

Review

Integrated Enzymatic–Microbial Systems for Textile Wastewater Detoxification: Mechanisms, Synergies and Industrial Perspectives

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Abstract

The textile industry generates dye-containing wastewater characterized by intense coloration, high salinity, elevated oxygen demand, and recalcitrant pollutants. Although conventional treatment technologies can remove color and reduce organic load, decolorization alone does not guarantee detoxification because toxic transformation products may persist or be generated during incomplete degradation. This review emphasizes the synergistic interaction between oxidative and reductive enzymes and microorganisms as a promising strategy for promoting extensive pollutant transformation and potentially enhancing mineralization. Particular attention is given to the complementary roles of laccases (LAC), azoreductases (AZOR), ligninolytic peroxidases, bacteria, fungi, and microbial consortia in sequential degradation pathways that can improve detoxification efficiency. Mechanisms including azo bond cleavage, aromatic amine transformation, and subsequent microbial degradation are discussed to demonstrate how integrated systems can overcome the limitations of enzyme- or microorganism-based treatments alone and may facilitate complete pollutant removal under appropriate conditions. The review also explores biomass-derived biocatalysts produced through solid-state fermentation of agro-industrial residues, demonstrating opportunities to integrate wastewater remediation with biomass valorization and circular bioeconomy principles. Finally, operational challenges, toxicity assessment, reactor design, enzyme immobilization, scale-up, and techno-economic considerations are discussed, and future research priorities are identified to support the development of sustainable and industrially applicable enzymatic–microbial detoxification technologies.



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1. Introduction

1.1. Global Impact of Textile Industry Waste

The textile industry plays a fundamental role in the global economy, contributing significantly to employment generation, industrial development, and international trade.

Countries such as China, India, Brazil, Bangladesh, and Pakistan account for a substantial share of global textile production, making this sector a major economic driver [1,2]. Despite its economic importance, the textile industry is recognized as one of the most environmentally impactful industrial sectors due to its intensive water consumption and the generation of large volumes of contaminated wastewater [1,3].

A significant portion of these environmental impacts originates from wet-processing operations, including desizing, bleaching, mercerization, dyeing, printing, and finishing. These processes require substantial amounts of water and involve the use of a wide range of chemical reagents, resulting in the generation of wastewater characterized by high organic and inorganic pollutant loads. In many regions, inadequate treatment and improper disposal of these effluents contribute to the degradation of water resources and aquatic ecosystems [1,2,4].

Textile wastewater (TW) has a complex composition, including intense coloration, high chemical oxygen demand (COD) and biochemical oxygen demand (BOD), elevated salinity, suspended solids, variable pH, and the presence of recalcitrant organic compounds [1,2]. Among these pollutants, attention has been paid to synthetic dyes due to their chemical stability, resistance to biodegradation, and potential toxicity [3]. Azo dyes account for approximately 60–70% of all dyes used in the textile industry and possess complex aromatic structures that enhance their environmental persistence [3,5]. Furthermore, a considerable fraction of these dyes fails to bind to textile fibers during the dyeing process and is subsequently discharged into wastewater streams [6].

In addition to dyes, textile effluents may contain inorganic salts, surfactants, detergents, chlorinated compounds, phenolic substances, and various other chemical additives employed throughout textile manufacturing processes [1]. Potentially toxic metals, including chromium, lead, cadmium, mercury, and arsenic, may also be present because of their application in dye and pigment formulations. These contaminants exhibit high environmental persistence and bioaccumulation potential, posing significant risks to ecosystems and human health (Figure 1) [1,6].

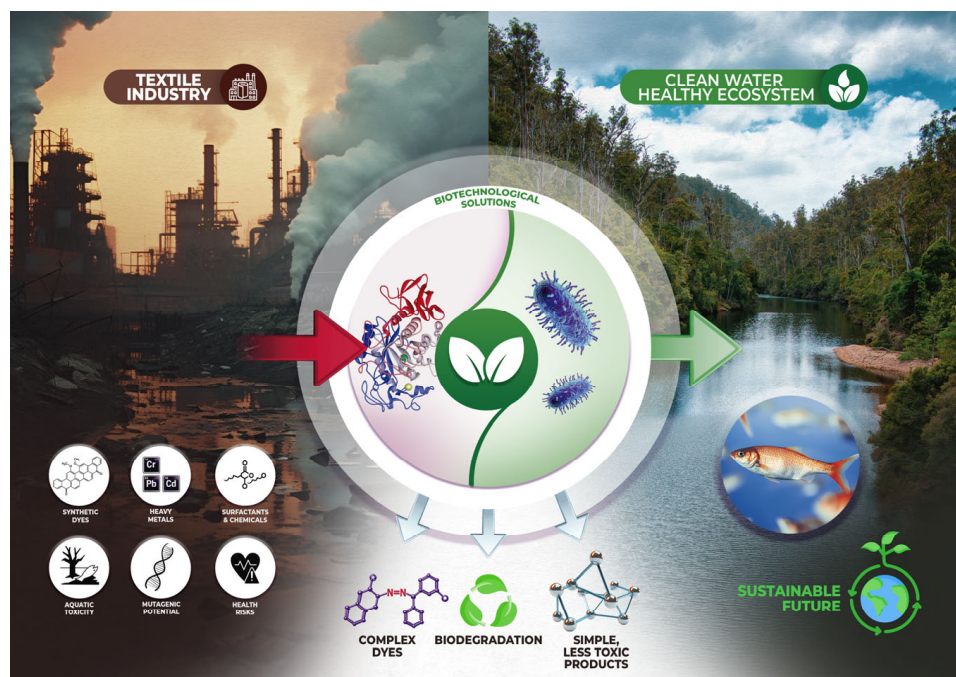


Figure 1. From industrial pollution to healthy ecosystems: integrated microbial–enzymatic bioremediation of textile effluents.

The environmental consequences associated with the improper discharge of textile wastewater (TW) are extensive. Intense coloration reduces light penetration into aquatic environments, impairing photosynthetic activity and decreasing dissolved oxygen levels, thereby adversely affecting aquatic flora and fauna [3,5]. Moreover, several synthetic dyes, particularly azo dyes, have been reported to exhibit mutagenic, cytotoxic, and carcinogenic properties and may generate toxic aromatic amines during conventional degradation processes [1,6]. Exposure to these compounds has been associated with dermatitis, respiratory disorders, neurological impairments, and various forms of cancer [3].

In this context, attention is given to alternative bio-based approaches for TW management. Between the available bio-based approaches, the production of biomass-derived biocatalysts through solid-state fermentation of agro-industrial residues is a growing research field, highlighting how biomass valorization can be integrated with TW detoxification within circular bioeconomy frameworks.

1.2. Limitations of Conventional Treatment Methods

The treatment of TW remains a major challenge due to the high chemical complexity of these effluents, which frequently contain synthetic dyes, salts, surfactants, potentially toxic metals, and recalcitrant organic compounds [7,8]. Although various physicochemical technologies have been widely applied for the removal of these pollutants, many of them present limitations related to treatment efficiency, operational costs, and the generation of secondary waste. Among the most employed conventional methods are coagulation/flocculation, adsorption, and oxidative processes.

Coagulation/flocculation is effective for the removal of suspended particles and colloidal matter and is therefore frequently applied as a pretreatment step. However, its efficiency decreases considerably when treating highly soluble dyes, such as azo, reactive, acid, and basic dyes. Furthermore, this process generates substantial quantities of sludge that require appropriate treatment and disposal, increasing the overall environmental and economic burden of wastewater management [7–10].

Adsorption has been extensively utilized due to its high dye removal capacity, particularly when activated carbon and other adsorbent materials are employed. Nevertheless, this process does not degrade contaminants but merely transfers them from the liquid phase to a solid matrix. In addition, the regeneration or replacement of adsorbents can significantly increase operational costs, limiting the economic feasibility of large-scale applications [7,8].

Oxidative technologies, including ozonation, UV/H₂O₂ systems, the Fenton reaction, and Advanced Oxidation Processes, have been widely investigated because of their ability to degrade persistent organic pollutants through the generation of highly reactive species. Despite their high treatment efficiencies, these systems generally require substantial energy inputs, continuous chemical reagent consumption, and strict operational conditions, all of which contribute to elevated implementation and operating costs [7,8,11]. Furthermore, some oxidative processes may generate potentially toxic by-products or secondary waste, such as the iron-rich sludge produced during Fenton-based treatments [7].

Therefore, although physicochemical methods play an important role in the current state of art for TW treatment, their standalone application is not always sufficient to meet increasingly stringent environmental requirements. These limitations have stimulated the development of more sustainable and efficient treatment strategies, particularly biotechnological approaches capable of achieving effective pollutant degradation while reducing chemical consumption, energy demand, and secondary waste generation.

1.3. Emergence of Biotechnological Alternatives

The limitations associated with conventional treatment technologies have driven the development of more sustainable alternatives for TW remediation. In this context, biotechnological processes based on microorganisms and enzymes have gained increasing attention due to their ability to degrade complex contaminants while requiring lower energy inputs and generating fewer secondary wastes than conventional physicochemical approaches [12,13].

Unlike many physicochemical methods, biological processes can promote degradation and, in some cases, the complete mineralization of recalcitrant organic pollutants. Bacteria, fungi, and yeasts have been extensively investigated for the removal of synthetic dyes and other contaminants commonly found in textile wastewater. These organisms employ diverse metabolic pathways capable of transforming complex molecules into less toxic compounds that can be more readily assimilated or further degraded within the environment [12].

Among the various biotechnological strategies, enzyme-based treatments have attracted particular interest due to their remarkable catalytic potential. Primarily produced by microorganisms, these biocatalysts operate under mild conditions and exhibit high substrate specificity, catalytic efficiency, and operational stability [13]. Enzymes such as azoreductases (AZOR (EC 1.7.1.6)), laccases (LAC (EC 1.10.3.2)), lignin peroxidase (LiP (EC 1.11.1.14)), and manganese peroxidase (MnP (EC 1.11.1.13)) have demonstrated the ability to degrade azo dyes and other persistent aromatic compounds frequently present in textile effluents [12,14].

Fungi are among the most promising organisms for these applications because they produce extracellular ligninolytic enzymes characterized by broad substrate specificity. This feature enables the degradation of a wide range of structurally complex organic pollutants, including recalcitrant synthetic dyes. Species such as *Phanerochaete chrysosporium* have demonstrated high decolorization efficiencies and significant reductions in the toxicity of textile wastewater, highlighting the potential of enzymatic processes for environmental bioremediation [14].

Beyond their effectiveness in pollutant removal, enzyme- and microorganism-based approaches are closely aligned with the principles of green chemistry and sustainable industrial development. The partial replacement of conventional chemical reagents with biological catalysts can reduce water and energy consumption, decrease the demand for hazardous chemicals, and minimize waste generation throughout the textile production chain [13].

Consequently, enzymes and microorganisms have emerged as promising tools for the development of cleaner and more efficient TW treatment technologies, particularly in response to the growing demand for environmentally sustainable and resource-efficient remediation processes [12,14].

1.4. Scope and Novelty

Over the past decade, numerous review articles have examined biological approaches for TW treatment, including the application of isolated enzymes, microbial degradation processes, fungal bioremediation, bacterial dye decolorization, and advanced oxidation technologies [15–18]. Most of these studies have focused primarily on pollutant removal performance, particularly color reduction and COD removal, or have addressed enzymatic and microbial processes as independent treatment strategies. While these contributions have significantly advanced the field, the integration of enzymatic and microbial systems for effective TW detoxification has received comparatively limited attention.

The distinction between decolorization and detoxification represents a critical challenge in TW treatment. Although color removal is often used as a primary indicator of treatment efficiency, the disappearance of visible color does not necessarily correspond to the elimination of toxicity. In many cases, the partial degradation of dyes, particularly azo dyes, can generate aromatic amines and other intermediate compounds that remain toxic, mutagenic, or persistent in the environment [19]. Consequently, treatment strategies capable of promoting not only dye transformation but also the subsequent degradation and mineralization of toxic intermediates are essential for achieving environmentally sustainable remediation.

In this context, integrated enzymatic–microbial systems have emerged as promising alternatives. Enzymes such as LAC, AZOR, LiP, MnP, and dye-decolorizing peroxidases (DyPs, (EC 1.11.1.19)) can rapidly initiate the transformation of complex dye molecules through oxidative and reductive reactions. However, enzymatic treatments alone may be limited by enzyme stability, operational costs, and incomplete degradation of reaction products. Conversely, microorganisms possess the metabolic versatility required to further transform and mineralize degradation intermediates, although microbial processes may exhibit slower kinetics and sensitivity to wastewater composition. The combination of enzymatic catalysis with microbial metabolism therefore offers opportunities for synergistic pollutant degradation, enhanced detoxification, and improved treatment efficiency.

Accordingly, this review provides a comprehensive and critical assessment of integrated enzymatic–microbial approaches for TW detoxification. Unlike previous reviews that primarily focus on isolated enzymatic or microbial treatments, this review integrates detoxification mechanisms, biomass-derived biocatalyst production, and circular bioeconomy perspectives into a unified framework. Emphasis is placed on the mechanisms governing enzymatic and microbial dye transformation, the role of microbial consortia, sequential and simultaneous treatment configurations, and the synergistic interactions that contribute to toxicity reduction. In addition, this review discusses the potential of solid-state fermentation and agro-industrial residues as platforms for producing low-cost biomass-derived biocatalysts, thereby linking TW remediation with waste valorization and circular bioeconomy strategies. Finally, operational parameters, scale-up challenges, reactor configurations, and future research directions are critically evaluated to identify pathways toward the industrial implementation of sustainable biocatalytic detoxification technologies.

1.5. Methodology

This comprehensive narrative review was conducted to critically evaluate the current advances in the detoxification of textile wastewater through integrated enzymatic–microbial systems, with particular emphasis on the synergistic interaction between oxidative and reductive enzymes, microbial consortia, and the direct application of solid-state fermentation (SSF)-derived biomass.

The literature search was performed using the Scopus, Web of Science, ScienceDirect, and Google Scholar databases. Publications from 2003 to 2026 were considered to encompass both the fundamental studies that established the field and the most recent technological developments. The search strategy employed combinations of the following keywords and Boolean operators: (“textile wastewater” OR “textile effluent”) AND (detoxification OR bioremediation) AND (“integrated enzymatic–microbial systems” OR “enzyme–microorganism synergy”) AND (laccase OR azoreductase OR peroxidase) AND (“microbial consortia” OR “solid-state fermentation” OR SSF) AND (“circular bioeconomy” OR biomass valorization).

The review prioritized original experimental studies reporting enzymatic, microbial, or integrated biological treatments for textile wastewater, particularly those investigating the complementary action of oxidative and reductive enzymes, microbial consortia, or SSF-derived fermented biomass. Studies addressing reactor configurations, continuous treatment systems, industrial scalability, and circular bioeconomy approaches were also included to provide a broader technological perspective.

The initial screening was based on titles and abstracts to identify thematic relevance. Potentially eligible publications subsequently underwent full-text evaluation. Review articles were consulted primarily to establish the theoretical framework and to identify additional relevant primary studies through backward citation analysis, whereas editorials, conference abstracts lacking experimental data, and studies unrelated to textile wastewater detoxification were excluded from the critical analysis.

For each selected study, information was extracted regarding the pollutant or dye investigated, biological catalysts employed (enzymes, microorganisms, or microbial consortia), reactor configuration, treatment strategy, decolorization efficiency, detoxification assessment, evidence of pollutant mineralization or degradation pathways, and reported operational limitations. Particular attention was given to studies distinguishing visual decolorization from effective detoxification, including the removal of toxic aromatic amines and other persistent transformation products, as well as investigations demonstrating synergistic interactions between enzymatic oxidation, microbial metabolism, and biomass valorization strategies. Finally, the collected evidence was comparatively analyzed to identify current technological trends, knowledge gaps, and future perspectives for developing scalable and sustainable integrated systems for textile wastewater detoxification within the framework of the circular bioeconomy.

2. Textile Dyes and Their Recalcitrance

2.1. Classification of Textile Dyes

Textile dyes comprise a diverse group of synthetic organic compounds designed to impart durable coloration to natural and synthetic fibers (Table 1). Their widespread use in textile manufacturing is associated with desirable properties such as color stability, resistance to washing, light exposure, oxidizing agents, and temperature fluctuations. However, these same characteristics contribute to their environmental persistence following discharge into wastewater streams [20,21].

Table 1. Major classes of textile dyes, representative structural characteristics, and environmental relevance.

