# Application of Enzyme-Nanoparticle-Polymer Composites in Wastewater Treatment

Subjects: Polymer Science

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Different water treatment technologies such as photochemical degradation, biodegradation, electrochemical degradation, reverse osmosis, and membrane separation have been used to get rid of water pollutants. Enzymatic treatments have received great attention due to several advantages compared to physical and chemical treatments, such as mild operating conditions and high catalytic efficiency without harsh side effects. Oxidase and peroxidase enzymes from different sources have been immobilized on metal and metal oxide-polymer composites and used in the degradation of pollutants.

Keywords: enzyme-nanoparticle-polymer composites ; wastewater ; pollutants

### 1. Introduction

In recent decades, the global community has increasingly recognized the formidable challenge posed by water pollution arising from the unregulated release of municipal and industrial waste  $^{[1][2]}$ . Many industries including petrochemical, paints and explosives, food, pharmaceutical, leather and textile, pulp and paper, and cosmetics have contributed to this cause  $^{[3][4]}$ . These discharges cause serious problems to aquatic life due to their high biochemical oxygen demand (BOD), chemical oxygen demand, and blockage of sunlight  $^{[5][6]}$ .

One of the industries producing the highest level of toxic chemicals from dyeing, printing, and finishing is the leather and textile industry <sup>[1]</sup>. The conversion of skin into leather in textile industries generates huge amounts of wastewater containing a variety of organic and inorganic chemicals such as dyes, neutral salts, phenols, and biogenic matter of skins  $^{[2][8]}$ . The complex aromatic structures of these chemicals, especially the dyes, make them highly soluble in water and stable against light, aerobic decomposition, and oxidizing reagents <sup>[9]</sup>. Therefore, their accumulation leads to serious environmental concerns for aquatic life and human beings due to their adverse effects of toxicity, carcinogenicity, and mutagenicity <sup>[10]</sup>. Another industrial sector that has developed rapidly in the last century is the pesticide industry, as it is an important component of modern global agricultural systems for controlling pests and increasing crop yield <sup>[11]</sup>. These pesticides are applied in much higher doses than those required to kill the pests, and end up accumulating in water bodies via run off and percolation <sup>[12]</sup>. Unfortunately, these agrochemical residues not only pollute the aquatic systems and damage biodiversity, they cause serious health hazards to humans and may even directly or indirectly lead to death <sup>[13]</sup>. Moreover, these compounds have very long half-lives and can remain in the environment for several decades <sup>[15][16]</sup>.

The growth of the pharmaceutical industry (veterinary and human medicines) in the past years has also led to rising amounts of drugs, antibiotics, and hormones. These medicines are not fully metabolized by living organisms and when these end up in wastewater treatment plants, they are difficult to biodegrade, since most of them are fat soluble [17][18][19]. For example a study conducted by Joss et al. [20] indicated that biological degradation of pharmaceuticals using activated sewage sludge from municipal wastewater could only degrade 4 out of 35 compounds by over 90% and 17 compounds by less than 50%. These compounds have increased in the environment due to their increased consumption and direct discharge into the environment. The presence of pharmaceuticals, cosmetics, and their metabolites in municipal waste and industrial effluents presents a significant challenge, as these compounds cannot be effectively eliminated using conventional techniques, and consequently are released to the receiving environment [21][22]. While in the environment, they accumulate or transform into metabolites under certain environmental conditions, and these secondary metabolites may even be more toxic than the parent compounds [12][23]. These make pathogenic organisms develop resistance against them over time, which is a high risk to human health [24].

The continued release, spread, and accumulation of persistent organic pollutants in the water environment from these industries, including polychlorinated biphenyls and polycyclic aromatic hydrocarbons from the petrochemical industries, have become a major threat to human health due to their toxic, mutagenic, and carcinogenic properties <sup>[25][26][27]</sup>. The emission of these pollutants occurs at the manufacturing stage, after consumption and disposal of unused products.

