table 1. There are also studies using plants as enzyme sources to produce laccase, polyphenol oxidase, and other enzymes for wastewater treatment [36–38], but are scarce. Compared to animals, plants grow faster, are widespread, and are easily accessible. However, some plant tissues may contain impurities that adversely affect enzyme activity and stability. For instance, the presence of phenolic compounds in plants can interact with proteins, forming a brown colour, changing protein structures, and decreasing protein solubility [56]. Plant cells, especially fruit cells, contain more soluble polysaccharides, which are difficult to separate. Moreover, plant cells are more difficult to disrupt than animal and microorganism cells due to their rigid cell walls and multicellular nature.

2.1.3. Microorganisms

Microorganisms are widespread enzyme sources commonly used for commercial enzyme production. Microorganisms serving as enzyme source has several advantages. Firstly, they are easy to culture and yield high due to their rapid growth rate. Secondly, microorganisms can be easily selected and modified compared to animals and plants due to their diverse species/functions and simple cell structures. Thirdly, microorganisms, as decomposers, can produce diverse enzymes to degrade different compounds in wastewater. *Candida rugosa*, *Pseudomonas aeruginosa* UKHL1, *Aspergillus oryzae*, and other microorganisms have been used as lipase sources for the degradation of oils and greases from wastewater [57–59]. Laccases are essential enzymes in wastewater bioremediation, with *Trametes versicolor* being the most common source of laccases (Table 2). Additionally, peroxidase can be derived from *Serratia* sp. AXJ-M, *Phanerochaete chrysosporium*BKMF-1767, *Streptomyces coelicolor*, and other species [60–62]. *Aspergillus niger* is a primary enzyme source for glucose oxidase production [63–65]. Garbage enzyme is a fermented product derived from a mixture of jaggery or molasses, organic waste (e.g., vegetable and fruit peels), and water,

Table 1
Typical animal and plant enzymes employed in wastewater treatment.

Enzyme types	Enzyme sources	Enzyme systems	Enzyme dosage	Applications	References
Lipase	*Porcine pancreas*	Free enzyme	50–2000 μL 4.76 wt% of lipase solution	Animal fat hydrolysis	[29]
Lipase	*Porcine Pancreas*	Immobilized enzyme	0.5 g of immobilized lipase	Azo dye removal	[34]
Laccase	*Malva parviflora*	Free enzyme	760 U/mL	Dye degradation	[36]
Laccase	*Rhus vernicifera*	Crosslinked with functionalized carbon nanotubes (FCNTs)	1 mg of laccase was added to 20 mL of FCNTs aqueous solution, and then 10 μL of the mixture was applied to each electrode	Biosensor, for *para*-cresol detection	[37]
Peroxidases and polyphenol oxidase	Green tea	Immobilized on nanoparticles	0.8 U/mL	Phenol removal	[38]
Peroxidase	Horseradish	Immobilized on azacalix[4]arene-based covalent organic framework (COF)	0.8 nM	7 kinds of emerging pollutants removal	[39]
Peroxidase	Horseradish	Immobilized on cationic maize starch	4U/mL	Azo dye decolorization	[40]
Peroxidase	Horseradish	Immobilized on a g-C_3N_4-based photocatalyst ACN_{45}	20 mg	Bisphenol A removal	[41]
Peroxidase	Horseradish	With mediator 2,2-Azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS)	0.6 U/mL	Tetracycline degradation	[42]
Peroxidase	Horseradish	Free enzyme	0.01–0.30 U/mL	Triclosan degradation	[43]
Peroxidase	Soybean	Free enzyme	0.01–0.30 U/mL	Triclosan degradation	[43]
Peroxidase	Soybean	Free enzyme	0.004–0.050 U/mL	Arylamines degradation	[44]
Peroxidase	Soybean	Free enzyme	For 3-Hydroxyquinoline: 0.1 U/mL; For 3-amino-quinoline: 5.0 U/mL	3-Hydroxyquinoline and 3-aminoquinoline degradation	[45]
Peroxidase	Soybean	Immobilized on silica-coated superparamagnetic iron oxide nanoparticles	9.02×102^{-9} M	Dye degradation	[46]
Peroxidase	*Prosopis juliflora*	Free enzyme	0.5 U/mL	Phenol removal	[47]
Peroxidase	*Raphanus sativus* var. *niger*	Immobilized enzyme	0.1 to 1.8 U/mL	Phenolic compounds removal	[48]
Peroxidase	Cabbage leaves	Immobilized enzyme	5 mg of immobilized enzyme	Dye decolorization	[49]
Peroxidase	*Cedrela fissilis* leaves	Free enzyme	10 mL of partially purified peroxidase was added to 100 mL of dye solution	Dye decolorization	[50]
Peroxidase	Cauliflower	Free enzyme	Initial enzyme activity was 0.4 U	Dye degradation	[51]
Peroxidase	*Zanthoxylum armatum* DC. fruit	Free enzyme	200 U/mL	Phenol removal	[52]
Peroxidase	Turkish black radish	Peroxidase-Cu^{2+} hybrid nanoflowers and free enzyme	The same concentrations of hybrid nanoflower (4.04 U/mg) and free enzyme (0.89 U/mg) were each added to a working volume of 3 mL, respectively	Dye decolorization	[53]
Peroxidase	*Brassica rapa*	Free enzyme	0.14 U/mL	Azo dye decolorization	[54]
Peroxidase	Ginger	Immobilized on polypyrrole-cellulose-graphene oxide nanocomposite	0.08 U/mL	Dye degradation	[55]

The data in this table are sourced from the ScienceDirect database. The search keywords are provided in the last paragraph of the Introduction. Only research articles published between 2014 and 2024 that directly applied animal and plant enzymes in wastewater treatment were considered.

typically in a ratio of 1:3:10 (v/w) under anaerobic conditions over approximately three months [66,67]. Rich in microbial enzymes such as lipase, amylase, protease, and other hydrolases [68], garbage enzyme has potential applications in wastewater treatment (Table 2).

Beyond these benefits, extremozymes produced by microorganisms exhibit exceptional stability and catalytic efficiency under harsh environmental conditions. For instance, thermophilic amylase can be produced by *Pyrococcus furiosus, Desulfurococcus mucosus, Pyrococcus woesei, Pyrodictium abyssi, Staphylothermus marinus, Thermococcus profundus, Dictyoglomus thermophilum,* and *Thermotoga maritima* [69]. Thermophilic lipase is produced by *Geobacillus* sp. *EPT9* and *Geobacillus* sp. *12AMOR1* [70], while metallophilic lipase is produced by *Ralstonia solanacearum* [71]. Additionally, alkaliphilic *Bacillus* strains can produce alkaline α-amylase and protease [72]. The remarkable resilience of these enzymes under extreme conditions highlights their potential for applications where conventional enzymes may lose activity.