Dye Class	Main Structural Feature	Major Applications	Environmental Relevance	Ref.
Azo	Azo bonds (-N=N-) linking aromatic rings	Cotton, wool, silk, synthetic fibers	Aromatic amine formation and high recalcitrance	[22]
Anthraquinone	Anthraquinone-derived aromatic structures	Cotton, polyester, acrylic fibers	High photochemical stability and biodegradation resistance	[23]
Reactive	Reactive groups (e.g., chlorotriazine, vinyl sulfone)	Cellulosic fibers	High water solubility and dye loss to effluents	[20]
Disperse	Nonionic aromatic compounds	Polyester and hydrophobic fibers	Difficult removal by conventional treatments	[21]
Basic	Cationic chromophoric structures	Acrylic fibers and paper	Potential ecotoxicity and cellular interactions	[21]

This table summarizes the main classes of textile dyes, their structural features, applications, and environmental relevance as reported in the literature.

The coloration properties of dyes are determined by chromophoric groups, including azo ($-N=N-$), quinonoid, nitro, and methine structures, which are frequently associated with conjugated aromatic systems. Auxochromic groups such as hydroxyl ($-OH$), amino ($-NH_2$), and sulfonic ($-SO_3H$) moieties further influence color intensity, solubility, and fiber affinity [20]. These structural characteristics are also closely related to the environmental behavior, degradation pathways, and recalcitrance of dye molecules (Table 1).

Textile dyes can be classified according to their chemical structure, application method, fiber type, or fixation mechanism. Among these, classification based on chemical structure is particularly relevant for environmental studies because it provides insight into degradation resistance and potential toxicity [20].

Azo dyes represent the largest class, accounting for approximately 60–70% of global dye production. Their widespread use is attributed to their broad color range, ease of synthesis, and relatively low production costs. Nevertheless, the reductive cleavage of azo bonds may generate aromatic amines, which are frequently associated with mutagenic and carcinogenic effects [22,23].

Other important dye classes include anthraquinone dyes, characterized by fused aromatic ring systems and high photochemical stability; reactive dyes, which form covalent bonds with textile fibers and often exhibit high water solubility; disperse dyes, commonly applied to hydrophobic synthetic fibers; and basic dyes, which are known for producing vivid coloration but may exhibit elevated ecotoxicity [20,21]. Despite differences in structure and industrial application, these dyes share a common characteristic: they were intentionally designed to resist degradation under operational and environmental conditions.

The structural diversity of textile dyes contributes significantly to the complexity of TW and represents a major challenge for remediation technologies. Differences in molecular architecture, solubility, and chemical stability influence their susceptibility to enzymatic and microbial degradation, highlighting the need for integrated treatment strategies capable of targeting a broad range of contaminants [21,22].

2.2. Recalcitrance of Textile Dyes

The environmental persistence of textile dyes is directly associated with the structural characteristics that make these compounds suitable for industrial applications. Dyes are intentionally designed to resist exposure to sunlight, temperature fluctuations, detergents, oxidizing agents, and repeated washing cycles. Consequently, the same properties that ensure color durability and stability also hinder degradation after discharge, promoting the long-term persistence of these compounds in aquatic environments [20,22].

Recalcitrance refers to the resistance of a substance to chemical, photochemical, and biological transformation processes. In textile dyes, this characteristic is primarily associated with highly stable molecular architectures that were engineered to maintain chromatic properties under harsh operational conditions [22]. Among the principal factors contributing to dye recalcitrance are extensive conjugated aromatic systems, stabilized chromophore groups, and the presence of functional groups that enhance chemical stability and environmental persistence [20,21].

Azo dyes, which represent the largest class of textile dyes, exemplify this challenge. Although azo bonds ($-N=N-$) can be cleaved under specific chemical or biological conditions, their stability contributes to the prolonged persistence of these compounds in the environment. Furthermore, incomplete degradation may result in the formation of aromatic amines, many of which are associated with mutagenic and carcinogenic effects [22,23]. Similarly, anthraquinone dyes and other compounds containing fused aromatic ring systems exhibit

remarkable resistance to biodegradation due to their highly stabilized molecular structures, often requiring specialized oxidative mechanisms for effective transformation [20,23].

The biodegradation of textile dyes is further constrained by their structural complexity. Many dyes possess high molecular weights, poorly accessible functional groups, and synthetic molecular architectures that lack natural analogs, limiting recognition and metabolism by conventional microbial enzymes [22]. In addition, sulfonated groups frequently incorporated to improve water solubility facilitate the dispersion of dyes in aquatic environments and reduce the effectiveness of conventional treatment technologies based on physical separation processes [20].

Importantly, the environmental concern associated with textile dyes extends beyond the persistence of the original compounds. Intermediate products generated during incomplete degradation may exhibit toxicity levels comparable to or even greater than those of the original dyes. Therefore, decolorization alone should not be considered evidence of successful remediation, as visual color removal does not necessarily indicate complete mineralization or effective detoxification [22,23].

Understanding the structural factors responsible for dye recalcitrance is essential for the development of advanced treatment strategies. These challenges have stimulated growing interest in integrated enzymatic–microbial systems capable of combining enzymatic transformation of complex dye molecules with microbial degradation and mineralization of intermediate products, thereby improving both pollutant removal and detoxification efficiency.

2.3. Toxicity and Environmental Risks

The environmental impacts associated with textile dyes extend far beyond the visual alteration of water bodies or the persistence of these compounds in aquatic ecosystems. A growing number of evidence has demonstrated that many synthetic dyes and their transformation products exhibit toxic properties capable of affecting aquatic organisms, disrupting ecological processes, and posing risks to human health [21,23].

The toxicity of textile dyes is associated not only with their molecular structures but also with the products generated during degradation processes. In many cases, chemical or biological transformation does not immediately result in harmless compounds. Color removal alone cannot be considered a reliable indicator of treatment efficiency, since a visually decolorized effluent may still contain hazardous substances capable of causing adverse environmental and health effects [22].

Azo dyes provide a representative example of this challenge. During reductive degradation, particularly under anaerobic conditions or through the action of specific microbial enzymes, azo bonds may be cleaved, generating aromatic amines. Although this reaction represents an important step in dye transformation, it does not necessarily result in complete mineralization. Many aromatic amines exhibit high chemical stability and have been associated with genotoxic, mutagenic, and carcinogenic effects, raising concerns regarding their persistence and environmental fate [22,23]. Compounds such as benzidine, aniline, and related derivatives are among the most extensively studied examples due to their potential to damage genetic material and interfere with normal cellular processes [23].

In addition to risks associated with human exposure, textile dyes and their degradation products can adversely affect microorganisms, algae, invertebrates, and fish. Ecotoxicological studies have reported growth inhibition, reproductive impairment, oxidative stress, cellular damage, and disruption of metabolic functions following chronic exposure to dye-contaminated waters [21]. Some degradation products may also undergo bioaccumulation and trophic transfer, increasing exposure risks for organisms occupying higher trophic levels and extending the ecological consequences of contamination [22].

These findings have important implications for wastewater treatment. Traditional performance indicators such as color removal, COD reduction, and decreases in organic load provide valuable information regarding process efficiency but do not necessarily reflect toxicity reduction. Consequently, the incorporation of ecotoxicological, genotoxicological, and mutagenicity assessments has become increasingly important for determining whether a treatment process achieves effective detoxification rather than simple pollutant transformation [22,23].

Therefore, the ultimate objective of TW treatment should extend beyond decolorization and pollutant removal to include the elimination of toxic intermediates and the reduction in ecological and human health risks. This challenge has stimulated growing interest in integrated enzymatic–microbial systems capable of coupling rapid dye transformation with the subsequent degradation and mineralization of potentially toxic metabolites, thereby improving the overall detoxification of textile effluents.

3. Biocatalytic Components of Integrated Detoxification Systems

Biological detoxification of TW relies on the catalytic activities of enzymes and microorganisms capable of transforming recalcitrant dye molecules into less toxic compounds or promoting their complete mineralization. These biocatalytic components constitute the fundamental building blocks of integrated detoxification systems, where enzymatic oxidation or reduction reactions are coupled with microbial metabolism to enhance pollutant removal and toxicity reduction.

Enzymatic catalysts frequently provide the initial transformation of dye molecules through oxidative or reductive reactions that destabilize complex aromatic structures and facilitate subsequent biodegradation. In contrast, microorganisms contribute broader metabolic capabilities that enable the assimilation, degradation, and mineralization of transformation products. Understanding the individual characteristics of these biocatalysts is therefore essential for evaluating their complementary roles in integrated enzymatic–microbial systems.

3.1. Enzymatic Catalysts

Enzymes have emerged as promising biocatalysts for TW treatment due to their high catalytic efficiency, substrate specificity, and ability to operate under relatively mild conditions. Unlike conventional chemical oxidants, enzymatic systems can promote selective transformations while minimizing secondary pollution and energy consumption. Among the enzymes most extensively investigated for dye degradation are LAC, AZOR, and ligninolytic peroxidases, each of which targets distinct structural features of textile dyes and contributes differently to detoxification processes.

3.1.1. Laccases (LAC)

Laccases (LAC (EC 1.10.3.2)) are multicopper oxidases widely distributed in fungi, bacteria, plants, and insects and represent one of the most extensively studied enzymatic systems for TW treatment. Their relevance in integrated detoxification processes stems from their ability to oxidize a broad range of phenolic and non-phenolic compounds, generating reactive intermediates that facilitate subsequent microbial degradation and mineralization steps [24,25]. The catalytic center of LAC contains four copper atoms organized into three functional sites: the Type 1 copper (T1) center, responsible for electron abstraction from the substrate, and the trinuclear T2/T3 copper cluster, where oxygen reduction occurs [24,26]. This process generates reactive radical intermediates that trigger subsequent bond-cleavage reactions, ultimately leading to the decolorization and detoxification of complex pollutants [27].

The versatility of LAC is largely attributed to their low substrate specificity, enabling the transformation of a wide variety of environmental contaminants [24,25]. In TW treatment, LAC have demonstrated high efficiency against recalcitrant synthetic dyes, including azo, anthraquinone, triarylmethane, and phthalocyanine dyes [25,28]. Beyond dye degradation, these enzymes have also shown the ability to transform emerging micropollutants [25,29], such as antibiotics (e.g., tetracycline), steroid hormones, organophosphate pesticides, and even certain microplastics, including polyethylene (PE), polyethylene terephthalate (PET), and polylactic acid (PLA) [29,30]. This broad catalytic spectrum positions LAC as key biocatalysts in sustainable bioremediation strategies [31].

To overcome thermodynamic limitations associated with the oxidation of non-phenolic substrates or compounds with high ionization potentials, laccase–mediator systems (LMS) have been extensively developed [25,32]. Synthetic mediators such as ABTS (2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) and HBT (1-hydroxybenzotriazole) act as electron shuttles, enabling the oxidation of pollutants that would otherwise be inaccessible to the enzyme alone [27,33]. Despite their effectiveness, synthetic mediators are often associated with high costs and potential ecotoxicological concerns [32]. Consequently, increasing attention has been directed toward natural mediators [33,34], including tea polyphenols such as epicatechin gallate (ECG) and epigallocatechin gallate (EGCG), as well as lignin-derived compounds such as syringaldehyde and vanillin. These compounds offer both environmental and economic advantages, with ECG reportedly outperforming ABTS in tetracycline degradation [30].

The differences between fungal and bacterial LAC largely determine their industrial applications [24,31]. Fungal LAC, particularly those produced by white-rot fungi such as *Phanerochaete chrysosporium* and *Trametes versicolor*, are characterized by high redox potentials, making them highly effective for the degradation of recalcitrant pollutants [24,31]. However, they generally exhibit optimal activity under acidic conditions (pH 3.0–6.0) and often possess limited thermal stability. In contrast, bacterial LAC, such as those from *Bacillus subtilis* and *Bacillus pumilus*, exhibit remarkable thermostability and enhanced tolerance to alkaline and saline conditions [24,28]. Furthermore, their relatively simple molecular architecture facilitates genetic engineering approaches aimed at improving catalytic performance and process robustness [24].

LAC offer several advantages as environmentally friendly biocatalysts, including operation under mild reaction conditions and the generation of water as the primary by-product [25,31]. Nevertheless, large-scale implementation remains constrained by enzyme instability and the high costs associated with production and purification [25,31]. To address these limitations, various immobilization strategies have been developed, including the use of porous supports, polymeric matrices, and magnetic nanoparticles, which improve enzyme stability, enable reuse, and reduce operational costs [24,27,28].

3.1.2. Azoreductases (AZOR)

Azoreductases (AZOR (EC 1.7.1.6)) plays a central role in the biodegradation of azo dyes by catalyzing the reductive cleavage of azo bonds (–N=N–), which constitute the primary chromophoric structures of the most widely used textile dyes worldwide. These enzymes are particularly important in integrated detoxification systems because they initiate dye decolorization and generate transformation products that can subsequently be degraded through aerobic microbial metabolism [9,35,36]. Recent experimental and computational studies suggest that although the enzyme catalyzes the initial reduction to a hydrazo intermediate, the final formation of aromatic amines may involve spontaneous non-enzymatic cleavage, provided that aromatic substituents are capable of stabilizing the resulting products through resonance effects [37].

The efficiency of azo bond reduction is strongly influenced by oxygen availability, with anaerobic conditions generally favoring the process because the dye serves as the terminal electron acceptor in microbial respiratory pathways [9,35,38]. In contrast, oxygen acts as a more favorable electron acceptor and competes directly with azo dyes, thereby inhibiting azoreductase activity [9,36]. However, complete dye degradation often requires a subsequent aerobic stage to mineralize the aromatic amines generated during the anaerobic reduction step, as these intermediates may exhibit toxic or carcinogenic properties if not further degraded [35,36].

AZOR activity is strictly dependent on reducing cofactors, particularly NADH and NADPH, which provide the electrons required for catalysis (Figure 2) [9,36,39]. Based on cofactor preference, AZOR are generally classified into three groups: Class I enzymes utilize NADPH, Class II enzymes preferentially use NADH, and Class III enzymes can employ both cofactors [36]. In optimized bacterial systems, NADH is considered the primary driving force for azo dye reduction and is generated mainly through glycolysis, supplemented by the tricarboxylic acid (TCA) cycle and fatty acid β -oxidation pathways [39].

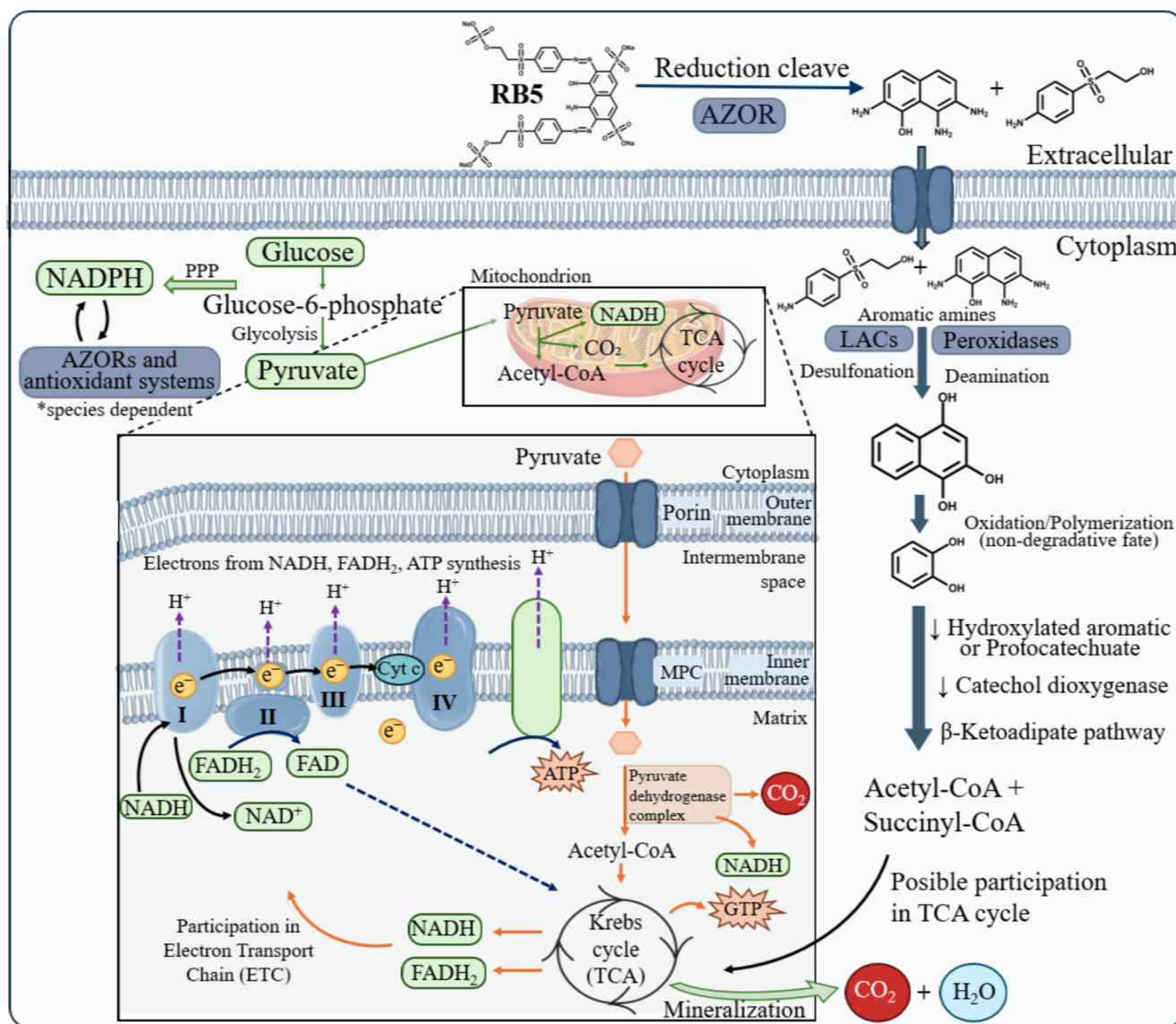


Figure 2. Integrated metabolic network for RB5 biodegradation: Pathways of cofactor regeneration, electron transfer, and central carbon assimilation. * Biochemical mapping of Reactive Black 5 (RB5) detoxification suggested for eukaryotes heterotrophs: Overview of metabolic integration between

central pathways (glycolysis and TCA cycle) and multi-enzymatic cascades. The diagram depicts the integration of dye RB5 biodegradation with central carbon metabolism. NADPH supports azoreductase-mediated azo bond cleavage, whereas aromatic intermediates are oxidatively metabolized through the β -ketoadipate pathway and funneled into the TCA cycle. Subsequent NADH and FADH₂ generation drives the electron transport chain, providing the energy required for complete mineralization of textile dyes into CO₂ and H₂O, establishing a mechanistic framework for effective textile wastewater bioremediation.