These products are hard to be tracked or controlled in most situations and are resistant to natural biodegradation <sup>[12][28]</sup>. Most of these compounds are phenolic and, therefore, bio-recalcitrant, carcinogenic, and easily accumulate in plants and animals. They should, therefore, be removed prior to wastewater discharge <sup>[17][29][30]</sup>.

Different water treatment technologies such as photochemical degradation, biodegradation, electrochemical degradation, reverse osmosis, and membrane separation have been used to get rid of these pollutants. However, these techniques are costly, consist of complicated procedures, do not entirely remove the pollutants, and lead to secondary contaminants that also need to be redisposed of <sup>[31][32]</sup>. Enzymatic treatments of these pollutants have received great attention due to several advantages compared to physical and chemical treatments, such as mild operating conditions and high catalytic efficiency without harsh side effects <sup>[33][34]</sup>. Hence, the use of biocatalysts in wastewater treatment has gained momentum due to their ability to target a wide range of pollutants <sup>[35]</sup>. Enzymes immobilized onto supports are often used in the treatment of wastewaters to ensure improved thermal and pH stability and repeatability, which is rarely achieved with free enzymes <sup>[36]</sup>. Various pollutants including drugs, dyes, pesticides, polycyclic aromatic hydrocarbons (PAHs), and even heavy metals have been degraded using enzyme/metal-polymer biocatalysts, as demonstrated in **Figure 1**. Oxidase and used in the degradation of pollutants, as observed in **Figure 1**.



Degradation of drugs, dyes, pesticides and heavy metals by enzyme/metal-polymer nanocomposites

**Figure 1.** Different pollutants that have been degraded by enzyme-nanoparticle-polymer composites. A—Laccase, B— Horse radish peroxidase, C—Lignin peroxidase, D—Chloroperoxidase, E—Glucose oxidase, F—Glucose oxidase/laccase, G—*S. cerevisiae* enzyme, H—Glycerophosphodiesterase, I—Manganese peroxidase, \* 0–6 h, # 6–24 h, ¢ over 24 h.

#### 2. Laccase-Based Nanocomposite Biocatalysts for Degradation of Pollutants

Laccase is the most explored enzyme in wastewater treatment due to its ability to degrade a wide range of micro pollutants including dyes, pharmaceuticals, and endocrine-disrupting chemicals [37][38][39]. Unlike other oxidoreductases, laccase does not require hydrogen peroxide or other cofactors for substrate cleavage [40][41][42] and its range of compounds for oxidation can be increased with redox mediators [43][44]. Laccase-based composite biocatalysts show great potential in wastewater treatment as they have demonstrated high pollutant degradation rates with high reusability (**Table 1**). For example, Laccase/Fe<sub>2</sub>O<sub>3</sub>/PEI biocatalyst completely degraded sulfa drugs (Sulfadiazine, Sulfamethazine and Sulfamethoxazole) within 30 min and could still degrade 82.8% after 10 cycles in the same time frame [24]. Laccase/Ca-

alginate beads degraded 99% bisphenol A <sup>[19]</sup> and dyes (aniline purple–86%, lanset grey G–85%, and reactive black 5–80%) <sup>[45]</sup> in 2 h and 24 h, respectively.

## Table 1. Application of enzyme-nanoparticle-polymer composites in degradation of organic pollutants for application in

wastewater treatment.