Microorganisms are promising enzyme sources but having some drawbacks. Although bacteria, fungi, and microalgae are all feasible as enzyme sources, the same enzyme type produced from different microorganisms has varying performance. Therefore, for specific industrial applications, it is necessary to identify suitable microorganisms from a large database. Sometimes, the isolated microorganism may exhibit unsatisfactory enzyme yield and ability. To address this issue, modification methods such as random mutation, genetic engineering, and optimizing culture conditions can enhance enzyme yield and ability [73,74]. However, using modified microorganisms could elevate safety concerns for the environment and living organisms due to the unintended consequences caused by the edited genes. Therefore, proper management is essential when dealing with genetically modified microorganisms. Moreover, preventing the contamination of other microorganisms during cultivation is necessary, as it can result in increased impurities and higher purification costs.

2.2. New enzyme sources

2.2.1. Organic waste

The utilization of organic waste as an enzyme source represents an innovative extension of traditional enzyme sources, offering sustainable solutions for wastewater treatment. Waste animals, waste plants, and waste microorganisms are all organic waste that can serve as enzyme sources, with waste plants and waste microorganisms as primary candidates. Waste plants mainly produce peroxidases, while enzyme mixtures are commonly made from waste microorganisms. Plant waste, such as luffa skin peels, potato peel, and soybean hulls, is viable for producing

Table 2
Microbial enzymes for wastewater treatment.

Enzyme types	Enzyme sources	Source of microbial	Enzyme systems	Enzyme dosage	Applications	References
Lipase	*Candida rugosa*	A gift sample	Free enzyme	0.2 % (w/v) enzyme loading	Diary wastewater treatment	[59]
Lipase	*Candida rugosa*	Provided by Biofuels by Biocatalysts Research Unit	Free enzyme	20.2 U/mL	Triglycerides degradation	[75]
Alkaline protease	*Bacillus pseudofirmus* SVB1	Isolated from tannery industry	Free enzyme	185 U/mL	Dairy industry and domestic sludge effluent treatment	[19]
Protease	*Lysinibacillus macroides*	Isolated from tannery soil-assisted tannery-total dissolved solids wastewater	Immobilized on EDA/ glutaraldehyde functionalized nanoporous activated carbon	294 U/g	Proteins degradation	[76]
Protease	*Bacillus Cereus* Strain KM201428	Did not mention	Free enzyme	50 mL working volume with 10 mL crude protease extract	Dye degradation	[77]
Laccase	*Trametes versicolor*	Isolated from a Park	Free enzyme	280 U/mL	Blue wastewater biodegradation	[78]
Laccase	*Trametes versicolor*	Purchased	Immobilized on $Ni_3(PO_4)_2$ hybrid nanoflowers	0.05 U/mL	Antibiotic degradation	[79]
Laccase	*Trametes versicolor*	Supplied by Labs	Free enzyme	Laccase concentration: 0.03–0.6 g/mL	Ibuprofen removal	[80]
Laccase	*Trametes versicolor*	Purchased	Immobilized on carbon nanotube nanocomposites (CNTs)	0.03 g/L	Dye removal	[81]
Laccase	*Trametes versicolor*	Purchased	Immobilized on 3D printed polylactide scaffolding	3D printed polylactide scaffolding with 20 mg laccase was added to 50 mL of wastewater	Estrogen removal	[12]
Laccase	*Myceliophthora thermophilia*	Purchased	Immobilized on acrylic modified kraft lignin microspheres	50 mg immobilized laccase	Dye degradation	[82]
Laccase	*B. amyloliquefaciens* LC02	Did not mention	Immobilized on magnetic zeolitic imidazolate frameworks	100 U/L	Dye decolorization	[83]
Peroxidase	*Streptomyces coelicolor*	Isolated from the mangroves of Jaladi Karnataka State, India.	Free enzyme	100 U/L	Dye decolorization	[61]
Peroxidase	*Serratia* sp.	Supplied by a lab	Free enzyme, mediated by ABTS	0.2 mg/mL	Textile wastewater treatment	[60]
Peroxidase	*P. chrysosporium* BKMF-1767	Purchased	Free enzyme	0–80 U/L	Sulfamethoxazole oxidation	[62]
Manganese peroxidase	*Meyerozyma caribbica* SSA1654	Isolated from termite gut symbionts	Free enzyme	23–27 U/mL	Dye degradation	[84]
Glucose oxidase	*Aspergillus niger*	Did not mention	Free enzyme	100 U/mL	Trace organic contaminants removal	[64]
Glucose oxidase	*Aspergillus niger*	Obtained from National Collection of Industrial Microorganisms (Pune, India)	Free enzyme	100–1000 U/L	Pharmaceutical degradation	[63]
Glucose oxidase	*Aspergillus niger*	Purchased	Immobilized on 2D planar nanobiocatalyst composed of graphene oxide (GO)	Maximum enzyme activity: 168 ± 7.94 units/mg-GO; Immobilized enzyme loading: 0.02–0.10 g-GO/m^2	Membrane antifouling	[85]
Garbage enzyme	Anaerobic microbial	Fermented mixture of jaggery, organic waste, and water in the ratio 1:3:10	Free enzyme	5–20 % (v/v) of total volume	Bioremediation of urban municipal landfill leachate	[86]
Garbage enzyme	Anaerobic microbial	Fermented mixture of jaggery, fruitpeels (pineapple, banana, orange and lemon) , and water in a ratio of 1:3:10	Free enzyme	5–20 % (v/v) of total volume	Bioremediation of landfill leachate	[87]

The data in this table are sourced from the ScienceDirect database. The search keywords are provided in the last paragraph of the Introduction. Only research articles published between 2014 and 2024 that directly applied microbial enzymes in wastewater treatment were considered.

peroxidase for wastewater treatment (Table 3). Moreover, enzymes can be extracted from waste microorganisms, primarily from activated sludge (Table 3). When enzymes derived from activated sludge are used in wastewater treatment, they are typically applied as cell lysates, which contain a variety of enzymes, including phosphatase, β-galactosidase, β-glucuronidase, and acetate kinase [88,89]. The rapid population growth produces more organic waste, making it an abundant enzyme source. Therefore, utilizing these wastes as enzyme sources does not exacerbate resource crisis and represents an environmentally friendly strategy to reduce material costs for enzyme production. It is crucial to analyse the presence of organic wastes and use them to replace traditional plant materials for commercial enzyme production.