In addition to direct enzymatic mechanisms, cellular regulatory networks play an important role in adaptation to anaerobic environments. In *Escherichia coli*, for example, azo dye reduction is regulated by the global anaerobic transcription factor FNR, which responds to oxygen limitation by stimulating hydrogen sulfide (H₂S) production through L-cysteine metabolism. This H₂S can chemically reduce azo bonds independently of the canonical AzoR protein, providing an alternative reduction pathway [40].

3.1.3. Peroxidases and Auxiliary Oxidative Enzymes

In addition to LAC and AZOR, several oxidative enzymes contribute to the degradation of recalcitrant textile dyes. Among the most important are LiP, MnP, Versatile peroxidase (VP (EC 1.11.1.16)), DyPs, and tyrosinases (TYR (EC 1.14.18.1)), which collectively expand the oxidative capacity of biological treatment systems and enhance the transformation of complex aromatic pollutants. Among these enzymes, LiP is distinguished by its exceptionally high oxidative capacity. LiP can oxidize non-phenolic lignin structures and high-redox-potential dye molecules that are often resistant to other oxidative enzymes. White-rot fungi such as *Phanerochaete chrysosporium* and *Phlebia radiata* are recognized as major producers of this enzyme [9,31].

Manganese peroxidase (MnP) is another key ligninolytic enzyme. In the presence of H₂O₂, MnP catalyzes the oxidation of Mn²⁺ to Mn³⁺. The resulting Mn³⁺ acts as a diffusible low-molecular-weight oxidant capable of penetrating dye structures and oxidizing phenolic compounds and aromatic fragments, thereby contributing significantly to azo dye mineralization [9,31].

Versatile peroxidase (VP) combines catalytic properties of both LiP and MnP, enabling the simultaneous oxidation of phenolic compounds, non-phenolic substrates, and manganese ions. This multifunctional catalytic behavior considerably broadens its substrate spectrum and enhances its effectiveness against different dye classes [31]. Furthermore, dye-decolorizing peroxidase (DyP; EC 1.11.1.19) has attracted increasing attention because of its remarkable ability to degrade azo and anthraquinone dyes while maintaining catalytic activity under acidic conditions and across a broad temperature range [31].

Tyrosinase (TYR) also contributes to dye biodegradation. These polyphenol oxidases catalyze the oxidation of phenolic compounds and aromatic amines using molecular oxygen and frequently act synergistically with peroxidases to promote the degradation and mineralization of potentially toxic intermediates generated during the early stages of oxidative treatment [9].

In addition, peroxidase activity is often sustained by aryl alcohol oxidase (AAO), an enzyme responsible for the continuous generation of H₂O₂ through the oxidation of aromatic alcohols. Together, these enzymes (Table 2) form an integrated oxidative system capable of transforming complex industrial dye molecules into less toxic products or achieving complete mineralization to carbon dioxide and water [31].

Table 2. Comparative characteristics of enzymatic systems used in dye biodegradation.

Property	LAC	AZOR	LiP	MnP	VP	DyPs	TYR
Active Site/Catalytic Center	Four copper atoms (T1 for substrate oxidation; T2/T3 cluster for O ₂ reduction).	FMN- or FAD-dependent flavoproteins.	Heme group (iron protoporphyrin IX).	Heme group with Mn(II)-binding site.	Hybrid catalytic sites for Mn(II), phenolic, and non-phenolic substrates.	Heme-containing dye-decolorizing peroxidases.	Copper-containing polyphenol oxidases.
Redox Potential	0.45–0.80 V (lower than peroxidases; often requires mediators for high-redox-potential substrates).	N/A (reductive electron-transfer mechanism).	1.1–1.26 V (capable of oxidizing highly recalcitrant non-phenolic compounds).	Moderate; acts through diffusible Mn ³⁺ as a natural mediator.	High; combines catalytic properties of LiP and MnP.	Effective against anthraquinone dyes under acidic conditions.	Similar to LAC; oxidizes phenols and aromatic amines.
Structure/Organization	Glycosylated monomer or homodimer (60–100 kDa).	Class I: tetrameric; Class II: dimeric; Class III: monomeric/versatile.	Monomeric glycoprotein.	Monomeric glycoprotein.	Structurally and functionally hybrid enzyme.	Four classes (A–D) based on sequence homology.	Oxidative enzyme complex.
Final Electron Acceptor/Cofactor	Molecular oxygen (O ₂), directly reduced to water (H ₂ O).	NADH or NADPH as e [−] donors;	Hydrogen peroxide (H ₂ O ₂).	Hydrogen peroxide (H ₂ O ₂).	Hydrogen peroxide (H ₂ O ₂).	Hydrogen peroxide (H ₂ O ₂).	Molecular oxygen (O ₂).
Oxygen Requirement	Aerobic (essential for the catalytic cycle).	Anaerobic or microaerophilic (FNR regulates anaerobic metabolism).	Aerobic.	Aerobic.	Aerobic.	Aerobic.	Aerobic.
Stability and Reusability	Excellent; retains 100% activity after nine cycles when immobilized in alginate; CLEAs often retain >80% activity.	Sensitive; performance depends on cellular viability and cofactor availability.	Stability significantly improved through immobilization	Similar to LiP; enhanced by immobilization.	High biotechnological efficiency in complex effluents.	Thermostable and active under acidic conditions.	Frequently co-immobilized with other oxidative enzymes.
Main Targets and Applications	Phenols, dyes, microplastics (PE, PET, PLA), and electrochemical biosensors.	Cleavage of azo bonds (-N=N-) in dyes and pharmaceuticals.	Lignin, non-phenolic dyes, and high-redox-potential micropollutants.	Phenolic and azo dyes; degradation and mineralization of intermediates.	Lignocellulosic substrates, Mn(II), and a broad spectrum of industrial pollutants.	Anthraquinone dyes and acidic textile effluents.	Mineralization of aromatic amines and toxic transformation products.
Reference	[24,25]	[9,36,39]	[9,31]	[9,31]	[9,31]	[31]	[9]

This comparative table summarizes structural, catalytic, and functional properties of major oxidative enzymes involved in lignocellulose and xenobiotic degradation, as reported in the literature. Enzymatic performance may vary depending on source organism, isoform, and operational conditions (pH, temperature, mediators, and immobilization strategy).

3.1.4. Advantages and Limitations of Enzymatic Catalysts

Enzymatic systems offer several advantages for TW treatment, including high catalytic efficiency, operation under mild reaction conditions, reduced chemical consumption, and the ability to transform structurally complex dye molecules. Oxidative enzymes such as LAC and peroxidases can rapidly attack aromatic structures that are often resistant to conventional treatment processes, whereas AZOR effectively initiate azo dye decolorization through reductive bond cleavage [9,24,25].

Despite their high catalytic efficiency, enzymatic systems face significant economic and operational bottlenecks. From an industrial perspective, the downstream processing of enzymes—including extraction, purification, and stabilization—is labor-intensive and can account for up to 70% of the total manufacturing cost. Specifically, Ndochinwa et al. (2024) [41] highlight that the high cost and limited accessibility of essential cofactors further increase the financial burden of large-scale treatments. Operationally, enzymes are susceptible to denaturation under harsh industrial conditions, such as extreme pH, temperature, and high salinity. This is further complicated by the stability-activity trade-off: catalytic residues are often naturally ‘stability weaknesses’ because conformational flexibility is required for functional turnover, making high efficiency structurally incompatible with long-term robustness [42]. Furthermore, isolated enzymes rarely achieve complete mineralization, often generating potentially toxic intermediate products that require further biological processing.

Despite these advantages, enzyme-based treatments also present important limitations. Enzyme production and purification can be costly, catalytic activity may decline under industrial conditions, and complete mineralization of dye molecules is not always achieved through enzymatic reactions alone. Furthermore, certain enzymatic transformations generate intermediate products, including aromatic amines, that require additional biological degradation to achieve effective detoxification [23,35].

These limitations highlight the importance of coupling enzymatic catalysis with microbial metabolism. While enzymes can rapidly transform recalcitrant dye structures, microorganisms possess broader metabolic capabilities that enable the degradation, assimilation, and mineralization of transformation products. Consequently, integrated enzymatic-microbial systems have emerged as promising strategies for achieving more complete detoxification of TW than either approach alone.

3.2. Microbial Catalysts

Microorganisms play a central role in the biological detoxification of TW due to their remarkable metabolic diversity and ability to transform, assimilate, and mineralize a wide range of recalcitrant organic pollutants. Unlike isolated enzymatic systems, microorganisms possess complex metabolic networks that enable the sequential degradation of dye molecules and their transformation products, contributing not only to decolorization but also to detoxification and mineralization processes.

Among the microbial groups investigated for TW treatment, bacteria and fungi have received particular attention. Bacteria are generally characterized by rapid growth rates, metabolic adaptability, and efficient degradation of aromatic intermediates, whereas fungi possess powerful extracellular oxidative enzyme systems capable of attacking highly recalcitrant dye structures. Together, these microbial catalysts constitute essential components of integrated detoxification systems.

3.2.1. Bacterial Systems

Bacteria are widely recognized for their capacity to remove contaminants from wastewater treatment systems. In TW treatment, dye removal represents a critical stage of the

remediation process. For instance, Azo dyes, which account for approximately 70% of the global dye market, are among the most recalcitrant synthetic colorants and are highly resistant to conventional photolytic and chemical degradation processes [43,44]. Despite their persistence, bacteria have emerged as key agents for their degradation due to their metabolic versatility, environmental adaptability, and rapid degradation rates [45]. In this case, their mineralization can mostly be done by synergistic and sequential action of two processes, anaerobic reductive and aerobic oxidative pathways, exploiting the distinct metabolic capabilities of bacterial communities under changing redox conditions [10,46].

The reductive cleavage of the azo bond ($-N=N-$) is the first step in the anaerobic reductive pathway [46], and its favored particularly under anaerobic or anoxic conditions since molecular oxygen acts as a highly competitive terminal electron acceptor, diverting reducing equivalents away from azo bond reduction [9,10]. Azo dye reduction is primarily catalyzed by AZOR, a diverse group of enzymes that are frequently flavin-dependent and may be localized in the cytoplasm or associated with the cell membrane [46]. During this process, reducing equivalents derived from intracellular electron donors such as NADH and NADPH are transferred to the dye molecule, resulting in cleavage of the azo linkage and rapid decolorization of the effluent [9].

Several bacterial genera commonly isolated from TW participate in this reductive stage. Among them, species of *Pseudomonas* and *Bacillus* have received particular attention due to their metabolic versatility and high decolorization efficiency. *Pseudomonas luteola*, *P. aeruginosa*, and *P. putida* have demonstrated effective reduction of azo dyes such as Reactive Yellow 145, Navitan Fast Blue S5R, and Congo Red under oxygen-limited conditions [47,48]. Similarly, several *Bacillus* species, including *B. cereus*, *B. subtilis*, and *B. albus*, produce AZOR and other oxidoreductive enzymes capable of rapidly decolorizing azo and non-azo dyes [44,49].

Despite its effectiveness for color removal, azo bond reduction typically generates aromatic amines that may exhibit greater toxicity, mutagenicity, and carcinogenicity than the parent dye molecules [9]. Although some anaerobic microorganisms can further transform these compounds, complete degradation is generally inefficient under anaerobic conditions, making a subsequent aerobic stage essential for mineralization.

The aromatic amines generated during anaerobic decolorization are subsequently subjected to aerobic degradation [10]. Under aerobic conditions, bacteria employ oxidative enzymes, particularly monooxygenases and dioxygenases, which catalyze hydroxylation, deamination, desulfonation, and aromatic ring-cleavage reactions [48]. Some bacterial species may additionally produce laccase-like enzymes and peroxidases that contribute to dye transformation and detoxification [49].

Among the bacteria involved in this stage, *Pseudomonas* species again are particularly noteworthy because of their ability to metabolize aromatic intermediates generated during azo dye reduction. Transcriptomic analyses of *Pseudomonas aeruginosa* have revealed the upregulation of genes associated with benzoate and aromatic compound degradation pathways, supporting its role in the catabolism of dye-derived aromatic metabolites [47]. Through pathways such as the β -ketoadipate route, these intermediates are converted into central metabolic precursors that ultimately enter the tricarboxylic acid (TCA) cycle and are mineralized to carbon dioxide and water.

Species of *Bacillus* also contribute significantly to aerobic detoxification through their diverse enzymatic repertoire. *Bacillus cereus*, for example, has been reported to degrade Reactive Green 12 and Acid Blue 113 through the coordinated action of AZOR, laccase-like enzymes, peroxidases, and NADH-DCIP reductases [44,50]. Furthermore, many *Bacillus* strains exhibit exceptional tolerance to alkaline pH, salinity, and heavy metals,

characteristics that are particularly advantageous for the treatment of real textile effluents containing multiple co-contaminants [51].

Consequently, sequential anaerobic–aerobic treatment systems are widely regarded as one of the most effective biological strategies for azo dye remediation because they combine rapid decolorization under reducing conditions with subsequent aerobic degradation of aromatic intermediates. Nevertheless, bacterial systems may exhibit reduced efficiency toward highly recalcitrant dye structures and frequently depend on carefully controlled redox conditions to achieve complete detoxification, highlighting the potential benefits of combining bacterial metabolism with complementary enzymatic processes.

3.2.2. Fungal Systems

Fungi rely on extracellular enzymatic systems to degrade complex substrates and acquire nutrients [52]. Unlike bacteria, which frequently depend on intracellular metabolic pathways for dye degradation, fungi often initiate pollutant transformation extracellularly through the secretion of oxidative enzymes capable of attacking large and structurally complex molecules before cellular uptake. This characteristic is particularly advantageous for the treatment of TW because many synthetic dyes possess high molecular weights, extensive aromatic conjugation, and limited bioavailability.

White-rot fungi are among the most extensively studied fungal groups for TW treatment due to their highly efficient ligninolytic enzyme systems. These systems are primarily composed of LiP, MnP, VP, and LAC, which collectively enable the oxidation of a broad range of recalcitrant organic pollutants, including azo, anthraquinone, triphenylmethane, indigoid, and heterocyclic dyes [7,53,54]. The low substrate specificity of these enzymes allows fungal systems to attack diverse dye structures through mechanisms analogous to those involved in lignin depolymerization.

The fungal degradation process is largely driven by extracellular oxidation reactions. LAC catalyze one-electron oxidation reactions that generate reactive radical intermediates, whereas peroxidases utilize hydrogen peroxide as an oxidizing agent to initiate the breakdown of complex aromatic structures. In the case of MnP, Mn(II) is oxidized to Mn(III), which subsequently acts as a diffusible oxidizing mediator capable of penetrating regions inaccessible to the enzyme itself. This Mn³⁺-mediated oxidation mechanism significantly expands the effective reaction zone and contributes to the degradation of structurally complex dye molecules [55].

