BILINGUAL PUBLISHING GROUP

Research in Ecology

https://journals.bilpubgroup.com/index.php/re

REVIEW

Advances and Feasibility of Biocatalytic Technologies for Dye Removal

Kuok Ho Daniel Tang

Department of Environmental Science, The University of Arizona, Tucson, AZ 85721, USA

ABSTRACT

The expanding dye and dye-related industries have led to the production of large volumes of dye-containing wastewater streams. Without adequate treatment, the wastewater could pollute the environment and give rise to health concerns. Biocatalytic technologies provide a channel of treating the wastewater. These technologies involve immobilizing dye-degrading enzymes particularly laccase and peroxidase, and microorganisms on or in suitable supports to enhance their activities, stability, efficiency, and recyclability. This review provides the latest advances in biocatalytic technologies for dye removal and their feasibility. Based on this review, laccase has been immobilized on supports comprising PEDOT-PPy-COOH/Pt, nanocellulose from quinoa husks, calcium alginate, delignified spent grain, polymeric membrane, and metal-organic frameworks to treat different dyes with efficiencies ranging from 39% to 100%. Peroxidase has been immobilized on calcium alginate, Fe_3O_4 nanoparticles, cationic maize starch, and graphene oxide-SiO_2 for treatment of various dyes with efficiencies in the range of 40–100%. The dye-degrading ability of azoreductase is often harnessed through immobilization of microbial cells which contain multiple enzymes in them and are frequently able to decolorize more than 90% of the dyes tested. An immobilized azoreductase has been successfully produced but showed lower dye-degrading efficiencies of 18.3–58.3%. The performance of biocatalysts can be affected by multiple factors, making optimization of the operating conditions important. The use of green support materials could reduce the cost of biocatalysts and the associated environmental concerns. A versatile biocatalyst or biocatalyst mixture is beneficial to degrade the complex pollutants in dye-containing wastewater.

Keywords: Azoreductase; Biocatalyst; Immobilization; Laccase; Peroxidase; Wastewater

*CORRESPONDING AUTHOR:
Kuok Ho Daniel Tang, Department of Environmental Science, The University of Arizona, Tucson, AZ 85721, USA;
Email: daniel.tangkh@yahoo.com

ARTICLE INFO
Received: 30 December 2023 | Received in revised form: 18 March 2024 | Accepted: 27 March 2024
DOI: https://doi.org/10.30564 / re.v6i2.6189

CITATION
Tang, K.H.D., 2024. Advances and Feasibility of Biocatalytic Technologies for Dye Removal. Research in Ecology. 6(2): 1–15. DOI: https://doi.org/10.30564 / re.v6i2.6189

1. Introduction

Dyes are used globally in multiple industries comprising tanneries, food, cosmetic, textile and medical sectors. The annual global production of dyes was estimated to be 1 million tons [1,2]. In 2022, the global market size of dyes and pigments was valued at USD 38.2 billion, with a projected compound annual growth rate of 5.3% from 2023 to 2030. By 2029, the market size of dyes and pigments will reach USD 56.91 billion [3]. Their larger production and market share correspond to increasing dye consumption by downstream sectors, which together, have led to increasing dye emissions into the environment. Dye-manufacturing industries release only 7% of the existing global dye effluents, forming a stark difference from the dye-consuming sectors, with textile industry contributing 55% of the dye effluents, the dyeing industries emitting 21%, as well as the paper and pulp industry emitting 9% [4].

Dyes are classified based on their chemical compositions or applications. The common types of dyes in the environment are azo dyes, anthraquinone dyes, nitro and nitroso dyes, indigoid dyes and cyanine dyes to name a few [5]. Azo dyes are the most widely used synthetic dyes containing an azo group (-N=N-) in their molecules and they are usually added to textiles, food products and cosmetics [6] Anthraquinone dyes are organic dyes derived from anthraquinone, typically used for dyeing textiles and paint production. Nitro and nitroso dyes contain nitro ($-NO_2$) and nitroso (-NO) groups, while indigoid dyes come from a natural dye called indigo. All these dyes are versatile and are used in textile and paint production [7]. The entry of dyes into water streams results in deleterious effects on aquatic lives. Dyes reduce light penetration in water, thus, negatively influencing photosynthesis. Lower photosynthesis causes lower dissolved oxygen levels in water which affects the respiration of aquatic flora and fauna [7]. The widespread use and presence of azo dyes, also known as azo group proliferation, causes health concerns as azo dyes can release aromatic amines, including benzidine, under certain conditions [8]. Benzidine is a known carcinogen. Three azo dyes comprising Direct Blue 6, Direct Brown 95, and Direct Black 38 could release benzidine upon partial degradation [8]. Azo dyes, together with anthraquinone dyes, and nitro and nitroso dyes, are also persistent and tend to stay in environment for extended periods. This increases exposure of organisms to dyes, giving rise to ecological hazards [9].

The removal of dyes from the environment has been a popular area of research due to their environmental and health implications. Adsorption has been proposed as a feasible method of dye removal due to its high efficiently, operational simplicity, and cost-effectiveness [10]. Adsorbents can be regenerated, making adsorption resource efficient. Carbon-based, metal oxide-based, and polymer-based adsorbents as well as metal-organic framework have been synthesized for this purpose [11]. Additionally, advanced oxidation processes provide another avenue for dye removal. The processes encompass ozonation, ultraviolet/hydrogen peroxide (UV/H_2O_2), Fenton, photocatalytic reactions, ultrasound and anodic oxidation [9]. Flocculation/coagulation involves the aggregation of dye molecules into larger clumps or flocs to facilitate their removal [12]. Biological oxidation makes use of microorganisms or plants to take up and break down dye molecules, whereas membrane filtration physically separates dye molecules from water according to their sizes [13–15] Except for advanced oxidation processes and biological oxidation, other removal methods do not degrade dye molecules and separate processes are required to do so [9]. Biological oxidation to remove dyes can be slow in comparison to physicochemical methods and may require specific microorganisms or plants targeting on certain dyes [13,15].