2.2.2. *Artifical enzyme*

Animals, plants, microorganisms, and organic waste can act as enzyme sources based on the foundation of a good understanding of

Table 3
Enzymes from organic waste for wastewater treatment.

Enzyme types	Enzyme sources	Enzyme systems	Enzyme dosage	Applications	References
Peroxidase	Potato peel	Free enzyme	0.1–1 U	Anthraquinone dye removal	[90]
Peroxidase	Soybean hull	Free enzyme	0.1–1 U	Anthraquinone dye removal	[90]
Peroxidase	Soybean hull	Free enzyme	Working volume: 15 mL; Peroxidase: 1.75 mL (0.373 U/mL)	Dye degradation	[91]
Peroxidase	Soybean hull	Immobilized on chitosan	0.012 ± 0.003 g (0.041 U/bead or specific activity 2.51 U/mg)	Phenolic compounds degradation	[92]
Peroxidase	luffa skin peels	Free enzyme	Working volume: 15 mL; Peroxidase: 1.75 mL (0.373 U/mL)	Dye degradation	[91]
Peroxidase	Onion dry scales	Free enzyme and immobilized on silica nanoparticles	Free enzyme: 20 mL of working volume with 5 mL of prepared enzyme extract Immobilized enzyme: reused for 50 times	Phenol removal	[93]
Peroxidase	Waste cabbage	Mediated by ABTS	0–8 mL of waste cabbage peroxidase was added to the reaction system	Phenol and synthetic dye degradation	[94]
Peroxidase	Soybean seed coat	Immobilized enzyme	135 g of beads containing 15 g immobilized soybean seed coat powder	Phenol removal	[95]
Enzyme mixture	Activated sludge	Free enzyme	13–15 μL	Micropollutants degradation	[89]
Enzyme mixture	Activated sludge	Free enzyme	20 μL of an organic micropollutant mixture was added to 100 μL of enzyme-containing lysate	Organic micropollutants degradation	[88]

The data in this table are sourced from the ScienceDirect database. The search keywords are provided in the last paragraph of the Introduction. Only research articles published between 2014 and 2024 that directly applied organic waste (e.g., wasted fruit, vegetable, and activated sludge) enzymes in wastewater treatment were considered.

cellular metabolisms. However, artificial enzymes may be a better option when there are unclear biosynthetic pathways and low production of targeted enzymes [96]. The synthesis of artificial enzymes can be accomplished by both protein engineering and *de novo* protein design. Artificial enzymes possess many advantages. Firstly, it can boost the yield of low-production enzymes. Secondly, new enzymes that have not been found can be designed to catalyse a specific reaction. For instance, *de novo* protein design and random mutation can be used for the development of new enzymes with desired functions [96]. Thirdly, the enzyme characteristics, such as selectivity, stability, and function could be modified, resulting in more stable structures and better catalytic abilities [96]. Fourthly, some studies aim at designing enzymes with more than one active site [97,98], which means one enzyme can catalyse more than one or one kind of reaction, leading to wider applications.

Despite experiencing a significant bloom recently, the development of artificial enzymes is hindered by some challenges. The process of enzyme synthesis is complex and hard to scale-up as it is associated with DNA-scaffold technology [99]. Sometimes, the synthetic enzymes may exhibit decreased enzyme activity, leading to lower catalytic efficiency [99]. The synthesis of artificial enzymes with multiple activity sites is a promising research direction. Unfortunately, there are only a few successful examples of artificial metalloenzymes [100–102] and more efforts are necessary.

Nanozymes, nanomaterials that mimic natural enzymes and have high catalytic ability, are also artificial enzymes [103,104]. These artificial enzymes can be easily synthesized, and exhibit the advantages of cost-effective, higher stability, easy surface modification, and simple preparation [104,105]. Due to these advantages, more researchers start to employ nanozyme for wastewater treatment. Current nanozymes that are used in wastewater treatment typically have mimicking activities of peroxidase, oxidase, and other enzymes. It has been reported that the Cu@SB nanozyme with peroxidase-like activities effectively activated H_2O_2 to catalyse amlodipine [106]. The nanogel-based artificial enzyme constructed by Shi et al. [107] exhibited mimetic activity similar to horseradish peroxidase. The activity of the nanozyme could be switched on/off through adjusting the operating temperature, and it is capable of degrading several azo compounds. Yang et al. [16] synthesized a nanozyme, CeO_2@ZIF-8, that exhibits excellent peroxidase-like activity. Despite nanozymes are promising in wastewater treatment, their activities are limited to peroxidase, oxidase, catalase, and oxidoreductase (Table 4). Normally, nanozymes exhibit lower activities than the natural enzymes, although there are some nanozymes possess higher activities than natural enzymes. Moreover, the narrow optimal working pH range also hinders the applications of nanozymes in wastewater treatment [108]. Therefore, more efforts are mandatory for the better applications of nanozymes in wastewater bioremediation.

3. Mechanisms and applications of typical enzymes in wastewater treatment

3.1. Lipases

Wastewater from animal production, meat production, slaughterhouses, the oil industry, and other sources typically contains many oils and greases, adversely affecting the aquatic environment if discharged without proper treatment [9]. Enzymes employed to biodegrade oil and grease-containing wastewater are lipases. Lipases exhibit various activities, such as aminolysis, acidolysis, interesterification, alcoholysis, and esterification [121]. However, the hydrolysis function of lipases is the key when treating wastewater. A study identified and isolated a lipase, PersiLipase1, from tannery wastewater. The results showed that this lipase biodegraded 91 ± 1 % of oil and grease from tannery wastewater [26]. After immobilizing the lipase from *Aspergillus oryzae* on an α-alumina membrane used for oily wastewater treatment, the membrane's ability for antifouling and self-cleaning was enhanced due to the hydrolysis ability of lipase [58].

Lipases catalyze the hydrolysis of ester bonds in triglycerides [122]. During this process, oils and greases are initially broken down into diacylglycerol by lipases, followed by further hydrolysis of diacylglycerol into monoacylglycerol by diacylglycerol lipases [123]. Finally, under the function of monoglyceride lipase, the oils and greases are converted into glycerol and free fatty acids (Fig. 1) [124]. Lipases can hydrolyze substrates containing short-, medium-, and long-chain fatty acid esters; however, they preferentially target long-chain fatty acid esters with more than ten carbon atoms [125]. (Fig. 1) [124]. Substrates containing short-, medium-, and long-chain fatty acid esters can all be hydrolysed by lipases, but lipases preferentially hydrolyse those with long-chain fatty acid esters exceeding ten carbons [125]. Lipases can be found in animals, plants, and microorganisms. However, studies on employing lipase for wastewater bioremediation are mainly

Table 4
Nanozymes in wastewater treatment.