A key feature of fungal ligninolytic systems is the participation of low-molecular-weight mediator compounds. These mediators act as electron shuttles that transfer oxidative equivalents from enzymes to target pollutants, thereby enhancing the oxidation of compounds that cannot directly access the catalytic site. Through these mechanisms, fungi promote aromatic ring oxidation, cleavage of conjugated chromophoric structures, and the formation of less complex intermediates that are more susceptible to subsequent biodegradation. Consequently, fungal degradation frequently extends beyond simple decolorization and contributes to the detoxification of textile effluents.

Following extracellular oxidation, the resulting aromatic intermediates may be assimilated by fungal cells and further metabolized through intracellular catabolic pathways. Ring-cleavage reactions generate smaller organic acids that can enter central carbon metabolism, including the tricarboxylic acid (TCA) cycle, ultimately contributing to complete mineralization. Therefore, fungal detoxification involves both extracellular ligninolytic oxidation and intracellular metabolic assimilation, allowing progressive conversion of complex dye molecules into carbon dioxide, water, and biomass.

The oxidative pathways exhibited by fungi can be illustrated by *Phanerochaete chrysosporium*, whose MnP oxidizes diverse synthetic dyes, including Poly B-411, Poly R-481, and

indigo trisulfonate, through Mn^{3+} -mediated reactions [55]. Similarly, *Trametes versicolor* produces a highly efficient ligninolytic enzyme system capable of degrading both direct and disperse textile dyes, with MnP and LAC playing central roles in aromatic ring oxidation and dye transformation [56]. Other fungal genera with demonstrated potential for TW treatment include *Pleurotus* [57] and *Ganoderma* [58], both of which possess extracellular oxidative systems comparable to those of white-rot fungi. Although white-rot fungi are primarily associated with extracellular oxidative degradation, other filamentous fungi contribute through complementary mechanisms including biosorption, bioaccumulation, partial biodegradation, and enzymatic transformation. In these organisms, pollutant removal frequently results from the combined action of cell-wall adsorption and intracellular metabolism rather than extensive extracellular ligninolytic activity.

Aspergillus species can produce enzymes for dye degradation and mineralization, but their ligninolytic enzyme expression is generally less pronounced than that observed in white-rot fungi. So, a common strategy for those species is the biosorption of the dyes [59]. Similar mechanisms have been reported for species belonging to the genera *Penicillium* [60,61] and *Fusarium* [62,63].

Processes relying primarily on biodegradation and mineralization generally require longer treatment times but can achieve more extensive conversion of pollutants into simpler molecules [61,64]. In contrast, biosorption does not require living cells and merely transfers dyes from the aqueous phase to fungal biomass. Therefore, toxicity may remain unchanged unless the adsorbed dyes are subsequently degraded or the spent biomass is appropriately managed [61].

One important advantage of fungi is their ability to thrive under the harsh conditions typically associated with TW [56]. Tolerant microorganisms maintain cellular viability and enzymatic activity under these conditions through mechanisms such as cell wall biosorption, extracellular enzyme secretion, osmotic regulation, antioxidant defense systems, and metal detoxification pathways [56,61,65,66]. This resilience is essential for achieving stable and efficient pollutant removal in real industrial wastewater streams.

Overall, fungi offer significant advantages for TW treatment due to their broad substrate specificity, extracellular oxidative enzyme systems, high tolerance to recalcitrant pollutants, and capacity for extensive dye transformation and mineralization [67,68]. However, their industrial application remains constrained by slow growth rates, oxygen requirements, mycelial morphology-related operational challenges, and sensitivity to process conditions. These limitations have stimulated increasing interest in immobilized fungal systems, enzyme-based treatments, and fungal–bacterial consortia that combine the strengths of multiple biological approaches [69,70].

3.2.3. Advantages and Limitations of Microbial Catalysts

Microbial systems offer several advantages for TW detoxification, including metabolic versatility, self-regeneration, adaptability to complex wastewater matrices, and the ability to achieve complete degradation and mineralization of dye molecules and their transformation products. Bacteria are particularly effective in the degradation of aromatic intermediates generated during dye reduction, whereas fungi possess extracellular oxidative systems capable of attacking highly recalcitrant aromatic structures. These complementary capabilities make microorganisms attractive candidates for sustainable wastewater treatment technologies.

However, microbial systems possess inherent limitations that restrict their standalone efficiency. A major drawback is the slow degradation kinetics, which often results in significantly prolonged hydraulic retention times compared to enzymatic processes [71]. Additionally, the initial high toxicity and heavy organic load of raw textile wastewater

can inhibit microbial growth and enzymatic expression, leading to inconsistent removal rates [72]. These kinetic and sensitivity barriers often prevent microbial populations from initiating the degradation of highly stable dye molecules without a prior catalytic ‘attack’.

Despite these advantages, microbial processes also present important limitations. Biological degradation is often slower than enzymatic oxidation or reduction, treatment performance may be influenced by fluctuations in pH, temperature, salinity, and contaminant composition, and complete detoxification may require carefully controlled operational conditions. Furthermore, some microorganisms exhibit limited capacity to initiate the degradation of highly stable dye molecules, while others generate intermediate metabolites that require additional biological or enzymatic transformation.

In summary, the high production costs and structural fragility of enzymatic systems, contrasted with the slow kinetics and toxicity sensitivity of microorganisms [71,72], create a technical and economic gap that standalone treatments cannot bridge. These critical limitations justify the transition toward integrated enzymatic–microbial approaches. In this framework, enzymatic catalysts provide the rapid initial cleavage of recalcitrant dye structures, overcoming chemical inertia, while microorganisms contribute the metabolic capacity necessary to further degrade and mineralize transformation products. By coupling fast catalytic conversion with long-term metabolic assimilation, integrated systems optimize both economic viability and detoxification efficiency, providing a robust and sustainable solution for the treatment of complex textile effluents [71,72].

4. Mechanisms of Dye Removal: Beyond Degradation

4.1. Enzymatic Cleavage Pathways

Dye removal in biological systems extends beyond simple physical removal mechanisms, such as biosorption and bioaccumulation, and involves complex enzymatic cleavage pathways that ultimately aim for the complete mineralization of pollutants [26,31,73]. While biosorption relies on passive physicochemical interactions occurring at the biomass surface and bioaccumulation depends on active intracellular transport, enzymatic pathways employ oxidoreductases to chemically transform stable chromophoric molecules into less complex metabolites [26,31,73].

Among enzymatic degradation pathways, the reduction of the azo bond ($-N=N-$) constitutes the initial and critical step in the decolorization of azo dyes, which represent the largest class of industrial dyes [39,73]. This process is primarily mediated by AZOR under anaerobic or microaerophilic conditions, using electron donors such as NADH, NADPH, or $FADH_2$ to cleave the nitrogen double bond [39,73]. Although the conventional mechanism describes a direct reductive cleavage, recent studies suggest that AZOR first convert the dye molecule into a hydrazo intermediate, whereas the subsequent formation of aromatic amines may occur through a spontaneous, non-enzymatic process driven by resonance stabilization of aromatic substituents [37]. In addition to enzymatic catalysis, low-molecular-weight redox mediators, such as endogenously produced flavins, can function as electron shuttles, facilitating extracellular reduction and overcoming membrane permeability limitations [39,73].

The subsequent breakdown of aromatic rings is essential for complete detoxification because the initial azo bond cleavage generates aromatic amines that, although colorless, are often more toxic, mutagenic, and carcinogenic than the parent dye molecules [73,74]. This stage is mainly driven by oxidative enzymatic systems, particularly LAC and peroxidases, including LiP and MnP, which operate synergistically under aerobic conditions [31]. LAC initiate degradation by abstracting electrons from aromatic rings, generating highly reactive phenoxy radicals that subsequently undergo ring cleavage or polymerization reactions [75,76]. Through symmetric or asymmetric cleavage pathways, these complex

aromatic structures are converted into smaller and less complex molecules, including maleic acid, isophthalic acid, and other low-molecular-weight carboxylic acids [31,76]. These degradation products may eventually enter central metabolic pathways, such as the tricarboxylic acid (TCA) cycle, ultimately leading to complete mineralization into carbon dioxide, water, and inorganic salts [74,76].

The metabolic network involved in the biodegradation of Reactive Black 5 (RB5) illustrates the coordinated integration of central metabolic pathways with a multienzyme detoxification cascade. This process relies on cofactor regeneration, with glycolysis serving as the primary metabolic pathway for the continuous production of cytoplasmic NADPH through the pentose phosphate pathway (PPP) [39]. In addition, the tricarboxylic acid (TCA) cycle and the degradation intermediates supply additional reducing equivalents, including NADH and FADH₂, which are essential for the electron transport chain (Figure 2) [51,77].

The initial decolorization step involves the transfer of reducing equivalents from NADH or NADPH to promote cleavage of the azo bond. Flavin-dependent azoreductases operate through a Ping-Pong Bi-Bi kinetic mechanism, in which NADH or NADPH first reduces a flavin cofactor (FMN or FAD), which subsequently transfers electrons to the azo dye, generating aromatic amine intermediates [51,78]. This electron transfer can be enhanced by endogenous redox mediators, such as riboflavin, which facilitate electron shuttling between the cytoplasm and the recalcitrant substrate [39].

To mitigate the toxicity and carcinogenic potential of these aromatic amines, the degradation network proceeds through an oxidative cascade mediated by enzymes such as laccases and peroxidases, which convert these intermediates into hydroxylated aromatic compounds, including catechol- and protocatechuate-type intermediates [51,77,79]. These compounds are subsequently funneled into the β -ketoadipate pathway, where aromatic ring cleavage generates central metabolic intermediates, primarily acetyl-CoA and succinyl-CoA. These metabolites enter the TCA cycle, coupling xenobiotic degradation with central carbon metabolism. The TCA cycle generates NADH and FADH₂, which fuel the mitochondrial electron transport chain for ATP synthesis, ultimately supporting the complete mineralization of the dye into CO₂ and H₂O and maintaining cellular energy homeostasis [77–79].

However, synthetic dyes represent only one fraction of the complex composition of textile wastewater. Anionic and nonionic surfactants, sizing agents, lubricants, emulsifiers, and various dyeing auxiliaries are also used during preparation, dyeing, and finishing, and many of these compounds remain in the wastewater after industrial processing [20]. Compared with aromatic dyes, these compounds are generally more susceptible to biodegradation. Nevertheless, because they are used at high concentrations, they account for a substantial proportion of the organic load and directly influence the metabolism of the microbial communities responsible for biological treatment [22].

Textile wastewater biodegradation therefore involves not only the transformation of aromatic dye structures but also the metabolism of diverse organic contaminants within the wastewater matrix. Surfactants, sizing agents, and dyeing auxiliaries are generally converted by hydrolytic and oxidative enzymes into lower-molecular-weight compounds, facilitating their assimilation through central metabolic pathways. Starch-based sizing agents, for example, are hydrolyzed by amylases to release glucose, which is metabolized through glycolysis into pyruvate and subsequently converted into acetyl-CoA. Similarly, polyvinyl alcohol (PVA), which is widely used in the textile industry, can be degraded by bacteria that produce specific enzymes, thereby reducing its environmental persistence and enabling its use as a carbon source [80,81]. Azo-dye degradation also generates aliphatic organic intermediates following aromatic-ring cleavage. These compounds are progressively metabolized by different microbial populations and incorporated into cen-

tral metabolism, simultaneously reducing the organic load, toxicity, and persistence of the wastewater [22].

In addition to undergoing mineralization, these aliphatic intermediates can serve as important carbon sources for microbial metabolism. Following their conversion into acetyl-CoA, pyruvate, and other tricarboxylic acid cycle intermediates, carbon flux may be redirected toward different biosynthetic pathways depending on the microbial species and operating conditions. Potential products include short- and medium-chain aliphatic carboxylic acids, such as acetic, propionic, butyric, and succinic acids, which are valuable platform chemicals for the chemical, food, and biotechnology industries [82]. From this perspective, carbon originally contained in textile wastewater contaminants is no longer regarded solely as waste to be removed but also as a potential substrate for bioconversion and waste valorization, consistent with the principles of biorefineries, bioremediation, and the circular bioeconomy. Although this strategy is not yet a primary objective of conventional wastewater treatment, recent advances in metabolic engineering and microbial platforms indicate that redirecting these intermediates toward carboxylic acid biosynthesis is a promising approach for adding value to biotechnological wastewater-treatment processes [82,83].

4.2. Adsorption and Biosorption

Microbial cell walls, particularly those of filamentous fungi, function as highly efficient natural biosorbents. They consist of a complex matrix of structural polymers, including chitin, chitosan, β -glucans, proteins, and lipids. These biopolymers provide a wide range of functional groups, such as amino, hydroxyl, carboxyl, and phosphate groups, which serve as binding sites for dye molecules. Chitin and chitosan are especially important because their amino groups enable strong electrostatic interactions with anionic dyes while also promoting hydrogen bonding and Van der Waals interactions. In systems employing dead or inactivated biomass, biosorption is a purely passive physicochemical process that does not require active metabolism or nutrient supplementation [26,31,84].

Unlike passive biosorption, bioaccumulation is an active and energy-dependent process occurring in living cells. In this mechanism, the cell wall and plasma membrane coordinate the transport of dye molecules into the cytoplasm or intracellular vacuoles. The affinity between dye molecules and surface functional groups constitutes the first step toward intracellular uptake. Although intracellular accumulation of synthetic dyes has been extensively documented in fungi such as *Aspergillus niger*, direct evidence of dye accumulation within white-rot fungi remains comparatively limited. Nevertheless, these organisms actively internalize aromatic degradation products generated during lignin breakdown [31,85].

Microorganisms in fermentation systems frequently develop as biofilms, producing extracellular polymeric substances (EPS) that form a protective and functional matrix that binds to diverse substances [86,87]. These polymers facilitate adhesion to solid substrates and contribute to the stabilization of extracellular enzymes, such as LAC and peroxidases, protecting them from adverse environmental conditions. In addition, low-molecular-weight compounds produced by microorganisms, including flavins, act as extracellular redox mediators. These compounds function as electron shuttles, transferring reducing equivalents (NADH/NADPH) from the intracellular environment to the extracellular medium, thereby enabling enzymatic cleavage of azo bonds in dye molecules that are too large or too polar to cross the cell membrane directly [39,73,88,89].

4.3. Coupled Processes

Pollutant removal efficiency can be substantially enhanced through coupled treatment processes that integrate adsorption and biodegradation into a single system. In these approaches, microbial biomass (fungal or bacterial) or enzyme-functionalized materials simultaneously operate as adsorbents and bioreactors. The treatment begins with the rapid adsorption of dye molecules onto the biomass surface through physicochemical interactions, including electrostatic attraction, hydrogen bonding, and Van der Waals forces. This initial concentration of pollutants at the solid–liquid interface minimizes mass-transfer limitations and facilitates the immediate catalytic action of extracellular enzymes, such as laccases (LAC) and peroxidases, accelerating dye oxidation and degradation [90,91].

The effectiveness of these integrated systems extends beyond rapid decolorization, being primarily determined by their ability to promote extensive pollutant mineralization and toxicity reduction. While isolated enzymatic treatments often result in only partial transformation of dye molecules, their integration with microbial consortia enables the sequential degradation of aromatic intermediates and the conversion of complex organic compounds into environmentally benign inorganic products, including ammonia, nitrite, and nitrate [90]. Evidence of this progressive degradation has been provided by Fourier-transform infrared spectroscopy (FTIR), which demonstrates the disappearance of characteristic absorption bands corresponding to azo bonds ($-N=N-$) and aromatic rings, confirming the breakdown of recalcitrant molecular structures [91].

This synergistic interaction provides significant operational advantages over the isolated application of adsorption or biodegradation. Whereas adsorption ensures rapid initial dye removal, subsequent enzymatic and microbial degradation converts the adsorbed pollutants into less complex and less toxic metabolites. This process promotes a biological self-regeneration mechanism, in which enzymatic degradation continuously re-releases occupied adsorption sites, preventing premature adsorbent saturation and extending the operational lifespan of the material. Furthermore, the use of immobilized living biomass, such as fungal or bacterial cells entrapped in porous matrices or sponges, combines passive biosorption with active bioaccumulation and intracellular biodegradation, resulting in higher mineralization efficiencies and improved treatment performance in real textile wastewaters [53,56].

Beyond chemical degradation, the environmental sustainability of these integrated systems is further supported by ecotoxicological assessments, which verify whether the degradation products exhibit lower toxicity than the parent dyes. Phytotoxicity assays using *Triticum aestivum* (wheat) and *Vigna unguiculata* (cowpea) have consistently shown that metabolites generated after treatment with microbial consortia do not inhibit seed germination or root and shoot development, in contrast to untreated textile dyes [92,93]. Similarly, cytotoxicity studies performed with CHO-K1 mammalian cells have reported high post-treatment cell viability, indicating that the bio-degradation products exhibit substantially reduced cytotoxicity compared with the original effluents [94].