The use of biocatalysts as a variant of biological oxidation has received increasing attention as it has certain advantages over conventional biodegradation. A biocatalyst is a catalyst of biological origin capable of activating or hastening biochemical reactions. Enzymes are a typical example of biocatalysts [16]. Biocatalysts like laccase have high catalytic activity and are more efficient than microorganisms in degrading dyes [16]. They may produce fewer toxic

by-products, have better stability and reusability, and can degrade a wider array of dyes [17,18]. Currently, few review articles are dedicated to presenting the latest advances of biocatalystic technologies for dye removal and their feasibilities. A review was conducted on the use of laccase as a green catalyst to degrade dyes, particularly after immobilization, which stabilizes its protein structure and enhances its functionality. The review was not extended to other biocatalysts [16]. Another review discusses the use of various adsorbents in removing dyes from contaminated water and the factors affecting dye adsorption. It does not include the use of biocatalysts [19] Bhattacharya et al. presented the techniques and mechanisms of using mesophilic bacteria for dye removal, and included enzyme-mediated biodegradation in the discussion as a mechanism of bioremediation [20] This paper, however, focuses on biocatalysts for dye removal solely, thus providing greater breadth and depth of the theme. It aims to systematically present the advances in biocatalytic technologies for dye removal and discuss their feasibilities. This review contributes to better understanding of the challenges, limitations, and opportunities of biocatalytic technologies for dye removal to provide a direction of future studies. It contributes to optimization of biocatalyst performance through insights into their immobilization techniques and operational conditions.

2. Enzymes for dye removal

Multiple enzymes have been identified to have dye-removing abilities. Azoreductases can break down azo bonds in synthetic dyes, such as mauveine and reduce them to aromatic amines [21]. They are produced by various bacteria, fungi, and plants. Laccases are produced primarily by white-rot fungi, and are capable of oxidizing phenolic and non-phenolic compounds in dyes, such as alizarin [18]. The oxidation yields radical intermediates which either polymerize or degrade. Peroxidases facilitate the degradation of dyes, such as anthraquinone and triphenylmethane, by reacting with hydrogen peroxide [18]. Dyes are usually degraded by peroxidases into less toxic products. Peroxidases are typically secreted by fungi and plants, and include manganese peroxidase, horseradish peroxidase and lignin peroxidase [22]. **Table 1** shows the optimal conditions for the activities of the enzymes and their dye removal efficiencies.

These enzymes can be separated from bacteria, fungi or plants through various methods comprising extraction, precipitation, adsorption, and membrane separation. In extraction, solvents such as water, organic solvents, or ionic liquids are used to extract the enzymes from the microbial cells or biomass. The extraction efficiency depends on the solubility, stability, and activity of the enzymes in the chosen solvent [21]. Precipitation utilizes salts, organic solvents, or polymers to precipitate the enzymes from aqueous solutions. The factors affecting precipitating efficiency are the same as those affecting extraction efficiency [24]. Adsorption uses solid materials, such as activated carbon, silica gel, or magnetic nanoparticles to adsorb the enzymes from solutions. Affinity of the enzymes for the adsorbent surface influences the adsorption efficiency. Enzymes can also be separated from the solution with membranes, particularly microfiltration, ultrafiltration or nanofiltration [23].

3. Enzyme immobilization techniques

Enzyme immobilization is a major biocatalytic technology which has found applications in the removal of a wide range of environmental

Table 1. Enzymes for dye removal, their sources, optimal conditions for activities and dye removal efficiencies.

Enzyme	Source	Optimal pH	Optimal temperature	Dye removal efficiency	Reference
Azoreductase	Bacteria, fungi, plants	5.5–7.5	25–40 °C	70–100%	[21,23]
Laccase	White-rot fungi, bacteria	3.0–5.0	30–60 °C	50–90%	[23,24]
Peroxidase	Fungi, plants	4.0–6.0	20–50 °C	60–95%	[23]

pollutants, including dyes. The common techniques of enzyme immobilization consist of microencapsulation, covalent bonding, adsorption, attachment, and entrapment (**Table 2** and **Figure 1**) [25,26]. Microencapsulation encloses enzymes in a polymeric or lipid shell that retains their activities and prevents them from degradation (**Figure 1**). Microencapsulation can be achieved through solvent evaporation, freeze-drying and spray-drying [25]. In solvent evaporation, a core material is dissolved in a solvent, which is subsequently evaporated under reduced pressure. The core material forms a thin film on the surface of the solvent droplets. Hot air or vacuum is used to dry the solvent droplets, leaving behind the core material and the solvent film, forming microcapsules [27]. Freeze-drying involves freezing the core material in a solution and then sublimating the ice crystals under reduced pressure. The core material remains intact and forms microcapsules with high encapsulation efficiency and low moisture content. Freeze-drying allows the activity and structure of enzymes to be better preserved than solvent evaporation. Spray-drying of core material dispersed in a liquid produces fine droplet. The core material forms microcapsules with high encapsulation efficiency and good flowability [28].

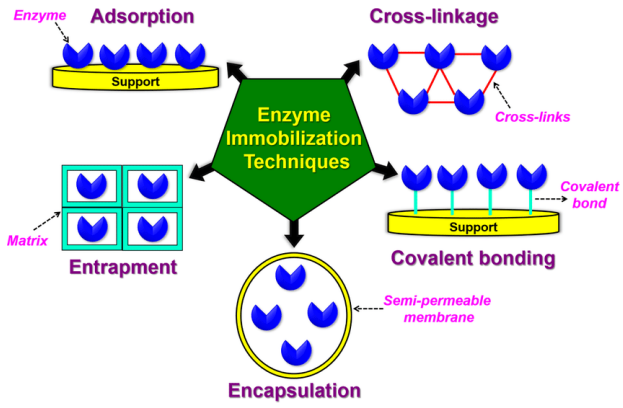

Figure 1. Methods of immobilizing enzymes.

Source: [29].

Alternatively, enzymes can be adsorbed to the surface of a support material through physical forces such as electrostatic or van der Waals attractions (**Figure 1**). Addition of co-solvents, surfactants, or other additives can increase the surface area and affinity of adsorbents, thus enhancing adsorption [30]

Attachment of enzyme centers on fusing or embedding an enzyme into a polymeric matrix or metal that confers mechanical strength and stability. Covalent bonding is a means of attaching an enzyme to a polymeric matrix or a solid surface [31] (**Figure 1**). Enzyme attachment can also be non-covalent in nature, involving electrostatic attraction, hydrogen bonds or hydrophobic interactions instead of the sharing of electrons. Non-covalent bonding is weaker and more reversible than covalent bonding, but it also preserves the enzyme structure and function better [31].