Nanozyme	Mimicking activity	Applications	References
Sulfur doped graphdiyne nanosheets	Peroxidase	The degradation of dyes and antibiotics; antibacterial	[21]
Nanogel-based artificial enzymes	Peroxidase	Dye degradation	[107]
Ferromagnetic chitosan nanozyme	Peroxidase	Phenol decomposition	[109]
Gold nanoparticles decorated MoS_2 nanosheets	Peroxidase	Sensing and degradation of carbamazepine	[17]
Amorphous cobalt hydroxide/oxide-modified graphene oxide	Peroxidase	Biosensor, for the detection of cyanide ions	[110]
A copper-containing nanozyme	Peroxidase	Organic dye degradation	[111]
CoZn zeolitic imidazolate framework@10-(diethylamino)-3-hydroxy-5,6-dihydrobenzo [c] xanthen-12-ium	Peroxidase	Biosensor, for the detection of detection of phosphate ions, o-phenylenediamine, and benzaldehyde	[112]
Pt deposited on sea urchin-like $CuCo_2O_4$ nanostructures	Peroxidase	Biosensor, for the detection of sulfide ions	[113]
CuS nanoparticles on two-dimensional nanosheets	Peroxidase	Biosensor, for the detection of ibuprofen	[114]
Nanozyme Ag/Fe3O4@hexagonal boron nitride	Peroxidase and oxidase	Dye degradation; As(V) removal and detection	[115]
Gold colloidal nanozymes/Co-doped iron oxide nanozymes	Peroxidase and oxidase	Dye degradation	[116]
Hollow C@MoS_2 nanotubes	Oxidase	Biosensor, for the degradation of detection of Hg^{2+} ions	[117]
Au-NiFe layered double hydroxide (LDH)/rGO nanocomposite	Oxidase	Organic mercury removal and SERS sensing	[118]
Gold nanoparticles	Oxidoreductase	Biosensor, for the determination of Cr (VI)	[119]
$Mn_4(P_2O_7)_3$ nanoflakes	Catalase	Dye removal	[120]

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derived from microorganisms, such as *Pseudomonas aeruginosa* UKHL1, *Aspergillus oryzae*, and *Candida rugosa*, while lipases from animals are scarce (Table 1). To our knowledge, there is no report on plant lipase-based wastewater treatment.

3.2. Proteases

Proteases are hydrolases that can hydrolyse the peptide bond in peptide and protein [126]. They are important biocatalysts in nearly all living processes, and have been used in food, pharma, detergent, leather, and other industries [127]. These enzymes have been used in wastewater treatment. They are mainly derived from microorganisms, such as *Bacillus pseudofirmus* SVB1 [19], *Enterococcus faecalis* [128], *Lysinibacillus macrolides* [76], and *Meiothermus taiwanensis* WR-220 [129].

Protease applications in wastewater treatment involve the biodegradation of protein contaminants and disinfection. It was reported that the immobilized protease from *Enterococcus feacalis* degraded 91 % of protein in synthetic tannery saline wastewater [128], while the immobilized protease from *Lysinibacillus macroides* completely hydrolysed the protein in tannery high total dissolved solids-containing wastewater under the conditions of 90 min, pH 6.0 and 30 ℃ [76]. Immobilized keratinase from *Meiothermus taiwanensis* WR-220 achieved a decolorization yield of 85–90 %. Additionally, it removed 61–70 % of chemical oxygen demand (COD) and 60–66 % of colorants in 5 days [129]. Moreover, proteases are also feasible for dye decolorization. A study employed crude protease from *Bacillus Cereus* KM201428 for the biotransformation of an azo dye Reactive Black 5. The results illustrated that the crude enzyme influenced the biodegradation and detoxification of azo dye Reactive Black 5, and the optimum conditions were 120 h, pH 9.0, and 25 ℃ [77]. The protease from *Aspergillus oryzae*, at a concentration of 88 mg/L, removed 4.0 % of the initial polyethylene bead mass under thermophilic conditions (55 °C), demonstrating its potential for wastewater treatment [130].

Furthermore, proteases could be utilized to enhance the performance of biofilm. Dosing with proteases, amylases, and lipases mixture to the anaerobic membrane bioreactor daily at the range from 0.9 to 1.8 mL/g of influent COD, the total and volatile suspended solids decreased by about 19 % and 22 %, respectively. Meanwhile, biogas production increased by approximately 26 % [131]. Huang et al. [132] utilized a protease and amylase combination in a 4:1 ratio to enhance the performance of aging biofilm. The results showed that the enzymatically treated biofilm improved COD and ammonia nitrogen removal performance.

Interestingly, proteases are also feasible for bacterial disinfection. A study reported that the alkaline proteases extracted from *Bacillus pseudofirmus* SVB1 could reduce the COD, biological oxygen demand, and total dairy and sewage sludge effluent solids. Also, it effectively lysed and suppressed the growth of both Gram-negative and Gram-positive bacteria, such as *Pectobacterium carotovorum*, *Bacillus coagulans*, *Wautersia eutropha*, and *Serratia marcescens* [19].

3.3. Laccases

Laccases are multifunctional enzymes that can oxidise many phenolic and non-phenolic compounds due to their low substrate specificity [133]. Belonging to the multi-copper oxidases family, they utilize oxygen as an electron acceptor in the catalytic process, generating water [31]. A laccase molecule contains four copper sites, namely type 1 (T1, one copper atom), type 2 (T2, one copper atom), and type 3 (T3, two copper atoms) [133]. The T1 site is the primary electron acceptor, and the reducing substrate is oxidised at this site. During this process, the Cu^{2+} is reduced to Cu^{+}; meanwhile, electrons are produced. The electrons are then transferred to the T2/T3 trinuclear site through a highly con-served His-Cys-His tripeptide [134], leading to a fully reduced enzyme state [133].