An additional advantage of coupled adsorption–biodegradation systems concerns the management of the spent biomass. In solid-state fermentation (SSF)-based platforms, lignocellulosic substrates function simultaneously as enzyme-production matrices, microbial supports, and biosorbents, allowing dye degradation to occur in situ through extra-cellular enzymes secreted during fungal colonization [92]. Rather than generating secondary hazardous waste, the residual biomass can be valorized within a circular bioeconomy framework. Recent studies have demonstrated that, after appropriate physicochemical characterization, this material meets environmental safety requirements and possesses suitable nutritional properties for application as a soil conditioner or biofertilizer, contributing to waste valorization and nutrient recycling [95].

5. Integrated Enzymatic–Microbial Systems for Textile Wastewater Detoxification

Integrated enzymatic–microbial systems have emerged as promising strategies for overcoming the limitations associated with stand-alone enzymatic or microbial treatment technologies. These systems combine the rapid catalytic activity of enzymes with the metabolic versatility of microorganisms, enabling sequential pollutant transformation, degradation, and mineralization. As a result, integrated approaches frequently achieve superior decolorization, detoxification, and organic matter removal compared with isolated biological treatments [10,96,97].

5.1. Why Integration Is Necessary

Enzymes and microorganisms contribute distinct but complementary functions during TW treatment. Oxidative and reductive enzymes such as LAC, peroxidases, and AZOR can rapidly attack recalcitrant dye molecules, initiating structural transformation through the cleavage of chromophoric groups and oxidation of aromatic structures [14,24,98]. These reactions frequently reduce color intensity and improve biodegradability. However, enzymatic systems may suffer from high production costs, operational instability, and limited catalyst lifetimes, while complete mineralization is rarely achieved through enzymatic reactions alone [98,99].

In contrast, microorganisms possess self-regenerating metabolic systems capable of assimilating and mineralizing complex organic compounds. Bacteria and fungi can utilize degradation intermediates as carbon and energy sources, contributing to long-term detoxification and pollutant removal [9,10]. Nevertheless, microbial degradation is often slower than enzymatic catalysis and may be inhibited by high dye concentrations, salinity, toxic intermediates, or fluctuating environmental conditions [46,100].

Consequently, integration creates a synergistic relationship in which enzymes rapidly transform recalcitrant pollutants into more biodegradable compounds, while microorganisms further degrade and mineralize the resulting intermediates. This complementary interaction reduces toxicity, improves treatment efficiency, and increases process robustness under complex wastewater conditions [96,97].

5.2. Sequential Enzymatic–Microbial Systems

Sequential enzymatic–microbial systems represent one of the most extensively investigated approaches for TW detoxification because they allow for the functional separation of enzymatic transformation and microbial degradation stages. In this configuration, enzymes initially act on recalcitrant dye molecules, whereas microorganisms subsequently degrade and mineralize the resulting transformation products [10,96]. This strategy is particularly attractive for TW treatment because many dyes, especially azo dyes, exhibit high structural stability and limited susceptibility to direct microbial degradation.

The first stage commonly employs oxidative enzymes such as LAC and peroxidases to modify complex aromatic structures and chromophoric groups. Through oxidation reactions, these enzymes reduce molecular complexity, increase substrate accessibility, and enhance the biodegradability of dye-containing effluents [14,98]. In addition to promoting decolorization, enzymatic pretreatment can partially reduce toxicity by transforming recalcitrant compounds that may otherwise inhibit microbial activity [96,100].

LAC are among the most widely investigated enzymes for this purpose due to their broad substrate specificity and ability to oxidize phenolic and non-phenolic aromatic compounds. Similarly, ligninolytic peroxidases, including LiP and MnP, contribute to the oxidation of highly recalcitrant dye structures, generating intermediates that are more amenable to subsequent microbial degradation [14,98].

Following enzymatic pretreatment, microbial polishing becomes responsible for the degradation of intermediate metabolites. Unlike isolated enzymatic systems, which often achieve only partial pollutant transformation, microbial communities possess broader metabolic capabilities that enable the assimilation and mineralization of degradation products. Bacteria and fungi can convert aromatic intermediates into less harmful compounds and ultimately into carbon dioxide, water, and cellular biomass through interconnected metabolic pathways [10,96,97].

This complementary interaction is particularly important because decolorization alone does not necessarily indicate effective detoxification. Enzymatic or reductive cleavage of dye molecules may generate aromatic amines and other transformation products that remain toxic even after visible color removal. The microbial stage therefore plays a critical role in reducing the accumulation of these intermediates and promoting more complete detoxification of textile effluents [10,49,96,97].

From an industrial perspective, sequential systems offer important advantages because each stage can be independently optimized according to the requirements of the biocatalysts involved. Enzymatic reactors may be operated under conditions that maximize catalytic efficiency, while subsequent biological treatment units can be designed to support microbial growth and degradation activity. This operational flexibility partially explains why sequential configurations remain among the most frequently proposed strategies for industrial implementation of integrated enzymatic–microbial treatment processes [96,98,99].

Nevertheless, sequential systems also present limitations. The use of multiple treatment stages may increase process complexity, reactor footprint, and operational costs. Furthermore, incomplete enzymatic transformation can still result in the accumulation of persistent intermediates if microbial degradation is insufficient. Despite these challenges, sequential enzymatic–microbial systems continue to represent one of the most promising approaches for coupling rapid dye transformation with effective detoxification and mineralization of TW [10,97].

5.3. Simultaneous Enzymatic–Microbial Systems

Simultaneous or one-pot systems integrate enzymatic catalysis and microbial metabolism within a single treatment unit, allowing pollutant transformation and biodegradation to occur concurrently [10]. In these systems, enzymatic reactions continuously generate transformation products that can be immediately assimilated by microbial populations, promoting cooperative degradation pathways and reducing the accumulation of intermediates.

The principal advantage of one-pot systems is process intensification. By combining multiple biological functions within a single reactor, these systems may reduce hydraulic retention times, simplify reactor design, and decrease operational costs [14,96]. However, successful implementation requires compatibility between enzymatic and microbial operating conditions. Differences in pH optima, temperature requirements, oxygen demand, and salinity tolerance frequently limit overall performance [98,99].

Despite these challenges, recent studies have demonstrated promising decolorization and COD removal efficiencies using adapted microbial consortia and robust enzymatic systems, highlighting the potential of one-pot configurations for future industrial applications [10,96].

5.4. Detoxification Cascades: From Dye Transformation to Mineralization

One of the most important advantages of integrated enzymatic–microbial systems is their ability to promote sequential detoxification cascades that extend beyond simple decolorization. In these cascades, enzymes and microorganisms perform complementary

metabolic functions that collectively transform recalcitrant dye molecules into less toxic products and ultimately into inorganic end products.

Azo dyes provide a representative example. During the initial treatment stage, AZOR or reductive microbial pathways cleave azo bonds ($-N=N-$), resulting in rapid decolorization. However, this process frequently generates aromatic amines that may remain toxic, mutagenic, or carcinogenic [22,23]. Subsequent oxidative enzymes such as LAC, LiP, MnP, and DyPs can further oxidize aromatic intermediates, increasing their susceptibility to microbial degradation (Figure 2) [24,75].

Following enzymatic transformation, bacterial and fungal populations assimilate intermediate compounds through central metabolic pathways, promoting ring cleavage, oxidation, and eventual mineralization to carbon dioxide, water, and biomass. Therefore, integrated systems reduce the risk of accumulating toxic metabolites while improving overall detoxification performance.

The distinction between decolorization and detoxification is discussed further in Section 8.

5.5. Role of Microbial Consortia in Integrated Systems

Microbial consortia have emerged as valuable components of integrated enzymatic–microbial systems due to their metabolic diversity, functional complementarity, and enhanced resilience under complex wastewater conditions. Unlike pure cultures, consortia combine the metabolic capabilities of multiple microorganisms, enabling the degradation of a broader range of pollutants and improving overall treatment performance [101–103].

A major advantage of microbial consortia is their metabolic complementarity. Individual community members frequently perform distinct but interconnected metabolic functions, allowing degradation pathways to be distributed among multiple species. Metabolites generated by one population can serve as substrates for another through cross-feeding interactions, facilitating the transformation of complex pollutants that are difficult to degrade by individual microorganisms alone [101,104]. In TW treatment, this characteristic is particularly important because dye degradation often requires multiple sequential biochemical reactions involving different enzymatic and metabolic activities.

In mixed-substrate systems, different compounds can enter the metabolic network at distinct points, improving the balance of carbon, energy, and cellular redox status. This combination can simplify multistep bioconversion processes, reduce metabolic stress and protein burden, and provide simultaneous access to multiple catabolic pathways by distributing metabolic functions across diverse cellular environments [105–107]. However, mixed substrates are not necessarily consumed simultaneously, as many microorganisms preferentially use specific compounds or exhibit diauxic growth due to native regulatory mechanisms. In *Pseudomonas putida*, for example, carbon catabolite repression and limited coordination among the pathways responsible for glucose, xylose, and arabinose utilization can impair synergistic substrate consumption until these pathways are rebalanced through metabolic engineering [105,108]. In this context, microbial co-cultures provide a valuable platform for overcoming carbon catabolite repression (CCR), as individual community members can be engineered or selected to grow specifically on different substrates [107].

Synergy may occur through co-feeding, in which one substrate supplies the ATP or NADPH required for the utilization of another; through cometabolism, in which a readily biodegradable carbon source activates enzymatic pathways capable of transforming recalcitrant pollutants; or through cross-feeding, in which metabolites released by one population are consumed by synergistic microorganisms. Such interactions can establish symbiotic “nutrient supply–detoxification” relationships that stabilize the microbial population and improve overall process efficiency [106,107]. This division of functions

reduces the biosynthetic burden on individual community members and may emerge through the exchange of metabolic intermediates or essential metabolites [109–111]. However, the nature of these interactions depends on substrate complexity: recalcitrant compounds tend to promote cooperation, whereas simple substrates may intensify competition between microorganisms [112].

In mixed cultures used for lignocellulose degradation, for example, the production of complementary enzymes and increased β -1,4-glucosidase activity enhanced substrate degradation and final culture density, although they did not necessarily increase the maximum growth rate [112]. Synergy is also influenced by the composition and proportions of the substrate mixture and by the inoculation ratio, which shapes the growth dynamics and metabolic capabilities of community members and may generate either synergistic or antagonistic effects. In co-digestion systems, specific mixing ratios have improved buffering capacity, prevented acidification, and promoted microbial community restructuring [78,107,113].

Therefore, in TW treatment, synergistic metabolism should be understood as a recurring principle underlying pollutant removal and conversion whenever metabolic pathways, cofactors, enzymes, or microbial species perform complementary functions. System performance consequently depends on the integration of cometabolism, cross-feeding, syntrophy, and appropriate substrate ratios rather than solely on the degradative capacity of an individual species [114].

Sequential degradation pathways constitute one of the principal mechanisms through which microbial consortia contribute to detoxification. In these systems, primary degraders initiate the transformation of dye molecules or their intermediates, generating compounds that are subsequently metabolized by other community members until complete mineralization is achieved [115]. Elnabi et al. (2025) [77] developed a microbial consortium composed of *Bacillus cereus*, *Proteus mirabilis*, and *Stenotrophomonas maltophilia* that could decolorize and detoxify Reactive Black 5 (RB5). Degradation involved the cleavage of azo bonds by azoreductase and NADH-DCIP reductase, followed by the oxidation of the resulting intermediates by laccases and peroxidases (Figure 2). The resulting less-toxic compounds were subsequently converted into TCA-cycle precursors and ultimately mineralized into CO_2 and H_2O . Such cooperative interactions improve degradation efficiency and reduce the accumulation of potentially inhibitory or toxic intermediates. Metagenomic studies have demonstrated that microbial consortia frequently harbor multiple complementary degradation pathways, increasing their capacity to respond to fluctuations in wastewater composition and operational conditions [116].

Within integrated enzymatic–microbial systems, microbial consortia play a particularly important role following enzymatic pretreatment. Oxidative enzymes such as LAC and peroxidases can transform recalcitrant dye molecules into smaller intermediates that become more accessible to microbial metabolism. Consortium members can subsequently degrade these transformation products through complementary metabolic pathways, promoting further detoxification and mineralization. This cooperative interaction between enzymatic catalysis and microbial metabolism represents one of the fundamental advantages of integrated treatment systems.

Detoxification cascades provide another important example of consortium-mediated cooperation. In these cascades, highly tolerant microorganisms initiate the transformation of toxic compounds, reducing their inhibitory effects and enabling more sensitive populations to participate in subsequent degradation steps [117]. Such mechanisms are particularly relevant in TW treatment, where dye degradation may generate aromatic amines and other intermediate compounds that require additional metabolic processing before complete detoxification is achieved. Through sequential metabolic interactions,

microbial consortia can reduce the persistence of these intermediates and enhance overall treatment effectiveness [118,119].

In addition to metabolic complementarity, microbial consortia generally exhibit greater robustness and functional stability than pure cultures. Functional redundancy among community members enables the maintenance of critical degradation processes even when environmental conditions fluctuate or specific populations experience temporary inhibition [102,120]. This resilience is particularly advantageous for industrial wastewater treatment, where variations in pH, salinity, dye composition, and organic load frequently occur.

Collectively, these characteristics make microbial consortia highly attractive partners for enzymatic treatment technologies. By combining complementary metabolic pathways, sequential degradation mechanisms, detoxification cascades, and enhanced process robustness, microbial consortia significantly contribute to the effectiveness of integrated enzymatic–microbial systems and support the transition from simple dye decolorization toward effective TW detoxification.

The implementation of integrated enzymatic–microbial systems depends on the availability of cost-effective and sustainable biocatalysts. One promising strategy involves the production of enzyme-rich biomass and microbial catalysts through solid-state fermentation of agro-industrial residues, thereby linking wastewater detoxification with biomass valorization and circular bioeconomy principles.

A comprehensive comparative assessment of different biocatalytic and microbial integrated systems, highlighting their performance across real and synthetic wastewater matrices, operational conditions, and detoxification efficiencies is summarized in Table 3.

Taken together, this comparative analysis demonstrates that no universal biocatalytic strategy can simultaneously provide high decolorization efficiency, substantial COD removal, comprehensive detoxification, operational robustness, and economic feasibility. Enzyme-based systems are distinguished by their rapid oxidation of dyes and potential to reduce toxicity [121]. However, they may be limited by incomplete mineralization, low COD removal, and, in some cases, dependence on redox mediators that increase operating costs [122]. In contrast, microbial consortia and integrated anaerobic–aerobic systems generally achieve greater mineralization and COD removal [44] and exhibit greater robustness when treating real textile wastewater. Nevertheless, their application may be constrained by high salinity, the presence of surfactants, and long hydraulic retention times [10].

Moreover, comparisons between synthetic and real wastewater indicate that the high performance frequently achieved under laboratory conditions is not always reproduced at the industrial scale because of the greater physicochemical complexity of real wastewater matrices [10]. Therefore, selecting the most appropriate strategy requires consideration not only of decolorization efficiency but also of mineralization capacity, detoxification, operational stability, and economic feasibility. Industrial implementation will ultimately depend on balancing these factors according to the specific characteristics of the wastewater to be treated.

Table 3. Critical and comparative evaluation of enzymatic and microbial systems in textile wastewater treatment.

Biocatalytic System/Organism	Wastewater Matrix and Dye Type	Operating Conditions (Temperature, pH, and Time/HRT)	Treatment Efficiency	Toxicity Assessment and Critical Analysis	Refs.
Microbial consortium in hybrid reactors (UASB + SBR)	Real textile wastewater	35 °C; pH 6.8–7.2; total HRT: 54 h (48 h anaerobic + 6 h aerobic)	Decolorization: 94%; COD removal: 98%	Anaerobic azo bond cleavage; aerobic aromatic amine degradation; potential mineralization.	[10]
Thermo-haloalkaliphilic consortium	Synthetic wastewater containing azo dyes, including Reactive Red 141 and Reactive Red 239	55 °C; pH 9.0–10.0; salinity: 0.5–10%; treatment time: 6–7 h	Decolorization: 96–99.9%; COD removal: approximately 92.7%	No cooling or neutralization required. Efficient under high-temperature, alkaline, and saline conditions.	[10]
Bacterial consortium (<i>Pseudomonas</i> sp. and <i>Providencia rettgeri</i>)	Synthetic wastewater containing Reactive Black 5, Direct Red 81, and other dyes	30 °C; salinity: 2%; HRT: 12–30 h under sequential anaerobic–aerobic conditions	Decolorization: 98–99%	High salt tolerance, short retention time, and reduced hazardous intermediate accumulation.	[44]
Immobilized spore-associated laccase from <i>Bacillus amyloliquefaciens</i> A1	Synthetic wastewater containing Crystal Violet, Direct Red 81, and Acid Black 24	30–60 °C; pH 6–7; treatment time: 24 h; immobilization in agarose gel	Decolorization: 68.8–94.5%	Reduced toxicity, eight reuse cycles, and potential need for additional mineralization steps.	[121]
Recombinant TP-Lac2 laccase from <i>Trametes polyzona</i>	Synthetic wastewater containing Malachite Green, Amido Black 10B, and Orange G	50 °C; pH 3.5–4.0; treatment time: 5 h; redox mediators including acetosyringone and ABTS	Decolorization: >75–94.6%	Rapid high-temperature dye degradation. Redox mediator requirement increases cost and chemical load.	[122]
Hybrid structured reactor (UAS) and biofilter (SAB)	Real textile wastewater with high salinity and linear alkylbenzene sulfonate (LAS) surfactants	30 °C; HRT: 24 h	Decolorization: 70%; sulfate removal: 87% with molasses supplementation	Molasses supplementation required; high LAS concentrations inhibit aromatic amine degradation.	[10]
Alkaliphilic microbial consortium in integrated anaerobic–aerobic reactors	Real and synthetic textile wastewater	25 °C; pH 10.2; total HRT: 54 h (18 h anaerobic + 36 h aerobic)	Decolorization: 96–99%; COD removal: 86–93.37%	Sequential anaerobic–aerobic treatment. Efficient in real wastewater without nutrient supplementation.	[9]

Overview of recent biocatalytic systems for textile wastewater treatment, emphasizing the complementary roles of enzymes and microorganisms under different operating conditions, together with their treatment performance, detoxification potential, and key operational limitations.