Entrapment, however, traps an enzyme in a porous polymer or metal (**Figure 1**). These structures permit mass transfer and diffusion of substrates for enzyme actions and the resultant products [32]. Entrapment can be achieved with extrusion and emulsification. Extrusion is a process where a liquid or semi-solid mixture of enzymes and a carrier material is forced through a nozzle or die to form a continuous strand or pellet. The extruded material is then dried or cut into desired sizes [31]. As for emulsification, a liquid or semi-solid mixture of enzymes and a carrier material is dispersed into another immiscible liquid, usually water or oil, to form small droplets. The droplets are then stabilized by adding an emulsifier or a surfactant [32]. The main difference between microencapsulation and entrapment is the size of the particles or droplets that contain the enzymes. Microencapsulation involves the formation of microscopic particles or droplets, usually ranging from 1 to 1000 µm in diameter, that are coated with a thin layer of polymer or other materials [27] Entrapment involves the incorporation of enzymes into larger structures, such as gels, fibers, or beads that have pores or channels for the diffusion of substrates and products [33]. Another difference is the degree of interaction between the enzymes and the carrier material. Unlike in microencapsulation where enzymes are physically separated from carrier material by the coating layer, enzymes in entrapment are in direct contact with the carrier material and this may affect their activity, stability, or specificity [34]. A summary of each of the immobilization techniques is presented in **Table 2**.

Table 2. The advantages and disadvantages of different enzyme immobilization methods.

Method	Advantages	Disadvantages
Microencapsulation	• Protects enzyme from external environment. • Preserves enzyme structure and function. • Allows controlled release of enzyme.	• Limits diffusion of substrates and products. • High cost of materials and process. • Reproducibility is less.
Attachment	• Provides strong and specific interactions. • Allows irreversible attachment through covalent bonding.	• Affects enzyme activity and conformation. • Requires reactive groups on support surface.
Adsorption	• Preserves enzyme structure and function. • Does not require reactive groups on support surface.	• Weak and reversible interactions. • Prone to enzyme leaching.
Entrapment	• Does not require interaction with enzyme. • Fast, cheap, and mild process. • Suitable for whole cell biocatalysis.	• Causes enzyme leaching or mass transfer limitations. • Weak mechanical strength of matrix. • Immunogenicity and uncontrolled degradation of matrix.

4. Enzyme immobilization for dye removal

Laccases are the most common enzymes used in dye removal and have been subjected to modifications including immobilization. Uygun et al. synthesized self-propelling laccase-based micromotors, which was tested on different dyes, such as Procion Red, Reactive Green 5, Reactive Green 19, Reactive Brown 10, Alkali Blue 6B. The micromotor unit was PEDOT-PPy-COOH/Pt [35] (**Table 3**). To enable self-propulsion, oxygen was generated by the Pt layer of the micromotor through the degradation of H_2O_2. The micromotor unit has carboxy terminals which serve to bind laccase after functionalization with 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide/N-hydroxysuccinimide [35]. This is a typical example of attaching an enzyme to a support, in this case a micromotor, via covalent bonding. The laccase-based micromotors demonstrated good stability across different pH (2–8) and temperatures (55–75 °C), with dye removal efficiencies of 76–94% recorded (**Table 3**). These efficiencies were significantly higher than those of the free enzyme, which are typically between 34 to 55% [35].

In another study, laccase was immobilized on the nanocellulose produced from waste quinoa husks [36]. The immobilization was achieved by dispersing the nanocellulose power in an enzyme solution, allowing the enzyme to adsorb or physically attach to the nano-carrier. The immobilized enzyme showed more superior ability to remove (removal has been used interchangeably with decolorization to show the reduction in dye concentration due to the actions of enzymes or bacteria) Malachite Green and Congo Red than nanocellulose alone, reaching an efficiency of 98% and 60%, in comparison to 54% and 12% of nanocellulose, respectively (**Table 3**). The immobilized enzyme removed the dyes through adsorption and degradation, while nanocellulose alone could only adsorb the dyes. However, the physical attachment between the enzyme and the nano-carrier means that it is possible for the enzyme to leak due to the relatively weaker bonding [36]. Additionally, laccase has been immobilized in calcium alginate beads for removal of aniline-based dye. The laccase was produced in the lab from solid-state cultivation of *Trametes versicolor* on barley husk and eggshell and was subsequently extracted for immobilization. The immobilization was achieved by entrapping the laccase in alginate beads [37] (see **Figure 2** for the process of entrapping enzymes in calcium alginate beads). Similar to laccase immobilized on nanocellulose, laccase in alginate beads removed aniline through degradation by laccase and biosorption by alginate beads, though the latter was the dominant mechanism in this study [37].

Figure 2. The process of immobilizing enzymes in calcium alginate beads.

There has been increasing interest in recycling waste organic materials for biocatalyst technologies either as the supports of immobilized enzymes or the cultures for enzyme production. Girelli et al. delignified spent grain using H_2SO_4/NaOH digestion and used it as a support for laccase immobilization [38]. The immobilization was accomplished through adsorption when the delignified spent grain was mixed with laccase solution. In a reactor, the immobilized enzyme was able to remove 66% of Methylene Orange and 100% of Methylene Blue (**Table 3**). Removal of the latter was primarily due to adsorption, as was the case of aniline biosorption by alginate beads reported by Tišma et al. [37]. The reason was because Methylene Blue as a cation dye was ionically attracted to the negative groups on the delignified spent grain support. Adsorption-facilitated removal was not observed for murexide, an ammonium salt of purpuric acid [38].

Laccase has also been successfully immobilized on a modified poly(vinylidene fluoride) membrane offering good mechanical strength and chemical stability [39]. Covalent bonding was involved in the immobilization, particularly glutaraldehyde crosslinking between laccase and the modified part of the membrane, namely the Fe_2O_3@SiO_2 cubes grafted on the polydopamine coated on the membrane. The immobilized enzyme was observed to have better storage stability and recyclability than free laccase with only 24% of the removal efficiency lost after five cycles of use [39]. Metal-organic frameworks have been used as supports for laccase immobilization. The frameworks were synthesized by adding metal nitrate solutions dropwise to 2-aminoterephthalic acid solutions until the respective solids were formed. The solids were isolated and vacuum dried. Laccase was immobilized on the solid frameworks through encapsulation [40]. The immobilized laccase was shown to remove Reactive Blue 171 and Reactive Blue 198, with laccase immobilized on Co metal-organic frameworks achieving a removal efficiency of 88% and 77%, respectively, when 6 mg of the biocatalyst was employed (**Table 3**). 18 mg of laccase immobilized on Cu metal-organic frameworks could remove 89% of Reactive Blue 171 but was not as effective against Reactive Blue 198 [40]. The activity of this immobilized enzyme was comparable to that immobilized on poly(vinylidene fluoride) membrane at the fifth cycle of use [39,40].