It should be noted that $4e^-$ is required to activate the enzyme, and the intramolecular electron transfer from T1 site to the T2/T3 trinuclear site is a rate-limiting step in this catalytic reaction. The reduction of the dioxygen molecule occurs at the T2/T3 site. Specifically, two electrons from the T3 site transfer to the dioxygen molecular, forming a peroxide intermediate. Then, the formed peroxide intermediate decays to an oxy radical and the O-O bond is cleaved with the participate of $2e^-$; meanwhile, a water molecular is released simultaneously. The diffusion of dioxygen to the T2/T3 trinuclear site is also another rate-limiting process in this reaction [133,135,136]. Finally, all four coppers in the enzyme are oxidized, and the O^{2-} is converted to the second water molecular. The overall reaction processes are illustrated in Fig. 2. Notably, the radicals generated during this process can participate in either synthetic or degradative reactions [137]. In the synthetic pathway, radicals facilitate the formation of homo- or heteropolymers. In the degradative pathway, they promote oxidation or degradation, often mediated by laccase mediators (e.g., ABTS and HBT) through electron or hydrogen atom transfer. This mechanism enhances the

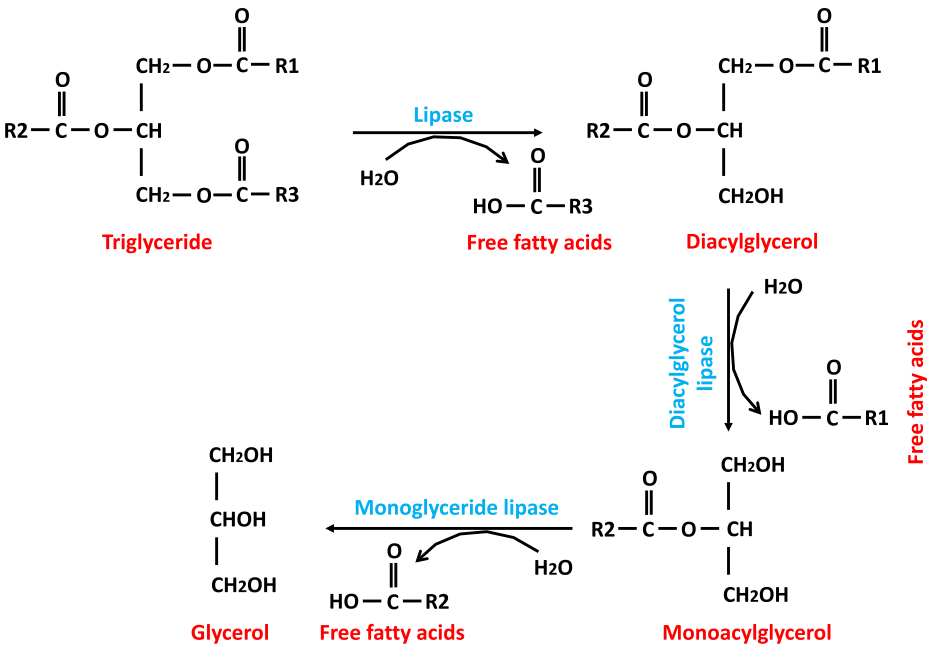

Fig. 1. The reaction scheme of the hydrolysis of triglycerides by lipase.

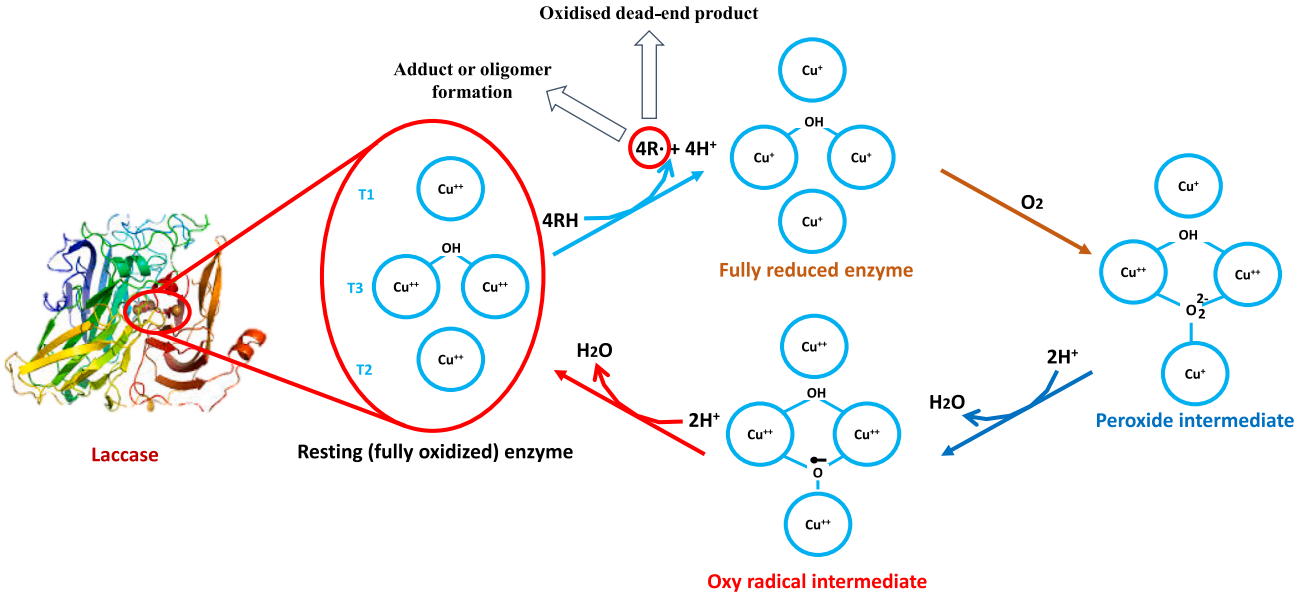

Fig. 2. The functional mechanisms of laccases. RH is the reducing substrate, while R· represents the by-products of RH reduction. Adapted from [133,137].

breakdown of refractory pollutants in wastewater via laccase-mediator systems.