Nanocomposite (NC)	Immobilization Method	Pollutants Removed	Degradation (%)	Degradation Time	Reusability	Ref.
TiO₂/polyvinylidene fluoride (PVDF)	Crosslinking of TiO <sub>2</sub> /PVDF membrane using APTES and glutaraldehyde followed by immersion in laccase solution	Bisphenol A	95	5 h	91.7% (96 h of continuous use)	<u>[46]</u>
TiO₂/bacterial cellulose (BC)	Physical adsorption of TiO <sub>2</sub> on BC followed by crosslinking with glutaraldehyde and immersion in laccase solution	Reactive red X- 3B in presence of ABTS	80	60 min	70% and 57% (6 and 10 cycles, respectively)	IJ
Calcium alginate	Physical entrapment of enzyme in nanocomposite	Fluoranthene in a fluidized bed reactor	81.06	8 h	66.845% (60 days of storage)	[ <u>27]</u>
Fe <sub>2</sub> O <sub>3</sub> /poly(ethylene glycol)/concovalin A	Chemical co- precipitation followed by crosslinking with glutaraldehyde and immersion in laccase solution	Sulfadiazine Sulfamethazine Sulfamethoxazole (all in presence of syringaldehyde mediator)	100	30 min	82.8% (10 consecutive cycles)	[24]
MNPs/chitosan	Physical mixing of NPs and chitosan followed by crosslinking	Reactive black 5 Evans blue	90 60	30 min 30 min	47% (10 ovalas)	[47]
	glutaraldehyde and immersion in laccase solution	Tryphan blue Direct blue 15	80 70	40 min 60 min	cycles)	
MNPs/polydopamine	Functionalized MNP- polydopamine NC with dialdehyde starch followed by immersion in	72 3 h 2,4- 7 dichlorophenol c 91 12 h	77% (8 cycles)	<u>[48]</u>		
Fe <sub>2</sub> O <sub>3</sub> /Cu-alginate	laccase solution		89.6	8 h		[4]
	Physical entrapment of enzyme in nanocomposite	Triclosan	53.2	8 h (wastewater) 8 h	86.9% (3 cycles in acetate buffer)	
		Remazol Brilliant Blue R (RBBR)	55	25 h (wastewater)		
			35	25 h (waste water)		

Nanocomposite (NC)	Immobilization Method	Pollutants Removed	Degradation (%)	Degradation Time	Reusability	Ref.
Cu (II)-chitosan-graft-poly (glycidyl methacrylate)/poly (ethylene imine)	Physical adsorption of laccase on nanocomposites	Phenol in presence of ABTS	80	4 h	50% (8 cycles)	<u>[30]</u>
MNPs/chitosan	Crosslinking with glutaraldehyde followed by immersion in laccase solution	2,4- Dichlorophenol 4-Chlorophenol	91.4 75.5	12 h	75.8% and 57.4% (2,4- DCP and 4- CP after 10 cycles)	[33]
					02.20/	
MNPs/SiO <sub>2</sub> /poly (glycidyl methacrylate)-S-SH	Physical adsorption of enzyme on the	Meloxicam Piroxicam	92 95	48 h	82.3%, 88.9%, and 87.5% (meloxicam, piroxicam and Cd <sup>2+</sup> , respectively, after 5	[21]
	nanocomposite	Cd <sup>2+</sup>	94			
MNPs/Poly(p- Phenylenediamine)	Covalent immobilization using glutaraldehyde for crosslinking	Reactive blue 19	80	1 h	cycles) 43% (8 cycles)	<u>[6]</u>
MNPs@MoS₂/polyethyleneimine		Malachite green	82.7		62% (10 cycles)	
	Physical adsorption of laccase on nanocomposite	<b>Bisphenol A</b>	87.6	Overnight		[25]
		Bisphenol F (all in presence of ABTS)	70.6	Overnight		-
		Fuschin blue	65 (HOBT)			
		Congo red	27 (ABTS)	4 h	100% and 95% (120 h continuous use and 15 days storage, respectively)	[8]
		Tryphan blue	51(syringaldehyde)			
	Physical entrapment of enzyme in nanocomposite	Malachite green	60 (ABTS)			
Cu-alginate		Erichrome black T	50 (HOBT)			
		Crystal violet (all in different mediators)	32 (HOBT)			
		Textile effluent in a continuous flow packed bed bioreactor	66 (colour) 90 (BOD) 98 (COD)			
	Physical entrapment of enzyme in presence of ionic liquid and ABTS	2,4- dichlorophenol	100	4 h	93.2% (for 2,4-DCP after 6 cycles)	
MNPs/chitosan		<b>Bisphenol A</b>	100	72 h		[ <u>49]</u>
		Indole	70.5	72 h		
		Anthracene	93.3	72 h		
MNPs/polyethylenimine	Crosslinking of NPs with PEI using glutaraldehyde followed by chelation of laccase with Cu(II)	Phenol in a fixed bed reactor	72.93% at a flowrate of 25 μL/min	-	-	[34]