As with laccase, the immobilization of peroxidase, another type of enzyme capable of degrading dyes, could be performed with similar techniques. Peroxidase is commonly sourced from horseradish. Urrea et al. (2021) used extrusion method to entrap horseradish peroxidase in calcium alginate beads [41]. Unlike the study of Tišma et al. (2020), the beads did not seem to adsorb Orange II dye significantly [37,41]. The reason was probably because the dye was quickly removed by horseradish peroxidase in the presence of hydrogen peroxide. The immobilized enzyme could remove 70-90% of Orange II in liquid phase through catalyzing oxidation by hydrogen peroxide (**Table 3**). Furthermore, a study immobilized peroxidase isolated from bioremediation products of textile wastewater on Fe_3O_4 magnetic nanoparticles [42]. The nanoparticles were synthesized from co-precipitation of Fe^{3+} and Fe^{2+} ions followed by hydrothermal treatment before modification with glutaraldehyde to enable covalent conjugation of peroxidase on the nanoparticles. The immobilized enzyme completely decolorized Direct Green dye and Reactive Red dye at 4 h and 6 h of treatment respectively (**Table 3**). The covalent bonding has conferred it good stability, with full activity even after 100 reaction cycles [42].

Table 3. Enzymes and microbial cells immobilized on different supports and the efficiencies of the biocatalysts in removing dyes.

Enzyme	Support	Dye	Removal duration	Removal condition	Removal efficiency	Reference
Laccase	PEDOT-PPy-COOH/Pt	Procyon Red, Reactive Green 5, Reactive Green 19, Reactive Brown 10, Cibacron Blue F3GA, Alkali Blue 6B, Brilliant Blue 6	10 min	pH = 2–4.5 Temperature = 45–75 °C	76–94%	[35]
Laccase	Nanocellulose from quinoa husks	Malachite Green	30 min	pH = 5 Temperature = 40–50 °C	98%	[36]
		Congo Red		pH = 6 Temperature = 50 °C	60%	
Laccase	Calcium alginate beads	Aniline-based dye	24 h	pH = 4 Temperature = 25 °C	100%	[37]
Laccase	Delignified spent grain	Methylene Blue	150 min	pH = 5 Temperature = 30 °C	100%	[38]
		Methyl Orange	180 min		66%	
		Murexide	300 min		40%	
Laccase	Poly(vinylidene fluoride) polymer membrane	Congo Red	3 h	pH = 7 Temperature = 35 °C	97.1%	[39]
Laccase	Co metal-organic framework	Reactive Blue 171	1 h	pH = 4.5 Temperature = 50 °C	76% (2 mg of biocatalyst) 82% (4 mg) 88% (6 mg)	[40]
		Reactive Blue 198			66% (2 mg) 73% (4 mg) 77% (6 mg)	
	Cu metal-organic framework	Reactive Blue 171		pH = 5 Temperature =50 °C	89% (18 mg of biocatalyst)	
		Reactive Blue 198			39% (18 mg)	
Peroxidase	Calcium alginate beads	Orange II – an azo dye	25 min	pH = 9 Temperature = 22 °C	70–90%	[41]
Peroxidase	Fe_3O_4 magnetic nanoparticles	Direct Green	4 h	pH = 6 Temperature = 20 °C	100%	[42]
		Reactive Red	6 h		100%	
Peroxidase	Cationic maize starch	Methyl Orange	1 h	pH = 7 Temperature = 50 °C	97%	[43]
Peroxidase	Amine-functionalized superparamagnetic iron oxide	Acid Black	Not specified	pH = 5.6 Temperature = 40 °C	Not specified	[44]
Peroxidase	Functionalized reduced graphene oxide-SiO_2	Methyl Green	35 min	pH = 7 Temperature = 25 °C	100%	[45]
		Phenol Red	35 min		100%	
		Coomassie Brilliant Blue (R250)	60 min		100%	
		Methyl Orange	60 min		100%	
		Bromophenol Blue	60 min		90%	
		Bromothymol Blue	60 min		80%	
		Bromocresol Green	60 min		70%	
		Methyl Red	60 min		45%	
		Reactive Black 5 (RB5)	60 min		40%	

Table 3 continued

Lysinibacillus fusiformis and *Mesobacillus jeotgal*	Calcium alginate beads	Congo Red	3 days	pH = 7 Temperature = 38 °C	96.0% (250 mg/L of dye) 93.7% (500 mg/L) 88.6% (1000 mg/L)	[46]
		Methylene Blue	3 days		91.6% (250 mg/L) 85.1% (500 mg/L) 80.8% (1000 mg/L)	
Bacillus subtilis	Superparamagnetic Fe_3O_4 nanoparticles	Congo Red	24 h	pH = 7 Temperature 37 °C	95%	[47]
Brevibacillus laterosporus and *Galactomyces geotrichum*	Calcium alginate	50 mg/L Remazol Red	20 h	Not specified	100%	[48]
	Polyvinyl alcohol		24 h		100%	
	Stainless steel sponge		11 h		100%	
	Polyurethane foam		15 h		100%	
Raoultella Ornithinolytica sp. A1	Magnetic Fe_3O_4 nanoparticles	Basic Blue 41 azo dye	24 h	pH = 8 Temperature = 37 °C	95.2%	[49]
Rhodococcus opacus 1CP azoreductase	Meso-cellular foams mesoporous silica (with amino groups)	Allura Red	18 h	Not reported	39.6%	[50]
		Brilliant Black			46.9%	
		Methyl Orange			18.3%	
		Reactive Orange			58.3%	

Note: Removal is used interchangeably with decolorization to indicate a reduction in dye concentration.

Mohamed et al. immobilized horseradish peroxidase on cationic maize starch, which was synthesized via deprotonating the hydroxyl groups in the starch, followed by etherification using (S)-1-amino-3-chloropropan-2-ol [43]. This modification enabled horseradish peroxidase to electrostatically adsorb onto cationic starch. The biocatalyst could decolorize 97% of Methyl Orange after 1 h of incubation (**Table 3**). Keshta et al. employed amine-functionalized superparamagnetic iron oxide for immobilization of horseradish peroxidase [44]. The enzyme was physically adsorbed onto the inorganic support, unlike that of Darwesh et al. (2019) involving covalent conjugation [42,44]. Both studies used Fe_3O_4 nanoparticles, but different modification methods where Keshta et al. performed amine-functionalization using (3-aminopropyl) triethoxysilane, instead of adding glutaraldehyde. The immobilized enzyme was reported to have significantly better ability to decolorize or remove Acid Black dye than free enzyme, and the removal efficiency was affected by factors such as amount of biocatalyst, pH, temperature, and hydrogen peroxide concentrations [44].