Due to the wide range of substrates, laccases are essential enzymes in wastewater bioremediation and can catalyse the degradation of dyes, phenolic compounds, personal care products, pharmaceuticals, and other micropollutants. The immobilized laccase removed 97 % of Congo red from synthetic wastewater under pH 7.0 and 35 ℃ [138]. A study combined laccase from *P. sanguineus* CS43 with electrooxidation (EO) and electrocoagulation (EC) processes to remove bisphenol A and triclosan from municipal wastewater. The results showed that the EO-laccase system removed 28 % and 93 % of bisphenol A and triclosan, respectively. At the same time, the removal of bisphenol A and triclosan were 76 % and 42 %, respectively, in the EC-laccase system [139]. Masjoudi et al. [140] combined an immobilized laccase from *Trametes hirsuta* with a mini-membrane reactor to remove carbamazepine and diclofenac from synthetic pharmaceutical wastewater. The results demonstrated that this system removed 27 % of carbamazepine in 48 h and 95 % of diclofenac in 4 h, respectively. Cai et al. [141] constructed a microorganism-sustained secreted laccase-straw oxidation system, which removed 99.9 % of As(III) when the initial As(III) concentration of 1.0 mg/L. The immobilized *Tramates versicolor* laccase removed 15 pollutants, including five analgesics, two non-steroidal anti-inflammatory drugs, three antibiotics, two antilipemics, and three pesticides from municipal wastewater at pH 7.0 and 20 ℃ [142]. The laccase demonstrated effective removal, achieving efficiencies ranging from 20 % to 99 %, particularly for 11 of the identified trace organic pollutants, such as acetaminophen, ibuprofen, naproxen, mefenamic acid, ketoprofen, indomethacin, carbamazepine, and others [142].

Laccases are prevalent, and insects, plants, and microorganisms like fungi and bacteria are all feasible laccase enzyme sources. Currently, laccases in wastewater treatment are mainly derived from microorganisms, such as *Trametes versicolor*, *Sulfitobacter indolifex*, *B. amyloliquefaciens* LC02, and *Myceliophthora thermophilia* (Table 2); subsequently, plants. For instance, the laccase from *Rhus vernicifera* was used as biosensor for the real-time analysis of *para*-cresol in wastewater [37]. However, studies that employ insect laccases for wastewater treatment are scarce [31].

3.4. Peroxidases

Peroxidases are essential enzymes in wastewater treatment since they can catalyse the oxidation of a wide range of halide/pseudohalide compounds [143], including dyes, pharmaceuticals, phenolic compounds, and other emerging pollutants (Table 1, 2, and 3). There are various members in the peroxidase family, and soybean peroxidase, horseradish peroxidase, manganese peroxidase, chloroperoxidase, and lignin peroxidase are commonly employed peroxidases in wastewater bioremediation [144]. Despite variations in substrate oxidation ability among different peroxidases, they share similar catalytic mechanisms (Fig. 3). Most peroxidases are heme proteins bearing iron protoporphyrin IX, magnesium, vanadium, selenium, or flavin groups in the active sites [145,146]. During the reaction, the ferric heme in the active site of peroxidase is initially oxidized by H_2O_2, forming the highly oxidized intermediate Compound I, while H_2O_2 is reduced to H_2O [146,147]. Compound I is then sequentially reduced through two one-electron transfer steps, oxidizing electron donors such as sulfonates, amines, indoles, aromatic phenols, and phenolic acids [148], ultimately restoring the enzyme to its native state. The first electron reduction step leads to the generation of Compound II and a free radical through the reduction of a reducing substrate, Then, Compound II returns to the resting state of the enzyme by oxidizing another reducing substrate, releasing free radicals in the second electron reduction step [143,146]. The radical products formed during the two sequential one-electron transfer reactions can polymerize into dimers, trimers, and oligomers or act as reducing substrates in subsequent reactions [148]. Notably, Compound I catalyzes the oxidation of halides and pseudohalides, whereas Compound II remains inactive in their oxidation [143].

Peroxidases have been found in animals, plants, bacteria, and fungi

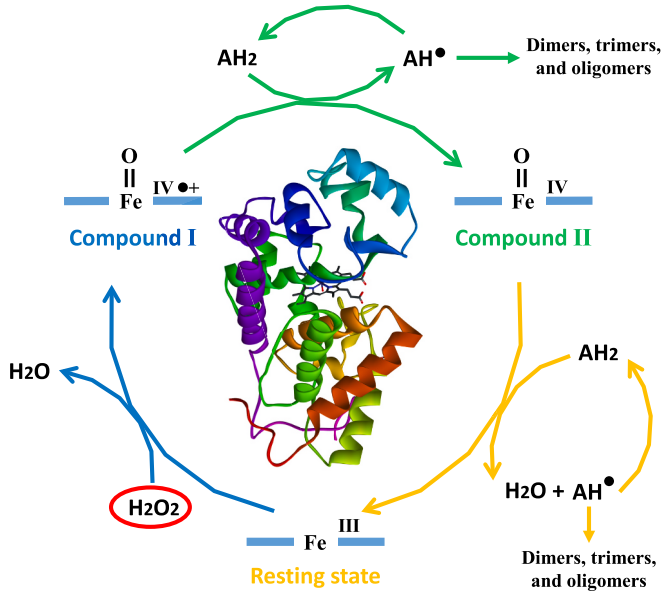

Fig. 3. The general catalytic cycle of horseradish peroxidase. AH_2 is the reducing substrate, while $AH^{\cdot}$ is its radical product.
Adapted from [148].

[147,149]. Still, peroxidases used in wastewater bioremediation are mainly from plants, such as horseradish, soybean, tea leaves, *Prosopis juliflora*, cabbage leaves, *Cedrela fissilis* leaves, cauliflower, *Zanthoxylum armatum* DC. fruit, Turkish black radish, ginger, *Brassica rapa*, etc (Table 1). Plant waste, including soybean hulls, luffa skin peels, waste cabbage, onion dry scales, potato peels, and banana peels are also feasible peroxidase sources used for wastewater treatment (Table 3). Although some studies that have explored the use of microbial peroxidases in wastewater treatment, their prevalence is lower compared to plant peroxidases. For example, the manganese peroxidase from *P. chrysosporium* BKMF-1767 transformed 87 % of sulfamethoxazole within 6 h under the conditions of pH 5, 40 U/L peroxidase activity, 0.2 mM H_2O_2 [62], while the peroxidase from *Streptomyces coelicolor* decolorized 75 % and 90 % of Direct Red 23 and Direct Blue 15, respectively, in the presence of lignin [61]. However, animal peroxidases have rarely been used for wastewater treatment.