Nanocomposite (NC)	Immobilization Method	Pollutants Removed	Degradation (%)	Degradation Time	Reusability	Ref.
		Malachite green	100 (ABTS)			
		Brilliant green	96.5 (ABTS)			
	In situ oxidation	Crystal violet	95.2 (ABTS)		89.4, 94.6,	
	of metal sait using PEG followed by	Azophloxine	97.7 (TEMPO)	120 min	76.5, 80.1, 74.6, and 66.1% (respectively, for the dyes after 10 cycles)	[ <u>10]</u>
	physical adsorption of	Red MX-5B	86.6 (ABTS)			
	laccase	Methyl orange	92.7 (VLA)			
		Reactive blue 19	96 (TEMPO)			
		Alizarin red	83.7 (TEMPO)			
		Alizarin red	61			
	Physical entrapment of	Tryphan blue	96		100% (14	[50]
TiO <sub>2</sub> /Zn-alginate	enzyme in nanocomposite	Malachite green	100	5 h	cycles)	1901
		Indigo carmine	100			
Ca-alginate	Physical entrapment with crosslinking of enzyme prior to entrapment	Bisphenol A	99	2 h	70% (10 successive cycles)	[ <u>19]</u>
Ca-alginate	Physical entrapment of enzyme in nanocomposite	Aniline purple	86.1	24 h	-	[ <u>51]</u>
	Physical	Reactive Red 180	67.2		-	<u>[52]</u>
Ca-alginate	enzyme in nanocomposite	Reactive Blue 21	88.05	11 days		
Ca-alginate	Physical entrapment of enzyme in nanocomposite	Reactive T. Blue	92	72 h	22.3% (6 cycles)	[53]
		RBBR	85	2 h	52.1% and	
	Physical	Reactive Black 5	80	24 h	70% (Bismarck	
Ca-alginate	entrapment of enzyme in nanocomposite	Bismarck Brown R	55	24 h	brown and all the	[ <u>45</u> ]
		Lancet Grey G	85	24 h	respectively)	
	Physical					
Cu-alginate	entrapment of enzyme in nanocomposite	Acid dye	38%	24 h	-	[ <u>54]</u>
MNPs/chitosan	Crosslinking with glutaraldehyde	Reactive yellow 2	85	10 h	_	[ <u>55]</u>
	followed by adsorption in laccase solution	Reactive blue 4	60	12 h		
	Polymer grafting with Cu	Reactive green 19	60		63%, 76%, and 59%	
MNPs/poly(GMA-MMA)/Cu- Poly(4-vinyl pyridine	chelation followed by adsorption of	Reactive red 2	88	18 h	(green, red, and brown dves	[ <u>56</u> ]
<b></b>	enzyme	Reactive brown	90	•	respectively)	
3. Horse Radish	Peroxidase	(HRP)-Base	d Nanocomj	posite Bio	ocatalysts	for

# Degradation of Pollutants

Another commonly explored peroxidase on nanoparticle-polymer composite materials is horse radish peroxidase (HRP), due to its ability to oxidize a wide range of phenolic compounds in the presence of hydrogen peroxide <sup>[59]</sup>. It oxidizes phenolic compounds by adding hydrogen peroxide to form corresponding radicals which spontaneously interact to form