In a separate study, horseradish peroxidase immobilization was achieved through covalent bonding, with a functionalized reduced graphene oxide-SiO_2 as the support [45]. The functionalized reduced graphene oxide was synthesized via hydrolysis of tetraethyl orthosilicate in an aqueous dispersion of graphene oxide. The biocatalyst was able to decolorize 100% of Methyl Green and Phenol Red in 35 min, while it removed 100% of Coomassie Brilliant Blue (R250) and Methyl Orange in 60 min. Besides, it could remove 90% of Bromophenol Blue, 80% of Bromothymol Blue and 70% of Bromocresol Green in 60 min (**Table 3**). As with other immobilized enzymes, the biocatalyst demonstrated less sensitivity to pH change and remained active at higher temperatures. After 10 reaction cycles, it still

retained 70% of its initial activity [45].

As for azoreductase, most of the relevant studies focus on immobilization of cells rather than the enzyme (**Table 3**). Subramaniam et al. isolated *Lysinibacillus fusiformis* and *Mesobacillus jeotgali* from termite mound soil and entrapped the cells in calcium alginate gel beads [46]. The immobilized cells were observed to decolorize 250, 500 and 1000 mg/L of Congo Red at the efficiencies of 96.0%, 93.7% and 88.6% respectively while they removed Methylene Blue of the same concentrations at the efficiencies of 91.6%, 85.2% and 80.8% respectively 3 days after inoculation (**Table 3**). The decolorization was attributed to the activity of azoreductase. Immobilization increased the activity of the enzyme from 0.089 units/mg to 0.093 units/mg. The immobilized cells were also found to have higher efficiencies of dye decolorization than free cells [46]. A separate study immobilized *Bacillus subtilis* on Fe_3O_4 nanoparticles, another popular support, through adsorption [47]. The biocatalyst can be separated by applying a magnetic field and recycled. It recorded a Congo Red decolorization rate up to 95% at pH 7 and could be reused for 7 cycles. The decolorization was due to biosorption by bacteria and enzymatic degradation by azoreductase. Azoreductase can be found in the intra- and extracellular compartments of bacteria [47].

Kurade et al. compared the efficiencies of a mixture of bacteria (*Brevibacillus laterosporus*) and yeast (*Galactomyces geotrichum*) immobilized in calcium alginate, polyvinyl alcohol, polyurethane foam, and stainless-steel sponge [48]. The authors reported that total removal of 50 mg/L Remazol Red was achieved by microbial consortia bound to the surface of polyurethane foam and stainless-steel sponge after 11 and 15 h of treatment, respectively, in comparison to 20 and 24 h of treatment for microbial consortia entrapped in calcium alginate and polyvinyl alcohol, respectively (**Table 3**). Microbial consortia immobilized in calcium alginate and polyvinyl alcohol demonstrated good recyclability with significant activities up to 5 cycles [48]. The microbial consortia secreted an array of enzymes for decolorization of the dye, comprising azoreductase, laccase, riboflavin reductase, tyrosinase, and veratryl alcohol oxidase. Furthermore, magnetic Fe_3O_4 nanoparticles were used again as supports for *Raoultella Ornithinolytica* sp. A1 [49]. The biocatalyst was also observed to remove 80.14% of the dye in continuous batch cycles with fresh dye added at 4 intervals of 24 h (**Table 3**). Azoreductase was deemed to be the enzyme contributing to dye removal by the bacteria [49].

While most studies on azoreductase biocatalyst are related to immobilization of bacteria probably due to greater challenges in immobilizing azoreductase since it lacks stability, there is at least one study that has successfully immobilized isolated azoreductase for this purpose [50]. The azoreductase was isolated from *Rhodococcus opacus* 1CP and has poor stability. Unsuccessful attempts to immobilize the enzyme using cross-linked enzyme aggregates and crystals were reported. In the study, azoreductase was immobilized on functionalized mesoporous silicas via covalent bonding, which significantly improved its stability [50]. The biocatalyst could decolorize 39.6% of Allura Red, 46.9% of Brilliant Black, 18.3% of Methyl Orange, and 58.3% of Reactive Orange over 18 h (**Table 3**). Compared to the dye removal efficiencies of immobilized laccase and peroxidase, these decolorization rates are relatively low.

5. Feasibility of biocatalysts in dye-containing wastewater treatment

Biocatalysts, particularly immobilized enzymes, and bacteria appear to be a promising option for treating dye-containing wastewater as they offer the advantages of high stability, reusability, and easy separation from reaction mixture. However, their efficiency depends on several factors, such as the type of enzyme, the immobilization method, the reaction conditions, and the characteristics of wastewater. Different immobilization methods have been proposed and they appear to confer varying effects on enzymes. Peroxidase covalently bonded to Fe_3O_4 nanoparticles retained their activities after 100

uses while laccase covalently bonded to a modified poly(vinylidene fluoride) membrane started losing activity after the fifth cycle [42]. This indicates that stability and recyclability could be enzyme- and support-dependent.

Biocatalysts can be packed in bioreactors for treatment of dye-containing wastewater [51]. The common bioreactors are batch, continuous, and membrane reactors [48]. Packed bed reactor, for instance, is a type of reactors that packs immobilized enzymes in the reactor bed where wastewater flows through and is continuously treated through contact with the immobilized enzymes [48]. Experimentally, soybean peroxidase immobilized on alginate membrane has been employed in packed bed reactor to treat wastewater containing phenol and the reactor attained a removal rate of 96.7% [52]. Additionally, 50 mg of immobilized laccase has been packed in a steel column for dye removal at bench scale in recirculation mode. Recirculation mode was observed to be more efficient than batch system in removing dyes [38]. With immobilization of enzymes on membranes, the use of membrane reactors for treating dye-containing wastewater has been made possible [39].