3.5. Glucose oxidases

Glucose oxidase is one of the widely employed oxidoreductase. It has been used in wastewater bioremediation as it can catalyse glucose to produce gluconic acid and H_2O_2 (Equation (1) [150], a highly reactive oxygen species that can be used for contaminant removal and disinfection of wastewater [63,64,85]. Currently, glucose oxidases are mainly derived from the microbial *Aspergillus niger* (Table 2). The glucose oxidase can degrade contaminants in wastewater through bio-Fenton reaction [63]. For instance, the glucose oxidase extracted from *Aspergillus niger* could partically remove 12 out of 15 pharmaceutically active compounds at 950 μg/L concentration [63]. A study produced glucose oxidase from *Aspergillus niger* cultured by *Casuarina equisetifolia* biomass removed 36–92 % of nine trace organic contaminants in domestic wastewater under the conditions of pH 7.0 and 360 mins [64]. The immobilized glucose oxidase on carbon felt successfully achieved a 93 % decolorization of wastewater containing Remazol Blue RR. Simultaneously, 34 % of the COD was effectively removed [151]. It was reported that the photocatalyst made by TiO and glucose oxidase was able to supress the bacteria *E. coli* and *B. subtilis*. Moreover, it degraded 70 % of methylene blue under UV irradiation after 1 h [20]. During the process of bio-Fenton reaction, the H_2O_2 produced by glucose oxidase (Equation (1) first decomposed by Fe^{2+} to generate hydroxyl radicals ($HO^{\cdot}$) (Equation (2). Then, the produced $HO^{\cdot}$ oxidizes the targeted contaminants (Equation (3) through hydroxyl addition, hydrogen abstraction, or electron transfer [63,152].

$$C_6H_{12}O_6 + H_2O + O_2 \xrightarrow{glucoseoxidase} C_6H_{12}O_7 + H_2O_2 \quad (1)$$

$$H_2O_2 + Fe^{2+} \rightarrow Fe^{3+} + HO^{\bullet} + OH^{-} \quad (2)$$

$$HO^{\bullet} + Pollutant \rightarrow OxidationProducts \quad (3)$$

Additionally, glucose oxidase can work with peroxidase to improve pollutant degradation [150,153]. H_2O_2 plays a crucial role in the enzymatic reaction of peroxidase as it serves as the oxidant (Fig. 3). Therefore, the addition of H_2O_2 is essential in a peroxidase-catalyzed system. However, a high concentration of H_2O_2 can irreversibly deactivate peroxidase [154], inducing intermittent addition. Nonetheless, the intermittent addition will result in a longer reaction time. Another alternative involves replacing H_2O_2 with *tert*-butyl hydrogen peroxide, which introduces extra organic impurity [65]. In light of these challenges, the integration of peroxidase and glucose oxidase emerges as a promising choice since glucose oxidase can continuously provide H_2O_2 to peroxidase (Fig. 4). Moreover, this cascade bi-enzyme reaction system not only reduces the adverse effects of H_2O_2 on both the environmental and the enzyme, but improves the efficiency of the catalytical reaction through decreasing the diffusion of H_2O_2 [155].

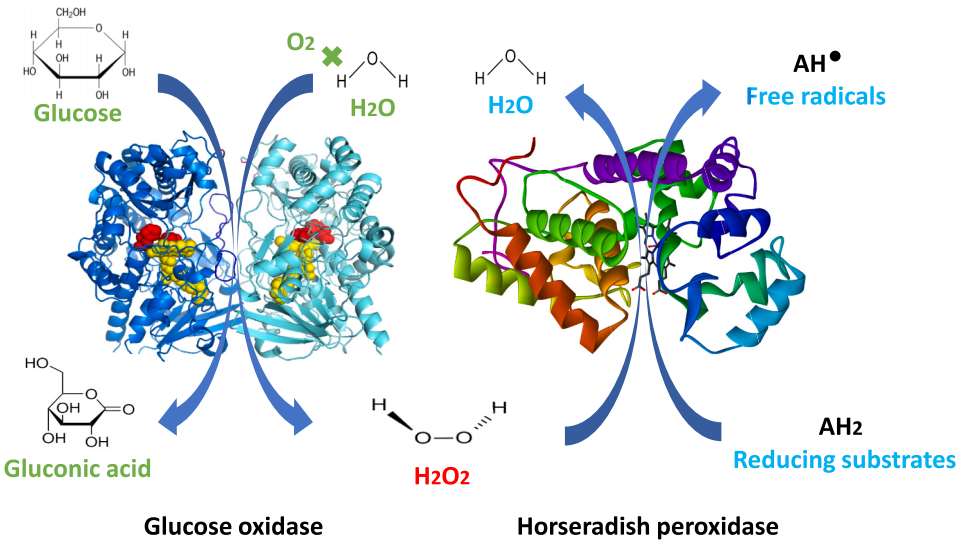

Fig. 4. The cascade system composed by glucose oxidase and horseradish peroxidase. Adapted from [150].

3.6. *Artificial enzymes*

Currently, most artificial enzymes used in real wastewater treatment applications are nanozymes. Various nanoparticles can act as nanozymes, such as sulfur-doped graphdiyne nanosheets, CoZn ZIF@HTD, hollow C@MoS_2 nanotubes, gold nanoparticles, and $Mn_4(P_2O_7)_3$ nanoflakes (Table 4). Those nanozymes typically have mimetic activities of peroxidases, catalase, oxidase, and oxidoreductase (Table 4). Interestingly, there are also bifunctional nanozymes. For instance, the nanozyme GOLDzyme/Co-MIONzyme exhibited both peroxidase and oxidase activities [116]. Another nanozyme, Ag/Fe_3O_4@h-BN, was synthesized with peroxidase- and oxidase-mimicking capabilities [115]. Those nanozymes have similar function mechanisms to natural enzymes and could catalyse the degradation of pharmaceuticals, phenolic compounds, dyes, and other micropollutants. For instance, it was reported that the CeO_2@ZIF-8 nanozyme, which exhibited peroxidase-like activity, successfully degraded over 80 % of methyl orange from natural dye wastewater after five successive cycles [16]. The nanozyme synthesized by Wang et al. [111] was capable of degrading methylene blue and methyl orange at the same time, and it had the potential to deal with wastewater that contains various organic pollutants. The nanozyme AuNPs/MoS_2 nanocomposites were used as biosensors to detect the content of carbamazepine in wastewater, and it showed a high efficiency in carbamazepine degradation [17]. Zhang et al. [21] synthesized a carbon-based nanozyme with peroxidase-like activities, and the nanozyme could degrade dyes and antibiotics in wastewater. Under optimal conditions, a ferromagnetic chitosan nanozyme removed more than 95 % of phenol from an aqueous solution within 5 h [109]. The nanozyme Au-NiFe layered double hydroxide/reduced graphene oxide nanocomposite removed 99.9 % of organic mercury in 2 h without secondary pollution by Hg^{2+} [118]. The nanozyme Ag/Fe_3O_4@h-BN achieved over 99.5 % removal of methyl orange, methylene blue, and rhodamine B, while the removal of As(V) was 63 % [115].