5.6. Synergistic Interactions in Microbial Consortia

Mixed microbial communities often outperform pure cultures because of metabolic cooperation and greater environmental resilience. In solid-state fermentation, a fungal consortium composed of *Daldinia concentrica* and *Xylaria polymorpha*, cultivated on common bean husks, achieved 97–98% removal of the reactive dye Cibacron Brilliant Red 3B-A within 48 h, with detoxification confirmed through phytotoxicity assays [92]. Similarly, a consortium of *Penicillium oxalicum*, *Aspergillus niger*, and *Aspergillus flavus* completely decolorized Acid Red 183, Direct Blue 15, and Direct Red 75 while minimizing the inhibition by degradation by-products frequently observed in single-culture systems [91]. However, synergy is not always achieved. In a binary consortium of *Trametes versicolor* and *Pleurotus pulmonarius*, competition for space resulted in lower decolorization efficiency (64.3%) than that obtained with the corresponding pure cultures (94%) [94].

In addition to fungal applications, native microbial consortia have demonstrated high efficiency in treating textile dyes and real effluents. A consortium of six bacterial strains, including *Bacillus cereus* and *Bacillus subtilis*, isolated from industrial drains in India, effectively decolorized and degraded Disperse Red 167.1, Disperse Red 54, and Disperse Blue 60, outperforming the individual strains [93]. Under extreme conditions, a thermoalkaliphilic consortium isolated from Ethiopian hot springs achieved more than 98% color removal and 92.7% COD reduction for Reactive Red 141 at pH 9.0 and 55 °C [90]. Finally, a native consortium of *B. subtilis* and *Pseudomonas aeruginosa*, isolated from batik-dye washing basins in Indonesia, effectively degraded the reactive dye Procion Red through NADH-dependent reductase activity [123].

6. Biomass-Derived Biocatalysts for Integrated Detoxification Systems

The large-scale implementation of enzymatic and microbial technologies for TW treatment is frequently constrained by the cost of biocatalyst production and the availability of robust biological systems capable of operating under industrial conditions. In this context, solid-state fermentation (SSF) has emerged as a promising platform for producing low-cost enzymes, fungal biomass, and microbial consortia using renewable agro-industrial residues as substrates. Beyond its role in waste valorization, SSF contributes directly to integrated detoxification systems by generating biomass-derived biocatalysts that can be applied as crude enzymatic extracts, immobilized enzymes, living fungal biomass, or mixed microbial cultures. Consequently, SSF establishes a direct link between biomass valorization, enzyme production, microbial biotechnology, and TW detoxification [88,124,125].

6.1. Solid-State Fermentation as a Biocatalyst Production Platform

Solid-state fermentation (SSF) is defined as a biotechnological process in which microorganisms grow on moist solid substrates in the absence or near absence of free water [88,124,126]. In this system, the solid material serves simultaneously as a nutrient source and a physical support, closely mimicking the natural habitat of many microorganisms, particularly filamentous fungi, thereby promoting intimate biological interactions [124,127]. SSF has emerged as a key strategy within the sustainable systems because it enables the valorization of organic residues by converting them into high-value bioproducts, including enzymes, biofuels, antioxidants, and other bioactive compounds [88,125,128].

Agro-industrial residues are the substrates most commonly employed in SSF due to their abundance, renewability, and low cost [124,127]. These materials can be categorized according to their chemical composition into lignocellulosic residues, such as wheat straw, sugarcane bagasse, and corn cobs; starch-rich residues, including rice bran and cassava peels; and substrates rich in soluble sugars, such as apple pomace and sugar beet

pulp [88,124]. Among these materials, wheat bran is frequently regarded as one of the most versatile supports because of its porous structure and inherent nutritional richness [126,127].

The success of SSF depends on the precise control of several operational parameters (Table 4). Moisture content and water activity (a_w) are considered the most critical factors, as they regulate gas diffusion and nutrient solubility within the solid matrix [88,127]. Temperature also plays a fundamental role by influencing microbial metabolism and enzymatic activity. However, metabolic heat generated during fermentation may create harmful thermal gradients, requiring appropriate control through aeration or cooling strategies [125,129]. Other important variables include pH, which affects protein stability and enzyme activity, and substrate particle size, which determines the available surface area and porosity for microbial colonization [127,128].

Table 4. Critical operational parameters in solid-state fermentation.

Parameter	Relevance and Effects	Typical/Recommended Conditions	Refs.
Moisture content (%)	Ensures cellular hydration and nutrient transport	Fungi: 40–60%; Bacteria: 60–85%.	[88,127]
Temperature (°C)	Influences enzymatic reactions and microbial growth	Mesophiles: 25–35 °C (20–55 °C for fungi); Thermophiles: up to 80 °C.	[127,129]
pH	Affects chemical equilibrium and reaction kinetics	Fungi: 3.0–6.0 (acidic); Bacteria: 7.0–9.0 (neutral to alkaline)	[88,126]
Aeration	Supplies oxygen, removes metabolic heat, and supports thermal regulation	Dependent on airflow and bioreactor design.	[88,125]
Particle size	Determines surface area, porosity, and microbial accessibility.	Preferably <1 mm; particles around 500 µm are commonly used	[127,128]

This table summarizes key operational parameters affecting solid-state fermentation and biocatalytic performance, including moisture content, temperature, pH, aeration, and particle size. Reported values represent typical or recommended ranges from the literature and may vary according to substrate type, microorganism, and process configuration.

6.2. Agro-Industrial Residues as Enzyme Production Substrates

The production of enzyme-rich biomass through solid-state fermentation (SSF) is predominantly driven by filamentous fungi due to their natural ability to colonize solid substrates and secrete large amounts of extracellular proteins [26,88,124]. Fungal systems, particularly those belonging to the genera *Aspergillus* and *Trametes*, are widely recognized for their robustness and versatility in converting agro-industrial residues into high-value bioproducts. These microorganisms employ hyphal growth to penetrate the solid matrix, facilitating the degradation of complex polymers such as lignin and cellulose [124,127].

LAC are primarily produced by white-rot fungi, with *Trametes versicolor* being one of the most extensively studied species because of its high redox potential and remarkable efficiency in degrading recalcitrant pollutants [25,26,31]. In addition to *Trametes*, species of *Pleurotus* and *Aspergillus* have demonstrated significant potential for LAC (EC 1.10.3.2) production when cultivated on substrates such as wheat bran, tomato pomace, and grape residues [26,31,88]. The production of these enzymes can be substantially enhanced through the addition of metallic inducers, particularly copper ions, as well as selected aromatic compounds that stimulate enzyme expression [25,31].

For cellulase and hemicellulase production, the genera *Aspergillus*, *Trichoderma*, and *Penicillium* are among the most prominent microbial producers [88,124,127]. *Trichoderma reesei* is frequently regarded as one of the primary industrial sources of cellulases, whereas several strains of *Aspergillus niger* are extensively employed for the production of xylanases

and other hydrolytic enzymes using substrates such as sugarcane bagasse and wheat straw [88,124,127]. Species of the genus *Neurospora* have also been investigated in SSF systems for cellulose degradation, particularly when cultivated on coffee pulp residues [88].

The selection of agro-industrial substrates, including rice bran, cassava peels, and corn cobs, plays a decisive role in determining the enzymatic profile of the resulting biomass [88,124,126]. These residues not only provide essential nutrients for microbial growth, but also induce the expression of multienzyme complexes that act synergistically in the deconstruction of lignocellulosic biomass [88,127]. Such technological integration enables the valorization of organic wastes by transforming them into low-cost sources of industrial enzymes while supporting the principles of the circular production [88,125].

The enzymes and fungal biomass generated through SSF are directly relevant to integrated detoxification systems. LAC, LiP, MnP, and other oxidative enzymes produced during fermentation can initiate the transformation of recalcitrant dye molecules, whereas the associated microbial biomass contributes additional biosorption, biodegradation, and mineralization capabilities. Therefore, SSF-derived biocatalysts provide a practical bridge between enzyme-based and microbial-based treatment approaches, facilitating the development of integrated detoxification platforms [25,26,31].

6.3. Direct Application of Fermented Biomass

The direct application of SSF-derived biomass represents one of the most promising approaches for integrating enzyme production and biological wastewater treatment. Rather than requiring extensive downstream processing, the fermented biomass can be applied directly as a multifunctional biocatalyst in which extracellular enzymes, fungal mycelia, and associated microbial communities act synergistically through biosorption, biodegradation, and detoxification mechanisms. This integrated functionality is particularly attractive for TW treatment because it combines rapid enzymatic transformation of dye molecules with subsequent biological degradation of intermediate products [26,125].

The comparison between crude enzymatic extracts and whole fermented biomass reveals distinct technical advantages. Whole biomass provides a robust platform combining adsorption capacity with intracellular metabolic pathways, whereas crude extracts enable an immediate and targeted catalytic response without the challenges associated with biomass recovery and disposal after treatment. Furthermore, crude multienzyme preparations generally outperform purified enzymes because they preserve the natural synergistic interactions among different enzyme classes, including LAC, LiP, and MnP, which are simultaneously produced during SSF. This enzymatic synergy enhances the breakdown of complex chemical bonds present in recalcitrant dyes. In addition, crude extracts derived from microbial co-cultures, such as *Aspergillus fumigatus* and *Pseudomonas fluorescens*, have demonstrated superior decolorization performance compared with extracts obtained from individual monocultures [26,31,130].

Recent experimental studies further demonstrate the practical feasibility of SSF-derived enzymatic systems and fermented biomass for dye removal using diverse fungal species and agro-industrial substrates. These investigations highlight the versatility of SSF platforms by integrating extracellular enzymatic oxidation, biosorption, and scalable treatment strategies. For instance, *Penicillium roqueforti* ATCC 10110 cultivated on an optimized substrate mixture consisting of rice husks (56%), coffee husks (30%), and cocoa shells (14%) produced a laccase oxidative multienzyme extract (LOME) capable of degrading methylene blue by 29.8% after 24 h without the addition of redox mediators, demonstrating the potential of low-cost lignocellulosic residues for enzyme production and dye remediation [131]. Similarly, *Penicillium commune* S6 grown on potato peel waste secreted laccases that achieved complete decolorization (100%) of Lanapel Red BM 143-PL (10 mg L⁻¹) within

120 min under alkaline conditions (pH 9.13), highlighting the applicability of SSF-derived enzymes under industrially relevant operational conditions [132]. Likewise, *Pleurotus ostreatus* cultivated on spent wheat bran generated a laccase-rich fermented biomass (66 U gds^{-1}) capable of removing more than 97% of malachite green and 95% of brilliant blue, further demonstrating the effectiveness of fungal SSF systems for treating structurally distinct synthetic dyes [95]. Collectively, these studies demonstrate that SSF enables the production of highly efficient enzymatic biocatalysts from inexpensive agro-industrial residues while reducing process complexity and supporting circular bioeconomy strategies.

Beyond enzyme extraction, increasing evidence indicates that whole fermented biomass can itself function as an effective multifunctional biosorbent while retaining its catalytic potential, thereby facilitating process integration and scale-up. Fermented cocoa bean shell colonized by *Penicillium roqueforti* exhibited a sixfold increase in surface area due to mycelial infiltration, enabling the removal of up to 95% of methylene blue through chemisorption mechanisms while simultaneously valorizing an abundant agro-industrial residue [133]. Likewise, *Trametes versicolor* cultivated on corn cob successfully degraded 96.26% of a binary mixture of Allura Red and Tartrazine after adsorption, and the process was subsequently validated in packed-bed bioreactors without compromising enzymatic performance, demonstrating the scalability and operational robustness of SSF-based treatment systems [94]. These findings reinforce that fermented biomass should not be regarded merely as a source of extracellular enzymes but rather as a multifunctional biocatalytic material capable of integrating biosorption, enzymatic oxidation, and microbial degradation into a single, economically attractive platform for textile wastewater treatment.

Economic advantages constitute one of the main drivers for the industrial implementation of SSF-based treatment systems. Eliminating downstream processing steps, including enzyme extraction, purification, and stabilization, significantly reduces production costs, as these operations may account for up to 70% of the total cost of enzyme manufacturing. The utilization of abundant and inexpensive agro-industrial residues, such as wheat bran, rice husks, and coffee-processing wastes, further decreases capital and operational expenditures. LAC produced through SSF have been estimated to reduce treatment costs by approximately 25% compared with conventional chemical processes, owing to lower energy requirements and the absence of harsh chemical reagents. Consequently, this approach not only reduces the pollutant load of textile effluents but also supports bioeconomy principles by transforming low-value residues into efficient biotechnological resources [76,125,130].

6.4. Waste Valorization Opportunities

The integration of solid-state fermentation (SSF) with biomass valorization represents an important component of resource recovery strategies by transforming low-value agro-industrial residues into functional biocatalysts for environmental applications. In this framework, SSF serves as a biotechnological platform capable of converting abundant agricultural by-products into enzyme-rich biomass and microbial catalysts suitable for TW treatment. By diverting these materials from landfills and uncontrolled disposal practices, SSF contributes to reducing environmental burdens while generating value-added products [88,125,126].

Lignocellulosic residues such as cereal straws, rice husks, coffee husks, sugarcane bagasse, and sawdust are particularly attractive substrates for SSF because they simultaneously function as physical supports for microbial growth and as sources of carbon and nutrients. During fermentation, microorganisms—especially filamentous fungi—colonize the solid matrix and secrete extracellular enzymes capable of degrading lignocellulosic polymers and other complex organic substrates [124,126,127].

A major advantage of this approach is the production of biomass-derived biocatalysts that can be directly integrated into TW detoxification processes. SSF supports the generation of oxidative and hydrolytic enzymes, including LAC, cellulases, and other extracellular enzymes, as well as active fungal biomass capable of contributing to biosorption, biodegradation, and detoxification mechanisms. Consequently, agricultural residues are transformed into low-cost biological resources that support both pollutant removal and resource recovery objectives [76,88,127,128].

Environmental advantages of SSF further reinforce its relevance for integrated detoxification systems. The use of inexpensive and widely available substrates reduces production costs and minimizes dependence on synthetic culture media, while the direct application of fermented biomass may decrease downstream processing requirements. Additionally, SSF generally requires lower water inputs than submerged fermentation systems and promotes the productive utilization of agricultural residues that would otherwise have limited economic value [88,125,127,128].

Overall, SSF should not be viewed solely as an enzyme production technology but rather as a platform for generating biomass-derived biocatalysts that support integrated detoxification systems. By simultaneously producing oxidative enzymes, fungal biomass, and microbial communities from low-cost agro-industrial residues, SSF contributes to both wastewater remediation and biomass valorization. These characteristics position SSF-derived biocatalysts as promising components of future integrated enzymatic–microbial treatment platforms capable of achieving effective TW detoxification while supporting resource recovery principles [88,125,127].

7. Process Scale-Up and Industrial Feasibility

7.1. Reactor Configurations

The selection of an appropriate operational mode is one of the most critical decisions during the development and scale-up of industrial bioprocesses. Reactor operation directly influences system productivity, microbial stability, resource consumption, energy efficiency, and overall production costs [134]. Although batch processes continue to dominate industrial biotechnology, particularly in applications involving enzymes, microbial metabolites, and wastewater biotreatment, continuous systems have attracted increasing attention due to their potential for process intensification and enhanced operational efficiency [134–137].