However, these technologies have not been able to progress beyond bench scale, thus greatly limiting their feasibility for industrial use, despite their good efficiency, stability, and recyclability. The following needs to be addressed to increase the industrial feasibility of biocatalysts:

- Wastewater from dye-related industries is composed of a complex mixture of contaminants instead of a single type of dyes. The presence of other contaminants could negatively affect the activities of biocatalysts [53].
- Biocatalysts are often specific in action and may only be effective in removing certain dyes. A mixture of biocatalysts may be required to enable concurrent removal of different dyes, but these biocatalysts may require different conditions to work optimally.
- Immobilization materials and methods may incur high cost and may not be economically feasible for large-scale production. Specifically, both the enzyme of interest and the substrate surface have to be modified by single or multi-step processes, which can increase reagent cost, operation time, and the complexity of immobilization [17].
- Biocatalyst leaching occurs over time. Biocatalysts also lose their activities over repeated use, some as soon as the fifth cycle. This means that constant replacement of biocatalysts may be needed, thus, further increasing operational cost.
- Leaching or leakage of enzymes or bacteria from the support matrix could pollute the environment. Similarly, certain support materials may pose environmental hazards, which have not been well-characterized [17,54].
- Reduced mass transfer and diffusion rates of substrates and products through support materials may affect the reaction kinetics and efficiency [55].
- Limited availability and diversity of enzymes and bacteria that can be immobilized may limit the range of applications and substrates.
- Dynamic operating conditions in the industry may lead to changing operational parameters which may not favor the activities of biocatalysts since they may have limited ranges of optimal pH and temperature.
- Biocatalysts may not result in mineralization of dyes. The dyes may be partially degraded and converted into products which still pose hazards to the environment.
- Using recombinant proteins for immobilized enzymes can put a strain on the enzyme during the immobilization process, leading to significant changes in its conformation and catalytic properties. This is due to certain harsh conditions during immobilization such as high temperatures and the use of organic solvents, as well as the potential involvement of similar amino-groups at the active site during the interaction of the enzyme with the matrix.

6. Conclusions

Biocatalytic technologies have emerged as an attractive alternative to remove dyes from textile wastewater due to their high catalytic activities,

hence efficiency. They are more stable than free enzymes and bacteria and remain functional over a wider range of pH and temperature. Their activities frequently last longer and they are recyclable. Laccase and peroxidase have been successfully immobilized on a myriad of supports, consisting of PEDOT-PPy-COOH/Pt, nanocellulose from quinoa husks, calcium alginate beads, poly(vinylidene fluoride) polymer membrane, metal-organic framework, Fe_3O_4 magnetic nanoparticles, cationic maize starch and functionalized reduced graphene oxide-SiO_2. Immobilization of azoreductase has not been as extensively conducted as laccase and peroxidase, probably because of its lack of stability and thus, microbial cells have been immobilized instead to harness the dye-degrading ability of azoreductase. Immobilization of microorganisms also confers the biocatalysts better stability, efficiency, and recyclability. There is an increasing trend of using green materials to synthesize the supports for immobilization. However, biocatalytic technologies have certain inherent limitations that need to be addressed to enhance their feasibility and practicality. The specificity of biocatalysts may limit their use for treating wastewater from dye-related industries which contains a mixture of pollutants. Biocatalyst technologies could be costly, and biocatalysts may be subjected to leaching and leakage, prompting their frequent replacement, thus, raising operational cost. The environmental hazards of biocatalysts have not been extensively studied. While enzymes are known to denature and degrade in the environment, the risks associated with the use of microorganisms, especially the genetically modified ones, as biocatalysts are not well characterized.

Therefore, this review recommends the use of low-cost and eco-friendly materials as enzyme supports, such as biochar, clay, chitosan, and cellulose. It recommends the development of a versatile biocatalyst or mixture of biocatalysts that can degrade a wider array of dyes commonly present in wastewater from dye-related industries and can withstand variations of operating conditions, particularly pH and temperature, over wider ranges. In fact, most biocatalysts can withstand larger pH and temperature variations than free enzymes or microorganisms and these characteristics can be optimized according to the operating conditions of the bioreactors they are employed in. The use of hybrid carriers such as alginate-gelatin-calcium can provide more efficient encapsulation of enzyme, thus preventing enzyme leakage, and provide increased mechanical stability. Genetic engineering techniques like site-directed mutagenesis and in-vitro evolution via gene shuffling can help in enzyme manipulation to exhibit the desired properties. Having said that, it is also necessary to design bioreactors that can accommodate the biocatalysts and allow them to function optimally. This may also require an understanding of the mediators or co-enzymes needed to enhance the performance of the biocatalysts and the combination of biocatalysts to achieve synergistic effect.

Conflict of Interest

The author declares that there are no known conflicts of interest.

Funding

Not applicable.

References

[1] Maheshwari, K., Agrawal, M., Gupta, A.B., 2021. Dye Pollution in Water and Wastewater. Springer Singapore: Singapore. pp. 1–25. DOI: https://doi.org/10.1007/978-981-16-2892-4_1

[2] Tang, K. H. (2023). State of the Art in Textile Waste Management: A Review. Textiles. 3(4), 454-467. DOI: https://doi.org/10.3390/textiles3040027

[3] Dyes And Pigments Market Size, Share & Trends Analysis Report by Product (Dyes (Reactive, Vat, Acid, Direct, Disperse), Pigment (Organic, Inorganic)), by Application, by Region, and Segment Forecasts, 2023–2030

[Internet]. Available from: https://www.grandviewresearch.com/industry-analysis/dyes-and-pigments-market

[4] Islam, T., Repon, M.R., Islam, T., et al., 2023. Impact of textile dyes on health and ecosystem: a review of structure, causes, and potential solutions. Environmental Science and Pollution Research. 30(4), 9207–9242.
DOI: https://doi.org/10.1007/s11356-022-24398-3

[5] Agaricales (Gilled Mushrooms) as Biosorbents of Synthetic Dye [Internet]. Available from: http://www.medic.upm.edu.my/upload/dokumen/2020112512045902_2020_0295.pdf

[6] Tang, K. H. D., Darwish, N. M., Alkahtani, A. M., et al. (2022). Biological Removal of Dyes from Wastewater: A Review of Its Efficiency and Advances. Tropical Aquatic and Soil Pollution, 2(1), 59-75.
DOI: https://doi.org/10.53623/tasp.v2i1.72

[7] Benkhaya, S., M'rabet, S., Lgaz, H., et al. (2022). Dyes: Classification, Pollution, and Environmental Effects . Springer Singapore: Singapore. pp 1–50.
DOI: https://doi.org/10.1007/978-981-16-5932-4_1

[8] Rehorek, A., Plum, A. (2007). Characterization of sulfonated azo dyes and aromatic amines by pyrolysis gas chromatography/mass spectrometry. Analytical and Bioanalytical Chemistry, 388(8), 1653–1662.
DOI: https://doi.org/10.1007/s00216-007-1390-0

[9] Piaskowski, K., Świderska-Dąbrowska, R., Zarzycki, P. K. (2018). Dye Removal from Water and Wastewater Using Various Physical, Chemical, and Biological Processes. Journal of AOAC INTERNATIONAL, 101(5), 1371–1384.
DOI: https://doi.org/10.5740/jaoacint.18-0051

[10] Salman, M., Demir, M., Tang, K. H. D., et al. (2022). Removal of cresol red by adsorption using wastepaper. Industrial and Domestic Waste Management, 2(1), 1–8.
DOI: https://doi.org/10.53623/idwm.v2i1.63