Additionally, nanozymes have other applications in wastewater treatment, such as bacterial disinfection and serving as biosensors. For one thing, nanozymes have been applied for disinfection. It was reported that the sulfur-doped graphdiyne nanosheets with peroxidase-mimicking activities were able to eliminate approximately 99 % of *Staphylococcus aureus* and *E. coli* after 60 min in the presence of H_2O_2 [21]. In addition, nanozymes can serve as biosensors for monitoring various pollutants in wastewater. Studies have shown that nanozymes are feasible sensors for the detection of metal ions like sulfide ions [113], cyanide ions [110], organic mercury [118], Cr(VI) [119], and Hg^{2+} ions [117]. Moreover, pharmaceuticals such as carbamazepine [17] and ibuprofen [114] could also be detected by nanozymes. Furthermore, a study reported that the nanozyme CoZn ZIF@HTD could be used as a biosensor for detecting phosphate ions, *o*-phenylenediamine, and benzaldehyde [112].

4. Large-Scale enzymatic wastewater treatment and the role of enzyme sources

Achieving large-scale enzyme-based wastewater treatment requires cost-effective enzymes that can be produced in substantial quantities. Additionally, strategies such as enzyme immobilization are crucial to minimize enzyme consumption and loss. While research on large-scale enzymatic wastewater treatment remains limited, existing studies align with these principles.

For instance, one study employed immobilized laccase from the *Thielavia* genus on fumed silica nanoparticles, combined with ultrafiltration, to remove bisphenol A from wastewater at a pilot scale. Under operating conditions of 13.2 ± 1.5 °C and an enzyme loading of 1.23 ± 0.11 kU/g of fumed silica nanoparticles, approximately 66 % of bisphenol A was removed, with the laccase retaining a significant portion of its enzymatic activity [32].

In another study, Steevensz et al. utilized crude soybean hull peroxidase for phenol removal from alkyd resin wastewater at a tote scale (700–1000 L). Under conditions of pH 7.0, 20 ± 2°C, 12.5 mM initial phenol, a peroxidase-to-initial phenol ratio of 1:1.5, 1.65 U/mL soybean hull peroxidase, and 800 mg/L Triton X-100 over 90 min, more than 99 % of the phenol was removed [33].

Additionally, a pilot-scale study investigated the production of xylanase from *Bacillus pumilus* MK001 and laccase from *Ganoderma* sp. rckk-02 for kraft pulp bleaching at a 10 kg scale. The enzymatic pretreatment reduced the requirement for chlorine dioxide, lowered the concentration of adsorbable organic halides (AOX) by 34 %, and increased the biochemical oxygen demand (BOD) by 13.8 % [156].

A wide range of commercial enzymes derived from various sources has been reported. Examples include lipase from porcine pancreas [157], peroxidase from horseradish [158], and laccase from *Trametes versicolor* [159]. According to a previous review, approximately 75 % of industrial enzymes are hydrolases [160]. Microorganisms have become the dominant source of industrial enzymes due to their rapid reproduction, low space requirements, cost-effective cultivation, and ease of

strain improvement [161]. Approximately 88 % of industrial enzymes are derived from microorganisms (e.g., fungi, bacteria, and yeast), while only about 10 % come from animals and plants [160].

Although enzymes from diverse sources can be used in wastewater treatment, microorganisms appear to be the most suitable choice. However, given the variability in wastewater composition and enzyme properties, further research is needed to explore commercial enzyme applications from alternative sources, such as animals, plants, and organic waste, in large-scale enzymatic wastewater treatment.

5. Future perspectives

The selection of enzyme sources is critical for enzymatic wastewater treatment. Among the five primary enzyme sources, all are viable for wastewater treatment; however, animal and plant enzymes—particularly those from animals—face limitations due to their low reproductive rates. A promising approach to overcome this limitation is the expression of animal and plant enzyme genes in microorganisms, which can significantly reduce production time.

Currently, enzymes such as lipase, protease, laccase, peroxidase, glucose oxidase, and nanozymes are widely used in wastewater treatment. As concerns over emerging pollutants like microplastics and pharmaceuticals grow, it is imperative to discover new enzymes capable of biodegrading these persistent contaminants and to identify optimal enzyme sources. Despite the availability of commercial enzymes from various sources, their large-scale application in enzymatic wastewater treatment remains limited.

To facilitate large-scale adoption, cost-effective enzyme production strategies must be developed, prioritizing enzyme sources with high yields and minimal impurities. Additionally, techniques such as enzyme immobilization can help reduce enzyme consumption and extend enzyme lifespan. Other approaches, including the use of additives like Triton X-100 to enhance enzyme efficiency [33] and enzyme engineering to improve catalytic activity, should be further explored to optimize enzyme utilization.

Ultimately, more large-scale studies on enzymatic wastewater treatment are essential to bridge the gap between laboratory research and industrial applications, enabling the widespread use of enzymatic processes in wastewater management.

6. Conclusion

Enzyme sources play a crucial role in enzymatic wastewater treatment, affecting enzyme types, activity, production methods, and overall cost. Animals, plants, microorganisms, organic waste, and artificial enzymes are feasible enzyme sources for wastewater treatment. Lipases, proteases, laccases, peroxidases, glucose oxidases, and nanozymes are commonly employed enzymes in waste stream bioremediation, each sourced from diverse origins and with distinct function mechanisms. However, research on scaling up enzymatic wastewater treatment remains limited. Greater emphasis should be placed on identifying enzyme sources capable of producing cost-effective, high-activity commercial enzymes. Challenges related to enzyme sources persist, necessitating future research in several key areas: genetic engineering to enhance the production of animal- and plant-derived enzymes, the discovery of new enzymes and their sources, large-scale application studies, cost-effective commercial enzyme development, and improved enzyme retention and utilization. Addressing these challenges is essential for advancing the industrial implementation of enzymatic wastewater treatment.

CRediT authorship contribution statement

Siran Feng: Writing – original draft, Resources, Investigation, Formal analysis. **Wenshan Guo:** Writing – review & editing, Supervision, Resources, Funding acquisition, Formal analysis. **An Ding:** Writing – review & editing, Resources, Formal analysis. **Seyed Masoud Parsa:** Writing – review & editing, Visualization. **Junting Pan:** Visualization, Resources, Formal analysis. **Dongle Cheng:** Writing – review & editing, Resources. **Tra Van Tung:** Visualization, Resources, Formal analysis. **Huu Hao Ngo:** Writing – review & editing, Supervision, Resources, Funding acquisition, Formal analysis.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

Data will be made available on request.

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