In batch systems, all components required for the process, including substrates, nutrients, and inoculum, are introduced into the reactor before cultivation begins. During operation, there is no significant inflow or outflow of material, and the system evolves dynamically until the process is terminated for product recovery [134]. This configuration offers considerable operational flexibility, allowing for the use of diverse feedstocks and process conditions without major modifications to the production facility. Consequently, batch reactors remain widely employed in processes utilizing agro-industrial residues or waste streams with highly variable compositions [134,136,138–140].

Within the textile industry, such flexibility is particularly valuable because wastewater characteristics often fluctuate substantially, exhibiting variations in pH, salinity, and azo dye concentrations depending on dyeing formulations and manufacturing processes [10]. Another important advantage of batch operation is its contribution to biosafety. Since each production cycle is completed before cleaning and sterilization procedures are performed, contamination events are generally confined to a single batch, thereby reducing the risk of prolonged production losses. Furthermore, periodic process interruption facilitates product quality assessment and operational adjustments between production cycles [134].

Despite these advantages, batch operation presents significant limitations when high industrial productivity is required. During cultivation, nutrient concentrations progres-

sively decrease while secondary metabolites and potentially inhibitory compounds accumulate within the medium. These changes may adversely affect cellular metabolism and reduce overall process yields. In addition, downtime associated with reactor loading, unloading, cleaning, and sterilization decreases volumetric productivity and equipment utilization [134,136,137].

In contrast, continuous systems operate through the constant addition of fresh substrate and simultaneous removal of processed culture broth. When properly controlled, these systems achieve a steady-state condition in which variables such as biomass concentration, substrate availability, and product formation remain relatively stable over extended periods [134]. This characteristic enables more efficient utilization of reactor capacity and minimizes operational interruptions.

The primary advantage of continuous biomanufacturing lies in process intensification. Maintaining microbial populations under near-optimal metabolic conditions for prolonged periods can significantly increase productivity per unit volume compared with conventional batch processes. Consequently, smaller reactors may achieve production capacities comparable to those of much larger discontinuous systems, contributing to reductions in capital investment, energy consumption, and waste generation [134–137,139].

Despite these benefits, industrial implementation of continuous bioprocessing still faces important technological challenges. Maintaining steady-state conditions requires advanced monitoring and control systems capable of rapidly responding to process fluctuations. Operational strategies such as chemostats, turbidostats, and automated data acquisition systems are frequently employed to ensure process stability [134]. Recent advances associated with the Bioprocessing 4.0 paradigm, including smart sensors, the Industrial Internet of Things (IIoT), and digital twins, have significantly enhanced real-time monitoring capabilities and facilitated the adoption of continuous systems on an industrial scale [134,135,137,140].

Another important limitation involves microbial contamination. Unlike batch processes, where contamination typically affects only a single production cycle, continuous systems may operate uninterrupted for weeks or even months [141]. Under such conditions, contaminating microorganisms can proliferate and compromise the entire production campaign. Furthermore, prolonged cultivation periods may promote physiological adaptation and phenotypic shifts within microbial populations, potentially affecting process stability and productivity over time [134].

From a scale-up perspective, another critical challenge is the formation of gradients in substrate concentration, dissolved oxygen, and pH within large-scale reactors. As reactor volume increases, mixing times often become significantly longer, creating heterogeneous microenvironments that can alter cellular metabolism and negatively impact process performance [139].

Recent studies have demonstrated that such heterogeneities may result in productivity losses, reduced biomass yields, and increased formation of undesirable by-products. To address these challenges, scale-down strategies have been widely adopted to reproduce, at laboratory scale, the environmental conditions encountered in industrial bioreactors. These approaches provide valuable insights into microbial responses to large-scale heterogeneities and support the development of more robust industrial processes [134,136,137,139].

Therefore, the choice between batch and continuous operation should simultaneously consider technical, economic, and operational factors. Processes utilizing heterogeneous feedstocks or highly variable waste streams generally benefit from the flexibility of batch systems. Conversely, mature processes characterized by stable feed composition and high production demand may achieve substantial efficiency gains through continuous operation. Consequently, both reactor configurations remain highly relevant in modern

biotechnology, with their selection depending on the specific objectives of the process and the characteristics of the production system [134–136,138,139].

7.1.1. Packed-Bed Reactors (PBRs)

Packed-bed reactors (PBRs) are widely employed in continuous bioprocesses due to their high capacity for retaining immobilized biocatalysts within a relatively small reactor volume. In this configuration (Figure 3), the substrate flows through a stationary bed composed of solid particles containing immobilized enzymes or microorganisms, enabling high volumetric productivity and continuous operation with relatively low energy consumption compared with other reactor systems. Their simple design and the limited exposure of biocatalysts to shear forces enhance operational stability and facilitate catalyst reuse, making PBRs particularly attractive for industrial biocatalysis and wastewater treatment applications [142–144].



Figure 3. Reactor configurations for textile wastewater bioremediation using solid-state fermentation-derived biocatalysts. Engineering perspectives on textile wastewater bioremediation: overview of input challenges, process intensification parameters, and scaling-up via diverse reactor configurations. The blue arrows indicate the sequential process flow, from the generation of textile wastewater and detoxification to treatment in different reactor configurations and the production of a bioremediated effluent. The green arrows and circular symbol represent the integration of on-site solid-state fermentation (SSF)-derived biocatalyst production with the circular economy concept, highlighting biomass valorization, resource recirculation, and closed-loop industrial implementation. The integration of on-site SSF provides a cost-effective pathway for biomass valorization, resulting in detoxified effluents and supporting the transition toward a circular bioeconomy.

In TW remediation, PBRs are especially suitable for the continuous operation of immobilized microorganisms or enzymes, such as AZOR and LAC, allowing sustained degradation of azo dyes while preventing biocatalyst washout into the treated effluent [10,144].

Despite these advantages, PBR performance is strongly influenced by mass transfer limitations within the packed bed, which frequently become the rate-limiting step of the process. Irregular particle packing may promote preferential flow channels and dead zones

characterized by poor fluid circulation, resulting in uneven substrate distribution and reduced contact efficiency between pollutants and biocatalysts [142,144]. Furthermore, although smaller particle sizes generally improve mass transfer, excessively fine particles can substantially increase pressure drop and favor operational clogging. Consequently, parameters such as flow velocity, bed porosity, and the physicochemical properties of immobilization supports play critical roles in determining reactor stability and performance on an industrial scale [142,144].

7.1.2. Fluidized-Bed Reactors (FBRs)

Fluidized-bed reactors (FBRs) operate through the suspension of solid particles by an upward flow of liquid or gas at velocities sufficient to fluidize the bed (Figure 3) [145,146]. Under these conditions, intense mixing between phases significantly enhances mass and heat transfer rates while promoting homogeneous distribution of nutrients, oxygen, and temperature throughout the reactor volume [145,147]. This dynamic behavior effectively mitigates limitations commonly encountered in packed-bed systems, including excessive bed compaction, dead-zone formation, and high pressure losses [146].

Continuous particle movement within the fluidized regime also reduces fouling and improves contact between the fluid phase and immobilized biocatalysts, making FBRs highly attractive for continuous industrial processes and environmental remediation applications [145,148]. This feature is particularly important for TW treatment, where high concentrations of suspended solids and residual textile fibers frequently cause rapid clogging of conventional fixed-bed systems [10].

However, maintaining fluidization requires greater energy input and may induce mechanical attrition of support particles, resulting in the formation of fines and loss of material through elutriation, which can compromise long-term operational stability [146,148]. In addition, hydrodynamic control becomes increasingly complex at large scale, especially in multiphase systems and media containing elevated solids concentrations [146,149].

7.1.3. Airlift Reactors (ALRs)

Airlift reactors (ALRs) rely on pneumatic circulation to promote mixing and oxygen transfer (Figure 3) without the need for mechanical agitation [150,151]. These systems consist primarily of two interconnected zones: the riser and the downcomer. Differences in gas holdup between these regions generate density gradients that drive continuous fluid circulation throughout the reactor [150,152].

This configuration provides efficient mixing while maintaining low shear stress levels, making ALRs particularly suitable for cultivating shear-sensitive organisms such as filamentous fungi, animal cells, and microorganisms involved in long-term fermentation processes [150,153]. Compared with mechanically stirred systems, ALRs generally exhibit lower energy consumption while maintaining high oxygen transfer and mass transfer efficiencies [150,151].

Nevertheless, reactor performance strongly depends on hydrodynamic and geometric parameters, including the height-to-diameter ratio and the efficiency of gas disengagement at the reactor top [150,152]. In highly viscous media, circulation velocity and mass transfer coefficients may decrease substantially, limiting bioprocess productivity. Despite these limitations, ALRs remain attractive for wastewater treatment, biopolymer production, and industrial fermentation processes because of their operational stability, scalability, and versatility [151,153,154].

7.2. Operational Challenges Enzyme Immobilization and Operational Stability

Enzyme stability remains one of the principal factors limiting the economic viability of industrial bioprocesses, as it directly determines catalyst consumption relative to product

formation. On an industrial scale, enzyme activity may be lost through several mechanisms, including thermal inactivation, mechanical shear stress generated by pumps, agitators, and valves, interfacial inactivation at liquid–air and liquid–solid interfaces, oxidation of sensitive amino acid residues such as cysteine and methionine, and inhibition caused by substrates or products that alter enzyme structure or microenvironment [119,155–158].

Several strategies have been developed to mitigate these limitations. Enzyme immobilization on solid supports can increase structural rigidity and protect enzymes from unfavorable interfaces. Protein engineering approaches, including directed evolution and rational design, enable the development of enzyme variants with enhanced tolerance to temperature, solvents, and extreme pH conditions. Additionally, stabilizing additives such as polyols (e.g., glycerol and sorbitol), sugars, salts, and protective substrates can improve enzyme longevity. In some cases, non-conventional reaction media, including green solvents, ionic liquids, and aqueous–organic biphasic systems, further reduce enzyme denaturation and inhibition [143,159,160].

A key parameter in process design and scale-up is the Total Turnover Number (TTN), defined as the number of moles of product generated per mole of enzyme throughout its operational lifetime. In many industrial applications, TTN values exceeding 10,000 are already considered economically attractive. Consequently, combined assessments of TTN, capital expenditures (CAPEX), and operating expenditures (OPEX) are commonly used to guide decisions regarding enzyme formulation, immobilization strategies, and process conditions [161,162].

Another important challenge involves the toxicity of metabolic intermediates. During fermentative processes and enzymatic cascades, accumulation of intermediate compounds may negatively affect both microbial viability and enzyme stability. Major toxicity mechanisms include membrane disruption caused by organic solvents, surfactants, and medium-chain fatty acids; intracellular acidification by weak organic acids that diffuse across membranes in their protonated forms; enzyme inactivation by reactive intermediates such as furfural and hydroxymethylfurfural (HMF), which covalently modify amino acid residues; and oxidative stress resulting from the formation of quinones and reactive oxygen species (ROS) [163,164].

To mitigate these effects, several approaches have been proposed, including in situ product removal (ISPR) through adsorbent resins, membrane-based separations, and biphasic extraction systems, which continuously reduce the concentration of toxic compounds. Additional strategies include systems metabolic engineering to minimize intermediate accumulation, fed-batch operation to avoid substrate and metabolite concentration peaks, adaptive laboratory evolution (ALE) to generate more tolerant microbial strains, and the use of co-substrates that reduce the formation of toxic organic acids [165–167]. In simultaneous saccharification and fermentation (SSF) processes, for example, the concurrent consumption of sugars by yeast reduces cellulase inhibition by glucose and cellobiose, illustrating how process integration can alleviate intermediate toxicity [168,169].

Effective control of process variables is equally essential for ensuring reproducibility, product quality, and operational safety. Critical variables include temperature, pH, substrate and product concentrations, agitation, aeration, and foam formation. Temperature affects both catalytic activity and enzyme stability and is typically regulated through heat exchangers and PID control systems. pH influences the ionization state of catalytic residues and is controlled through buffer systems and acid/base addition. Substrate and product concentrations are managed through feeding strategies and ISPR technologies to minimize inhibition effects. Agitation and aeration determine oxygen transfer rates and shear levels, while foam formation, which may promote operational problems and interfacial enzyme

inactivation, is commonly controlled through reactor design modifications and antifoaming agents [170,171].

Modern bioprocesses increasingly incorporate Process Analytical Technology (PAT) tools, including real-time monitoring through spectroscopy, chromatography, and biosensors. Advanced control systems combine in-line measurements of pH, dissolved oxygen, and off-gas CO₂ with soft sensors based on mechanistic or data-driven models capable of estimating variables that cannot be directly measured, such as biomass concentration or substrate consumption rates [172,173]. Model Predictive Control (MPC) has emerged as a particularly powerful strategy for managing multivariable systems operating under complex regulatory and operational constraints. Together, PAT and advanced control systems support the Quality-by-Design (QbD) paradigm, reducing process variability and improving the robustness of industrial biomanufacturing processes [172,174,175].

7.3. Techno-Economic Considerations

Energy demand is one of the most important parameters in both techno-economic assessments (TEA) and life cycle assessments (LCA) of bioprocesses, as it directly influences operating costs, environmental performance, and overall process sustainability. In enzymatic and microbial processes, the major energy-consuming operations include heating and cooling, mixing and aeration in aerobic reactors, lignocellulosic biomass pretreatment, and, most notably, downstream processing. Unit operations such as distillation, evaporation, drying, and freeze-drying are particularly energy-intensive and may account for the largest fraction of total process energy consumption, as observed in bioethanol production and other large-scale biomanufacturing systems [176,177].

Energy performance is commonly evaluated using indicators such as Cumulative Energy Demand (CED), Energy Return on Investment (EROI), and exergy efficiency. These metrics enable comparisons among alternative process routes and facilitate the identification of energy bottlenecks throughout the production chain. Based on these assessments, several strategies have been proposed to reduce energy consumption, including heat integration, waste heat recovery from process streams, replacement of conventional distillation with membrane-based separation technologies, process automation and advanced control systems, and combined heat and power (CHP) generation using residual biomass [178,179].

One of the major advantages of enzymatic technologies is their ability to operate under milder conditions than conventional chemical processes, often requiring lower temperatures and pressures. This characteristic can substantially reduce the energy demand associated with heating operations. However, such benefits must be balanced against the potential kinetic advantages of operating at elevated temperatures, particularly when thermostable or thermophilic enzymes are employed, as higher reaction rates may offset part of the additional energy requirements [180,181].

8. Decolorization Versus Detoxification

Color removal is one of the most frequently reported indicators of treatment efficiency in studies involving TW remediation. High decolorization percentages are often interpreted as evidence of successful contaminant removal, particularly when enzymatic, microbial, or integrated biological systems are employed. However, increasing evidence indicates that decolorization and detoxification are not synonymous processes. Although decolorization reflects the disruption of chromophoric structures responsible for visible coloration, it does not necessarily indicate the elimination of toxic compounds or the complete mineralization of dye molecules [22,23].

This distinction is particularly important for azo dyes, which represent the largest class of dyes used in the textile industry. As discussed in previous sections, azo dyes

are characterized by azo bonds ($-N=N-$) linking aromatic structures that are responsible for color generation. Enzymatic systems such as AZOR and various bacterial reductive pathways efficiently cleave these bonds, frequently resulting in rapid decolorization of textile effluents [35,36,182]. However, this initial transformation often generates aromatic amines as intermediate products. Although the visible color disappears, these compounds may remain chemically stable and can exhibit mutagenic, genotoxic, or carcinogenic properties [22,23].

Consequently, treatment processes that achieve high decolorization efficiency do not necessarily guarantee environmental safety. In some cases, incomplete degradation may even increase toxicity due to the accumulation of intermediate metabolites. This limitation is particularly relevant for systems based exclusively on reductive dye cleavage, where the transformation of chromophoric groups occurs more rapidly than the subsequent degradation of aromatic intermediates [23,35].

The limitations of decolorization-oriented treatment strategies help explain the growing interest in oxidative enzymes such as LAC, LiP, MnP, and DyPs. Unlike simple azo bond cleavage, these enzymes can promote further oxidation of aromatic structures and contribute to the transformation of compounds that are often resistant to conventional biological degradation [24,75]. Nevertheless, enzymatic reactions alone may not always achieve complete mineralization, and residual transformation products can remain in the treated effluent.

Similarly, microbial systems possess broader metabolic capabilities that enable the degradation and assimilation of aromatic intermediates generated during enzymatic or reductive processes. Bacteria are frequently involved in the degradation of aromatic amines under aerobic conditions, whereas fungi contribute through extracellular oxidative enzyme systems capable of attacking recalcitrant aromatic structures. However, microbial treatments may be limited by slower degradation rates, operational conditions, and the complexity of industrial wastewater matrices [22,182].