[11] Zhou, Y., Lu, J., Zhou, Y., et al. (2019). Recent advances for dyes removal using novel adsorbents: A review. Environmental Pollution, 252, 352–365.
DOI: https://doi.org/10.1016/j.envpol.2019.05.072

[12] Teh, C. Y., Budiman, P. M., Shak, K. P. Y., et al. (2016). Recent Advancement of Coagulation–Flocculation and Its Application in Wastewater Treatment. Industrial & Engineering Chemistry Research, 55(16), 4363–4389.
DOI: https://doi.org/10.1021/acs.iecr.5b04703

[13] Tang, K. H. D., Awa, S. H., Hadibarata, T. (2020). Phytoremediation of Copper-Contaminated Water with Pistia stratiotes in Surface and Distilled Water. Water, Air, & Soil Pollution, 231(12), 573.
DOI: https://doi.org/10.1007/s11270-020-04937-9

[14] Tang, K.H.D., 2019. Phytoremediation of soil contaminated with petroleum hydrocarbons: A review of recent literature. Glob J Civil Environ Eng. 1: 33-42.
DOI: https://doi.org/10.36811/gjcee.2019.110006

[15] Mishra, A., Takkar, S., Joshi, N.C., et al., 2022. An Integrative Approach to Study Bacterial Enzymatic Degradation of Toxic Dyes. In Frontiers in Microbiology (Vol 12).
DOI: https://doi.org/10.3389/fmicb.2021.802544

[16] Naseem, S., Rawal, R. S., Pandey, D., et al. (2023). Immobilized laccase: an effective biocatalyst for industrial dye degradation from wastewater. Environmental Science and Pollution Research, 30(36), 84898–84917.
DOI: https://doi.org/10.1007/s11356-023-28275-5

[17] Morsy, S. A. G. Z., Ahmad Tajudin, A., Ali, M. S. M., et al. (2020). Current Development in Decolorization of Synthetic Dyes by Immobilized Laccases . In Frontiers in Microbiology (Vol 11).
DOI: https://doi.org/10.3389/fmicb.2020.572309

[18] Harish, B.S., Thayumanavan, T., Nambukrishnan, V., et al., 2023. Heterogeneous biocatalytic system for effective decolorization of textile dye effluent. 3 Biotech, 13(6), 165.
DOI: https://doi.org/10.1007/s13205-023-03586-z

[19] Dutta, S., Gupta, B., Srivastava, S.K., et al. (2021). Recent advances on the removal of

dyes from wastewater using various adsorbents: a critical review. Materials Advances, 2(14), 4497–4531.
DOI: https://doi.org/10.1039/D1MA00354B

[20] Bhattacharya, S., Mazumder, A., Sen, D., et al., 2022. Bioremediation of Dye Using Mesophilic Bacteria: Mechanism and Parametric Influence. Springer Singapore: Singapore. pp. 67–86.
DOI: https://doi.org/10.1007/978-981-16-5932-4_3

[21] Dave, B., Sanghvi, G., 2022. Enzyme Action for Dye Degradation. Springer Singapore: Singapore. pp. 141–163.
DOI: https://doi.org/10.1007/978-981-16-5932-4_6

[22] Mishra, S., Maiti, A., 2019. Applicability of enzymes produced from different biotic species for biodegradation of textile dyes. Clean Technologies and Environmental Policy. 21(4), 763–781.
DOI: https://doi.org/10.1007/s10098-019-01681-5

[23] Singh, G., Dwivedi, S. K., Mishra, J. (2020). Role of Fungal Enzymes in the Removal of Azo Dyes. Springer Singapore: Singapore. pp. 231–257.
DOI: https://doi.org/10.1007/978-981-15-1710-5_9

[24] Bhandari, S., Poudel, D.K., Marahatha, R., et al., 2021. Microbial enzymes used in bioremediation. Journal of Chemistry, 2021, 8849512.
DOI: https://doi.org/10.1155/2021/8849512

[25] Khan, M. R. (2021). Immobilized enzymes: A comprehensive review. Bulletin of the National Research Centre, 45(1), 207.
DOI: https://doi.org/10.1186/s42269-021-00649-0

[26] Tang, K.H.D., Lock, S.S.M., Yap, P.S., et al., 2022. Immobilized enzyme/microorganism complexes for degradation of microplastics: A review of recent advances, feasibility and future prospects. Science of the Total Environment. 832, 154868.
DOI: https://doi.org/10.1016/j.scitotenv.2022.154868

[27] Bodade, R. G., Bodade, A. G. (2020). Chapter 17 - Microencapsulation of bioactive compounds and enzymes for therapeutic applications. Elsevier: Amsterdam. pp. 381–404.
DOI: https://doi.org/10.1016/B978-0-12-816897-4.00017-5

[28] Marikar, S.N., El-Osta, A., Johnston, A., et al., 2022. Microencapsulation-based cell therapies. Cellular and Molecular Life Sciences. 79(7), 351.
DOI: https://doi.org/10.1007/s00018-022-04369-0

[29] Naresh, V., Lee, N., 2021. A review on biosensors and recent development of nanostructured materials-enabled biosensors. In Sensors 21(4).
DOI: https://doi.org/10.3390/s21041109

[30] Karthik, V., Senthil Kumar, P., Vo, D.V.N., et al., 2021. Enzyme-loaded nanoparticles for the degradation of wastewater contaminants: A review. Environmental Chemistry Letters. 19(3), 2331–2350.
DOI: https://doi.org/10.1007/s10311-020-01158-8

[31] Thangaraj, B., Solomon, P.R., 2019. Immobilization of Lipases – A Review. Part I: Enzyme Immobilization. ChemBioEng Reviews, 6(5), 157–166.
DOI: https://doi.org/10.1002/cben.201900016

[32] Reshmy, R., Philip, E., Sirohi, R., et al., 2021. Nanobiocatalysts: Advancements and applications in enzyme technology. Bioresource Technology. 337, 125491.
DOI: https://doi.org/10.1016/j.biortech.2021.125491

[33] Imam, H.T., Marr, P.C., Marr, A.C., 2021. Enzyme entrapment, biocatalyst immobilization without covalent attachment. Green Chemistry. 23(14), 4980–5005.
DOI: https://doi.org/10.1039/D1GC01852C

[34] Nguyen, H. H., Kim, and M. (2017). An Overview of Techniques in Enzyme Immobilization. Applied Science and Convergence Technology, 26(6), 157–163.
DOI: https://doi.org/10.5757/ASCT.2017.26.6.157