insoluble polymers that can be e Nanocomposite (NC) have been explored in the degra	ลล์ไม <sub>่อได้</sub> การณังดีว่า froma สันชิมเอศ of phenols, e	ଲିଲ୍ଲ୍ଲ୍ଲ୍୍ର୍ରେstewater <mark>(60 D ଅନୁଅନ୍ଟ୍ରି</mark> and endocri	<sup>2]</sup> . HRP/nanopa egradation (%) ine-disrupting	articl <b>e-polymer</b> com <b>Fioun</b> ds, a	composite bioca Reusability s illustrated in Ta	atalysts Ref. able 2
For example, HRP/MNPs/polyv	inyl alcohol/poly ac	rylig agid gould o	completely deg	grade estrone	after 40 min [1	<sup>8]</sup> , and
HRP/TiO <sub>2</sub> /polvdopamine comple	etelvaremoved 2.4-c	dic <b>holidion</b> enol in	Zhaohe wast	- ewater sample	es in only 30 m	nin <sup>[61]</sup>
Interestingly the HRP/TiOs/polyc	doentrapment of atalys	ntaining tannic	nd 90% deara	dation activity	after 1 <b>55% (8</b> 25 r	- 
resease stively	enzyme in	forulic acid, 070 a	nu 30 <b>75</b> ucgia	dation agentity	cycles)	Cuaus
respectively.	nanocomposite	esorcinol, and				
		pyrogallol		<b>.</b> .		
Table 2. Application of enzyme	e-nanoparticle-polym	ner composites in	degradation of	organic polluta	ants for application	on in
		stewater treatmer	nt.			
	membrane					
	Immobilization	Pollutants	Degradation	Degradation		
Nanocomposite (NC)	solvothermal Method	Removed	(%)	Time	Reusessey/Hath/d	Ref.
FScubes/PDA@PVDF	followed by	Congo red	97.1	3 h	and 5 cycles.	58
	covalent				respectively)	
	immobilization				100% 90%	
	of laccase using on				and 63.6%	
TiO <sub>2</sub> /polydopamine	as crocelowedrby	2,4- dichloronhenol	100	30 min	(15, 25, and	[ <u>61</u> ]
	covalent	ulemorophenor			40 reuses,	
	crosslinking of				respectively)	
	glutaraldehyde					
		phenol	86	2 h	84% (8	
		• • •			weeks), 92%, and 79%	
	Crosslinking of				(phenol and	
MNPs/poly(glycidylmethacrylate-	enzyme and	n ahlaranhanal			p-	[3]
(poly(GMA-MMA))	beads using	p-chlorophenol (in the			chlorophenol,	
(,,(	glutaraldehyde	presence of	59		respectively,	
		H <sub>2</sub> O <sub>2</sub> )			continuous	
					use)	
	Crosslinking of	<b>Disphanal A in</b>				
Fe <sub>2</sub> O <sub>3</sub> /poly (amido amine)	nanocomposites	presence of	80	120 min	_	[62]
(PAMAM)/silk fibroin	using	H <sub>2</sub> O <sub>2</sub>				
	glutaraldehyde					
	Dhysical					
	entranment of				Can be	
Calcium alginate	enzyme in	Acid blue 113	76	240 min	recycled up to	Ľ
	nanocomposite				3 times	
	0					
	Crosslinking of	Phenol in				
Aluminosilicate halloysite	nanocomposites	presence of	98.8	30 min	60% (4	[ <u>63]</u>
nanotubes/chitosan	using	nyarogen			cycles)	
	glutaraldehyde	peroxide				
	Crosslinking of					
	enzyme with					
MNPs/polyacrylonitrile	nanocomposites	Phenol	85.2	-	52% (5 cycles)	[ <u>29]</u>
	using				cyclesy	
	glutaraldehyde					
	Physical					
MNPs/poly(vinyl	adsorption of	Estrone	100	40 min	56.2% (7	[18]
alcohol)/poly(acrylic acid)	enzyme on	250 016	100		cycles)	
	nanocomposites					
	Physical	Phenol in				
MNPs/nolymethyl methacrylata	entrapment of	presence of	55	50 min	_	[64]
and sporymenty memorylate	enzyme in	hydrogen	55	50 mm	-	
	nanocomposite	peroxide				
		Phenol	86	2 h		
					91% and 79%	
	Crosslinking of	р-			(phenol and	
MNPs/poly(glycidylmethacrylate-	enzyme with	Chlorophenol			chlorophenol,	[3]
co-metnyimetnacrylate) (poly(GMA-MMA))	heads using	(in presence of hydrogen	59		after 48 h of	
	glutaraldehyde	peroxide in a			continuous	
	-	fluidized bed			operation)	
		reactor)				