These limitations highlight the importance of integrated enzymatic–microbial systems. By combining the rapid transformation capacity of enzymes with the metabolic versatility of microbial communities, integrated systems can promote sequential degradation pathways that extend beyond decolorization toward effective detoxification. In such systems, enzymes initiate the breakdown of complex dye molecules, while microorganisms subsequently degrade intermediate products and contribute to their mineralization. This complementary interaction reduces the risk of accumulating toxic metabolites and improves the overall environmental performance of treatment processes.

Therefore, the evaluation of TW treatment technologies should not rely exclusively on color removal, COD reduction, or other conventional physicochemical indicators. Whenever possible, treatment performance should also be assessed through toxicity-related endpoints, including ecotoxicological, phytotoxicity, genotoxicity, and mutagenicity evaluations. Such approaches provide a more realistic assessment of treatment effectiveness and are essential for determining whether a process achieves true detoxification rather than simple decolorization [22,23].

The distinction between decolorization and detoxification represents a fundamental challenge for the future development of TW treatment technologies. Ultimately, the success of integrated enzymatic–microbial systems should be measured not only by their ability to remove color but also by their capacity to eliminate toxic intermediates and reduce environmental risks associated with textile effluents.

9. Circular Bioeconomy Perspectives

Integrated enzymatic–microbial systems align closely with circular bioeconomy principles because they combine wastewater remediation with biomass valorization and resource recovery. Rather than treating wastewater management and biomass utilization as independent processes, these approaches establish connections between agricultural residues, enzyme production, microbial biotechnology, and environmental remediation.

Within this framework, agro-industrial residues can serve as substrates for biocatalyst production, enzyme-rich biomass can be applied to wastewater detoxification, and treated effluents may potentially support water reuse strategies. Such integration contributes to the development of more resource-efficient and environmentally sustainable industrial systems while reducing dependence on non-renewable inputs [183,184].

Consequently, the future development of TW treatment technologies is expected to increasingly emphasize integrated platforms capable of combining detoxification performance with biomass valorization and circular resource utilization, thereby strengthening the role of biotechnology within emerging circular bioeconomy models.

10. Future Perspectives and Research Gaps

10.1. Integration with Other Technologies

Although enzymes and microorganisms have demonstrated considerable potential for the degradation of textile dyes, growing evidence indicates that stand-alone biological systems are not always capable of achieving complete contaminant mineralization or eliminating toxic intermediates generated during treatment. Consequently, the integration of biological processes with complementary technologies has emerged as one of the most promising directions for advanced TW treatment. Among these strategies, the combination of Advanced Oxidation Processes (AOPs) with biological treatments has received particular attention. AOPs are highly effective in the initial breakdown of recalcitrant compounds, reducing the structural complexity of dye molecules and enhancing their biodegradability. However, numerous studies have shown that chemical oxidation does not necessarily result in complete detoxification and may generate intermediate by-products with significant ecotoxicological potential. In this context, subsequent biological treatment stages play a crucial role by removing these intermediates, promoting further mineralization, and reducing the residual toxicity of the treated effluent [97].

Another important research direction involves the integration of AOPs with membrane-based separation technologies. According to Mirza, 2020 [185], hybrid AOP–membrane systems can combine the chemical degradation of persistent contaminants with the physical retention of residual compounds, resulting in more robust treatment processes with reduced formation of undesirable by-products. Nevertheless, most studies remain at the laboratory scale, and significant challenges persist regarding reactor design, continuous operation, process integration, membrane fouling control, and overall economic feasibility.

Emerging bioelectrochemical technologies, particularly Microbial Fuel Cells (MFCs), have also attracted increasing interest as components of integrated treatment systems. Recent investigations have demonstrated that these platforms can simultaneously promote dye degradation and energy recovery, thereby improving the sustainability of wastewater treatment processes. Despite these promising results, further advances are still required to enhance operational stability, optimize electron transfer mechanisms, and ensure reliable performance under real industrial conditions [185].

More broadly, the current scientific consensus suggests that the future of TW treatment will rely less on individual technologies and more on hybrid systems capable of exploiting the complementary advantages of biological, chemical, and physicochemical processes.

However, optimizing these integrated approaches and validating their performance under realistic operating conditions remain major scientific and technological challenges.

10.2. Challenges to Commercialization

Despite the large number of studies published over the past two decades, the commercial implementation of advanced TW treatment technologies remains relatively limited. Most reported results have been obtained under controlled laboratory conditions using synthetic dye solutions that fail to fully replicate the complexity and variability of real industrial effluents. Consequently, treatment efficiencies achieved at the laboratory scale are often difficult to reproduce in pilot-scale and industrial systems [186].

The transition from laboratory research to industrial-scale application represents one of the most significant barriers to technology deployment. Zhang et al. (2021) [8] highlighted that, although numerous AOPs exhibit excellent performance in bench-scale studies, relatively few have undergone comprehensive validation in full-scale industrial facilities. Additional investigations are required to assess operational costs, energy consumption, process monitoring strategies, maintenance requirements, and long-term performance under realistic operating conditions.

Another major challenge concerns the long-term stability of treatment systems. In biological processes, fluctuations in wastewater composition, pH variations, the presence of toxic compounds, and changing operational conditions can negatively affect microbial activity and compromise degradation efficiency. Similarly, enzyme-based systems continue to face limitations associated with catalytic activity loss, enzyme denaturation, restricted operational lifetimes, and the need for frequent biocatalyst replacement. These issues remain significant obstacles to large-scale industrial implementation [186].

Beyond operational concerns, an important research gap relates to the environmental safety assessment of treated effluents. Numerous studies have demonstrated that color removal and reductions in chemical oxygen demand (COD) do not necessarily indicate effective detoxification. In some cases, partial degradation of dye molecules may generate intermediates that are more toxic than the parent compounds. For this reason, recent authors have emphasized the need for future investigations to incorporate systematic ecotoxicological assessments, detailed monitoring of degradation by-products, and long-term environmental impact analyses alongside conventional treatment performance indicators [97].

Economic viability also remains a critical factor influencing technology adoption. Although many advanced treatment systems exhibit high pollutant removal efficiencies, their implementation may be constrained by capital investment requirements, operational expenditures, energy consumption, and maintenance costs. Consequently, future research should place greater emphasis on techno-economic assessments, life cycle analysis, and cost-benefit evaluations to identify treatment configurations that are both environmentally sustainable and economically competitive.

Furthermore, advances in synthetic biology, metabolic engineering, artificial intelligence, and process automation offer new opportunities to overcome current limitations. The development of engineered microbial consortia, robust enzyme formulations, smart bioreactors, and predictive process-control systems may significantly improve treatment efficiency, stability, and scalability. Integrating these emerging technologies with existing wastewater treatment platforms could accelerate the transition from laboratory-scale proof-of-concept studies to commercially viable industrial solutions.

Overall, while biotechnological and hybrid treatment systems represent highly promising approaches for the sustainable remediation of TW, their widespread commercialization will depend on the development of processes that are more robust, economically fea-

sible, and operationally stable over extended periods. Future efforts should prioritize industrial-scale validation, comprehensive life cycle assessments, techno-economic analyses, and continuous ecotoxicological monitoring to bridge the remaining gap between laboratory achievements and practical implementation. By addressing these challenges, next-generation treatment technologies may play a pivotal role in advancing sustainable water management and supporting the transition toward a circular bioeconomy.

10.3. Artificial Intelligence and Synthetic Biology: Interdisciplinary Perspectives for Next-Generation Textile Wastewater Detoxification

The growing demand for more sustainable solutions, together with the high complexity of textile wastewater detoxification systems, highlights the need for approaches capable of integrating biological information across different levels of organization. Biological treatment processes involve complex interactions among microbial communities, metabolic pathways, and enzymatic activities, making it increasingly difficult to predict system behavior using conventional experimental strategies alone. In wastewater treatment systems, microbial ecology is influenced by factors such as the presence of uncultured microorganisms, functional redundancy, and spatiotemporal variation, which limit our understanding of the contributions of individual microbial groups to overall system performance [187]. In this context, emerging computational approaches and synthetic biology strategies have been proposed as tools for integrating large volumes of biological information and improving the prediction and control of complex biological systems [187,188].

Artificial intelligence (AI) and machine learning (ML) have emerged as important computational strategies for extracting functional information from the growing volume of available genomic and metagenomic data. Although high-throughput sequencing technologies have substantially expanded the sequence space available for enzyme discovery, the functional interpretation of these data remains challenging when based solely on conventional homology- and rule-based approaches [189]. Machine-learning models can capture complex sequence-function relationships, predict enzymatic properties, and prioritize potential biocatalysts from diverse biological sources. These models have been applied to enzyme function annotation, substrate-specificity prediction, and the estimation of properties such as kinetic parameters, solubility, thermostability, and optimal pH [189].

AI-based approaches have also advanced the analysis of microbial communities involved in wastewater treatment. Multi-omics strategies integrating metagenomics, transcriptomics, proteomics, and metabolomics can relate microbial composition to metabolic activity and system performance. However, a substantial proportion of the identified genes remain functionally unannotated, limiting the interpretation of these microbial ecosystems. Integrating multi-omics datasets with AI-based ecological models may help identify microbial groups associated with treatment-system resilience or instability Renganathan [188]. Similarly, AI and ML models have demonstrated the potential to predict microbial dynamics, treatment performance, and effluent characteristics by integrating microbial, physicochemical, and environmental data [187].

Beyond predictive performance, recent AI developments have sought to improve the interpretability of biological analyses. Song [190] proposed the Multi-Scale Chain-of-Thought Fusion (MS-CoTF) framework to overcome the limitations of single-scale biological reasoning by integrating information at the molecular, cellular, tissue, and system levels. Through bidirectional information flow and cross-scale attention mechanisms, this framework combines information from different biological hierarchies and improves the interpretability of AI-generated predictions. The model has been applied to protein-function prediction, molecular-interaction inference, and biological-mechanism analysis, producing biologically plausible reasoning chains supported by interpretable evidence [190]. Because textile wastewater detoxification encompasses processes ranging from enzyme–substrate

interactions to microbial community behavior, approaches that integrate information across biological scales offer a valuable direction for future mechanistic studies.

Synthetic biology also provides strategies for designing and optimizing microbial systems with improved functional properties. These strategies can not only be applied to individual microorganisms, but also at community level, enabling the control of inter-species interactions and the coordination of metabolic activities [191]. Synthetic microbial consortia composed of strains with complementary catabolic activities can broaden substrate utilization, reduce the accumulation of toxic metabolic intermediates, and improve pollutant-removal efficiency compared with approaches based on a single engineered strain [191]. Such consortia are particularly promising for degrading complex xenobiotic mixtures because different microorganisms can perform complementary metabolic functions. The incorporation of fungi may provide additional oxidative capabilities, as species such as *Trametes versicolor* and *Aspergillus niger* can degrade dyes and other xenobiotics through relatively nonspecific redox catalysis [188].

At the community level, synthetic biology enables the development of microbial platforms with coordinated and programmable functions. Strategies that regulate intercellular communication, including quorum-sensing-based mechanisms, have been investigated to dynamically control microbial interactions and metabolic activities [191]. These approaches aim to produce microbial systems capable of maintaining stability under changing environmental conditions, optimizing metabolic functions, and simultaneously acting on different contaminants [191]. Genome-editing technologies have further expanded the possibilities for engineering microorganisms for bioremediation. Among these tools, CRISPR–Cas9 has received particular attention because of its precision, efficiency, versatility, and applicability to introducing genetic modifications associated with desirable microbial functions [192].

The integration of AI and synthetic biology therefore represents an interdisciplinary pathway for advancing biological textile wastewater detoxification. Computational strategies can support enzyme discovery, biological-function prediction, and the analysis of complex microbial systems, whereas synthetic biology provides tools for engineering microorganisms and microbial communities with controlled metabolic properties. Synthetic biologists can develop engineered microbial consortia, computational researchers can construct predictive models of biological interactions and degradation mechanisms, and environmental engineers can integrate these approaches into wastewater treatment systems. Combining these disciplines may enable more predictive and rationally designed detoxification strategies, moving beyond exclusively empirical approaches toward data-driven solutions based on engineered biological systems [188,191].

The following take-home messages emerge from this review:

- (I) Treatment performance must be confirmed through transformation-product analysis and ecotoxicological assessment rather than inferred from color and COD removal alone.
- (II) Integrated enzymatic–microbial systems offer complementary functions. Enzymes enable rapid pollutant transformation, whereas microorganisms support the subsequent degradation of toxic intermediates and potential mineralization.
- (III) Microbial consortia should be designed around metabolic division of labor. Substrate partitioning, cross-feeding, co-metabolism, and sequential degradation can improve the treatment of complex textile wastewater matrices.
- (IV) Synthetic and computational biology can accelerate system development. Engineered microbial communities, multi-omics, and AI-assisted models can support enzyme discovery, pathway prediction, toxicity assessment, and process optimization.

- (V) Industrial translation requires evidence beyond laboratory efficiency. Future studies should prioritize real textile wastewater, continuous reactors, long-term stability, mass balances, techno-economic analysis, and life-cycle assessment.

11. Conclusions

Textile wastewater (TW) treatment remains a major environmental challenge due to the recalcitrance of synthetic dyes and the potential formation of toxic intermediates during incomplete degradation. This review demonstrates that effective remediation requires approaches that move beyond simple decolorization toward true detoxification. Enzymes and microorganisms provide complementary functions in this context: enzymatic catalysts enable rapid transformation of complex dye structures, whereas microbial systems contribute metabolic versatility for the degradation and mineralization of transformation products. Integrated enzymatic–microbial systems therefore represent a promising strategy for coupling fast pollutant conversion with long-term detoxification and process robustness. The incorporation of microbial consortia further enhances treatment performance through complementary metabolic pathways and sequential degradation cascades. In parallel, solid-state fermentation and agro-industrial residues offer sustainable routes for producing low-cost biomass-derived biocatalysts, linking wastewater remediation with biomass valorization and circular bioeconomy principles. Future progress will depend on industrial-scale validation, improved operational stability, comprehensive toxicity assessments, and techno-economic analyses. Advancing these areas will be essential for translating integrated biocatalytic detoxification systems from laboratory research to practical and sustainable industrial applications.

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Abbreviations

The following abbreviations are used in this manuscript:

AAO	Aryl alcohol oxidase
ABTS	2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)
ALRs	Airlift reactors
AOPs	Advanced Oxidation Processes
aw	Water activity
AZOR	Azoreductases
BOD	Biochemical oxygen demand
CAPEX	Capital expenditures
CED	Cumulative Energy Demand
CHP	Combined heat and power
CLEAs	Cross-linked enzyme aggregates
COD	Chemical oxygen demand
DyPs	Dye-decolorizing peroxidases
EC	Enzyme Commission (numerical classification system)
ECG	Epicatechin gallate
EGCG	Epigallocatechin gallate
EPS	Extracellular polymeric substances
EROI	Energy Return on Investment
FAD	Flavin adenine dinucleotide
FADH ₂	Flavin adenine dinucleotide (reduced form)
FBRs	Fluidized-bed reactors
FMN	Flavin mononucleotide
FNR	Global anaerobic transcription factor
HBT	1-hydroxybenzotriazole
HRT	Hydraulic Retention Time
HMF	Hydroxymethylfurfural
H ₂ O ₂	Hydrogen peroxide
H ₂ S	Hydrogen sulfide
IIoT	Industrial Internet of Things
ISPR	In situ product removal
LAC	Laccases
LAS	Linear Alkylbenzene Sulfonate
LCA	Life cycle assessment
LiP	Lignin peroxidase
LMS	Laccase–mediator systems
MFCs	Microbial Fuel Cells
MnP	Manganese peroxidase
MPC	Model Predictive Control
NADH	Nicotinamide adenine dinucleotide (reduced form)
NADPH	Nicotinamide adenine dinucleotide phosphate (reduced form)
OPEX	Operating expenditures
PAT	Process Analytical Technology
PBRs	Packed-bed reactors
pH	Potential of Hydrogen
PE	Polyethylene
PET	Polyethylene terephthalate
PID	Proportional–integral–derivative (control)
PLA	Polylactic acid
ppm	Parts per million
QbD	Quality-by-Design

ROS	Reactive oxygen species
SAB	Submerged Aerated Bio-filter
SBR	Sequencing Batch Reactor
SSF	Solid-state fermentation
T	Temperature
T1	Type 1 copper center
T2/T3	Trinuclear copper cluster
TCA	Tricarboxylic acid cycle (Krebs cycle)
TP-Lac2	Trametes polyzona laccase isoform 2
TEA	Techno-economic assessments
TTN	Total Turnover Number
TW	Textile wastewater
TYR	Tyrosinases
UAS	Up-flow Anaerobic Structured-bed reactor
UASB	Up-flow Anaerobic Sludge Blanket
VP	Versatile peroxidase ABTS: 2,2'-azinobis (3-ethylbenzthiazoline-6-sulfonate)

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