[35] Uygun, M., Asunción-Nadal, V. de la, et al. (2021). Dye removal by laccase-functionalized micromotors. Applied Materials Today, 23, 101045.
DOI: https://doi.org/10.1016/j.apmt.2021.101045

[36] Ariaeenejad, S., Motamedi, E., Salekdeh, G.H.,

2022. Highly efficient removal of dyes from wastewater using nanocellulose from quinoa husk as a carrier for immobilization of laccase. Bioresource Technology. 349, 126833.
DOI: https://doi.org/10.1016/j.biortech.2022.126833

[37] Tišma, M., Šalić, A., Planinić, M., et al., 2020. Production, characterisation and immobilization of laccase for an efficient aniline-based dye decolourization. Journal of Water Process Engineering. 36, 101327.
DOI: https://doi.org/10.1016/j.jwpe.2020.101327

[38] Girelli, A.M., Pambianco, E., Scuto, F.R., 2021. Sustainable recycling of spent grain for laccase immobilization as dyes removal tool. Journal of Environmental Chemical Engineering. 9(6), 106653.
DOI: https://doi.org/10.1016/j.jece.2021.106653

[39] Zhu, Y., Qiu, F., Rong, J., et al., 2020. Covalent laccase immobilization on the surface of poly(vinylidene fluoride) polymer membrane for enhanced biocatalytic removal of dyes pollutants from aqueous environment. Colloids and Surfaces B: Biointerfaces. 191, 111025.
DOI: https://doi.org/10.1016/j.colsurfb.2020.111025

[40] Birhanlı, E., Noma, S.A.A., Boran, F., et al., 2022. Design of laccase–metal–organic framework hybrid constructs for biocatalytic removal of textile dyes. Chemosphere. 292, 133382.
DOI: https://doi.org/10.1016/j.chemosphere.2021.133382

[41] Urrea, D.A.M., Gimenez, A.V.F., Rodriguez, Y. E., et al. (2021). Immobilization of horseradish peroxidase in Ca-alginate beads: Evaluation of the enzyme leakage on the overall removal of an azo-dye and mathematical modeling. Process Safety and Environmental Protection, 156, 134–143.
DOI: https://doi.org/10.1016/j.psep.2021.10.006

[42] Darwesh, O.M., Matter, I.A., Eida, M.F., 2019. Development of peroxidase enzyme immobilized magnetic nanoparticles for bioremediation of textile wastewater dye. Journal of Environmental Chemical Engineering. 7(1), 102805.
DOI: https://doi.org/10.1016/j.jece.2018.11.049

[43] Mohamed, S. A., Elaraby, N. M., Abdel-Aty, et al. (2021). Improvement of enzymatic properties and decolorization of azo dye: immobilization of horseradish peroxidase on cationic maize starch. Biocatalysis and Agricultural Biotechnology, 38, 102208.
DOI: https://doi.org/10.1016/j.bcab.2021.102208

[44] Keshta, B.E., Gemeay, A.H., Khamis, A.A., 2022. Impacts of horseradish peroxidase immobilization onto functionalized superparamagnetic iron oxide nanoparticles as a biocatalyst for dye degradation. Environmental Science and Pollution Research. 29(5), 6633–6645.
DOI: https://doi.org/10.1007/s11356-021-16119-z

[45] Vineh, M.B., Saboury, A.A., Poostchi, A.A., et al., 2020. Biodegradation of phenol and dyes with horseradish peroxidase covalently immobilized on functionalized RGO-SiO2 nanocomposite. International Journal of Biological Macromolecules. 164, 4403–4414.
DOI: https://doi.org/10.1016/j.ijbiomac.2020.09.045

[46] Subramaniam, S., Velu, G., Palaniappan, M., et al., 2023. Decolourization of azo dyes using immobilized bacterial isolates from termite mound ecosystem. Total Environment Research Themes. 6, 100041.
DOI: https://doi.org/10.1016/j.totert.2023.100041

[47] Nadi, A., Boyer, D., Charbonnel, N., et al. (2019). Immobilisation of bacteria onto magnetic nanoparticles for the decolorisation and degradation of azo dyes. IET Nanobiotechnology, 13(2), 144–149.
DOI: https://doi.org/10.1049/iet-nbt.2018.5026

[48] Kurade, M. B., Waghmode, T. R., Xiong, J.-Q., et al. (2019). Decolorization of textile industry effluent using immobilized consortium cells in upflow fixed bed reactor. Journal of Cleaner Production, 213, 884–891.
DOI: https://doi.org/10.1016/j.jclepro.2018.12.218

[49] Bekhit, F., Farag, S., Attia, A.M., 2022. Characterization of immobilized magnetic Fe3O4

nanoparticles on raoultella ornithinolytica sp. and its application for azo dye removal. Applied Biochemistry and Biotechnology. 194(12), 6068–6090.
DOI: https://doi.org/10.1007/s12010-022-04076-3

[50] Qi, J., Anke, M.K., Szymańska, K., et al., 2017. Immobilization of Rhodococcus opacus 1CP azoreductase to obtain azo dye degrading biocatalysts operative at acidic pH. International Biodeterioration & Biodegradation. 118, 89–94.
DOI: https://doi.org/10.1016/j.ibiod.2017.01.027

[51] Zdarta, J., Jankowska, K., Bachosz, K., et al., 2021. Enhanced wastewater treatment by immobilized enzymes. Current Pollution Reports. 7(2), 167–179.
DOI: https://doi.org/10.1007/s40726-021-00183-7

[52] Rezvani, F., Azargoshasb, H., Jamialahmadi, O., et al., 2015. Experimental study and CFD simulation of phenol removal by immobilization of soybean seed coat in a packed-bed bioreactor. Biochemical Engineering Journal. 101, 32–43.
DOI: https://doi.org/10.1016/j.bej.2015.04.019

[53] Dwevedi, A. (2016). Basics of Enzyme Immobilization. Springer International Publishing: Cham. pp.21–44.
DOI: https://doi.org/10.1007/978-3-319-41418-8_2

[54] Li, Y., Liu, C., Yang, H., et al., 2024. Leaching of chemicals from microplastics: A review of chemical types, leaching mechanisms and influencing factors. Science of the Total Environment. 906, 167666.
DOI: https://doi.org/10.1016/j.scitotenv.2023.167666

[55] Sirisha, V.L., Jain, A., Jain, A. (2016). Chapter Nine - Enzyme Immobilization: An Overview on Methods, Support Material, and Applications of Immobilized Enzymes. Academic Press: MA. pp. 179–211.
DOI: https://doi.org/10.1016/bs.afnr.2016.07.004