# 4. Other Oxidase and Peroxidase-Based Nanocomposite Biocatalysts for Degradation of Pollutants

Other enzymes such as chloroperoxidase, manganese peroxidase, and lignin peroxidase immobilized on composite materials, though not very popular, prove that they can offer wonderful materials for pollutant degradation (**Table 3**). For example, when lignin peroxidase was immobilized on MNPs@SiO<sub>2</sub>/polydopamine, it was able to degrade tetracycline and other phenolics such as 5-chlorophenol, phenol, and dibutyl phthalate completely within 24 h <sup>[32]</sup>. Manganese peroxidase immobilized on MNPs/chitosan degraded 96% of methylene blue in synthetic wastewater in just 50 min <sup>[2]</sup>, glucose oxidase immobilized on NiFe2O4/tannin could degrade 98.6% of indigo carmine in presence of UV light within 90 min <sup>[31]</sup>, and chloroperoxidase/TiO<sub>2</sub>/polydopamine nanocomposites degraded over 95% of aniline blue and crystal violet in 2 min <sup>[61]</sup>.

 Table 3. Application of enzyme-nanoparticle-polymer composites in degradation of organic pollutants for application in wastewater treatment.

Nanocomposite (NC)	Enzyme	Immobilization Method	Pollutants Removed	Degradation (%)	Degradation Time	Reusability	Ref.
iO <sub>2</sub> /polydopamine	Chloroperoxidase (CPO)	Covalent crosslinking of enzyme with nanocomposites using glutaraldehyde	Aniline blue	97.58	2 min	90.3%, 78.2%, and 53.71% (10,	[ <u>61</u> ]
			Crystal violet	98.98	2 min	reuses, respectively)	
NiFe₂O₄/tannin	Glucose oxidase	Physical adsorption of enzyme on nanocomposite	Indigo carmine in presence of UV light	98.6	90 min	85.57% (5 cycles)	[ <u>31</u> ]
		Physical	Methylene blue	82.13			
MnFe <sub>2</sub> O <sub>4</sub> /calcium alginate	Glucose oxidase and Laccase	adsorption of enzymes on the	Indigo	25.09	1 h	-	[ <u>9]</u>
		nanocomposite	Acid red 14	20.42			
MNPs/PAMAM	Glycerophosphodiesterase (GpdQ)	Crosslinking of enzyme with nanocomposites using glutaraldehyde	Organophosphate pesticide	44.5	120 days	Used as a filter in a Pasteur pipette between two layers of sand	[ <u>14</u> ]
			Tetracycline	100	24 h	80.3% and 67.5% (7 and 14 days of storage), 70% and 30% (4 and 8 cycles, respectively)	[ <u>32</u> ]
	Lignin peroxidase	Physical adsorption of enzymes on the nanocomposite	Dibutyl phthalate	100	24 h		
			5-chlorophenol	100	24 h		
MNPs@SiO <sub>2</sub> /polydopamine			Phenol	100	24 h		
			Phenanthrene	79	24 h		
			Fluoranthene	73	24 h		
			Benzo(a)pyrene	65	24 h		
MNPs/chitosan	Manganese peroxidase	Crosslinking of enzyme with nanocomposites using glutaraldehyde	Methylene blue	96		91.7% and 86.7% (5 cycles- methylene blue and reactive orange, respectively)	[2]
			Reactive orange 16	98	50 min		
Fe <sub>2</sub> O <sub>3</sub> /chitosan	Saccharomyces cerevisiae enzyme	Adsorption of chitosan on the NPs surface followed by crosslinking with enzyme using glutaraldehyde	Cu(II)	96.8	60 min		<u>[65</u> ]

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