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Review

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Review

Filamentous Fungi as Bioremediation Agents of Industrial Effluents: A Systematic Review

Fernanda Maria Rosa [1], Thaís Fernandes Mendonça Mota [1], Cleverson Busso [2], Priscila Vaz de Arruda [2], Patrícia Elena Manuitt Brito [1], João Paulo Martins Miranda [1], Alex Batista Trentin [2], Robert F. H. Dekker [3] and Mário Antônio Alves da Cunha [1,4,*]

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Abstract: The industrial sector plays a crucial role in driving global economic growth by creating employment opportunities, generating local income, and significantly contributing to each country's Gross Domestic Product (GDP). However, the manufacturing industry is responsible for producing a substantial amount of polluting effluents, which can harm the environment and compromise the life quality of future generations if not appropriately treated. Various methods, including physical, chemical, and biological processes, have been employed in the treatment of industrial effluents. Filamentous fungi, in particular, have garnered attention as effective bioremediation agents due to their ability to produce enzymes capable of degrading recalcitrant compounds and adsorb different pollutant molecules. This study aimed to assess the collective body of research on using white and brown rot fungi to remove phenolic compounds from industrial effluents. The research employed a systematic review, incorporating scientometric analysis, with the primary goal of constructing a comprehensive overview of the evolution of this technology over time. The analysis also aimed to scrutinize its geographical distribution and identify gaps and trends within these research inquiries. Data for the study were obtained from the Web of Science (WoS) database using specific search terms (("white rot fung*" OR "brown rot fung*") AND (phenol* OR phenoloxidase OR "polyphenol oxidase*") AND (wastewater OR effluent* OR bioremediation)). The time frame considered ranged from 1945 to 2023, resulting in 464 publications. After refinement, 109 studies were selected for further analysis, focusing on using filamentous fungi in the bioremediation of industrial effluents. The findings revealed that white rot fungi were the most studied in industrial effluent remediation processes, accounting for 96.3% of the studies assessed, followed by brown rot fungi at 2.7%. Filamentous fungi demonstrate significant potential for treating industrial effluents due to their high capacity to degrade phenolic compounds, especially in wastewater from olive mills, paper production industries, and distilleries. Among the investigated species, *Phanerochaete chrysosporium*, *Pleurotus ostreatus*, *Trametes versicolor*, and *Lentinula edodes* have been the most extensively researched for this application. Notably, cultures with free cells (64.15%) stood out compared to cultures with immobilized cells, and cultures with isolated fungi surpassed microbial consortia. Geographically, Italy, Spain, Greece, India, and Brazil emerged as the most prominent countries in publications related to this area during the evaluated period.

Keywords: environmental detoxification; microbial bioconvertion; phenol biodegradation; scientometric analysis; systematic review

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1. Introduction

The economic progress and prosperity of a nation is primarily determined by its industrial development. Industries constitute essential elements of modern civilization, supplying vital materials to humankind and creating employment opportunities [1]. Despite the importance of the industrial sector for developing a country, it is essential to note that industries commonly generate a lot of effluent, which can cause environmental damage if discharged into receiving bodies without adequate prior treatment [2].

Industrial effluents are broadly divided into three categories, depending on the type of industry: a) waste from organic processes (e.g., dairies, chemicals, pharmaceuticals, textiles, foods, and brewing, among others); b) waste from inorganic processes (chemical and mining industry); and c) chemical waste (insecticides, acids, bases, dyes, fertilizers, pharmaceuticals, among others) [3]. Among the pollutants found in industrial effluents are dyes, suspended solids, heavy metals, hydrocarbons, sulfur compounds, chlorinated compounds, fatty acids, surfactants, and phenolic compounds [4]. Phenolic compounds are genotoxic, promote endocrine-disruptive effects, have acute toxicity, and are one of the primary environmental contaminants, especially in water [5]. They can be also released on a smaller scale under natural conditions from decomposing organic matter, but also due to anthropogenic actions by their high use in industry [6]. These compounds are essential in producing chemicals, plastics, oils, pharmaceuticals, dyes, and explosives, among other applications. Consequently, the effluents from these industries exhibit high concentrations of various phenolic compounds [7].

Phenol is the simplest compound in the phenolic family, characterized by a singular aromatic ring and an isolated phenolic hydroxyl group [8]. It presents limited solubility and adsorption to particulate matter. Consequently, the primary modes of transformation for these compounds in the environment involve biodegradation and redox reactions. However, this intricate process can often lead to the formation of compounds that are more environmentally hazardous [9,10]. Common phenol derivatives with high toxicity and low biodegradability such as chlorophenols, aminophenols, bisphenols and nitrophenols have been detected in waters [11]. The elevated concentrations of phenolic compounds and their toxic derivatives in the environment induce notable alterations in aquatic biota and microbiota, showing significant risks to human health [12].

In many countries, effluent release into the ecosystem needs to be better regulated. Many industrial effluent treatments could be more efficient, and some treatments are costly, which makes correct disposal by small and medium-sized industries difficult [3,13]. In this sense, several organizations are working to develop sustainable industrial effluent treatment technologies [3], and some techniques have been considered effective in removing pollutants from industrial effluents, such as membrane filtration, adsorption, advanced oxidation processes and bioremediation [14,15].

Bioremediation is a pollutant cleanup technique focused on biological processes to degrade, reduce, eliminate, modify, detoxify, or transform pollutants into a non-toxic or harmless state [16,17]. Biological treatment with microorganisms has become a preferred method for treating and reducing toxic organic compounds in industrial effluents [18]. Numerous microorganisms have been recognized for their potential in the removal of phenolic compounds from industrial effluents. These include well-established environmental actors, such as bacteria from the genera *Pseudomonas* and *Bacillus*, alongside filamentous fungi, such as *Aspergillus*, with particular attention to white and brown rot fungi [19–21].

White and brown rot fungi, prevalent in nature, are distinguished for their robust lignin-degrading capabilities [22]. These fungi exhibit resilience and proficiency in degrading various recalcitrant compounds, including phenolic compounds. Their significant production of extracellular enzymes such as peroxidases, laccases, and phenol oxidases enables the effective reduction of phenolic compounds into simpler, less ecotoxic molecules [23–25]. Studies have underscored the remarkable potential of white and brown rot fungi in phenol reduction, achieving the removal of up to 100% of phenolic forms in industrial effluents [26,27].

The global relevance of using adequate and efficient effluent treatments to maintain life on the planet, as well as the high degree of recalcitrance and toxicity of industrial effluents rich in phenolic

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compounds, combined with the potential of using filamentous fungi in detoxification of these effluents stimulated the present study. This study aimed to evaluate the collective body of research concerning utilizing white and brown rot fungi in removing phenolic compounds from industrial effluents. The investigation employed a systematic review incorporating scientometric analysis. The overarching goal was to delineate a comprehensive picture of the evolution of this technology over time, examine its geographical distribution, and identify gaps and trends within the scope of these research inquiries.

2. Scientific Literature Indexing Tool for Data Collection

The Web of Science (WoS) is currently the most comprehensive and reliable database of academic information. In scientometric studies, this database is recommended as a literature indexing tool for data collection [28,29]. CiteSpace is an information visualization software, widely used in scientometric reviews, which uses the WoS textual data format [30]. The data of this study were obtained from WoS, using the following words: ((“white rot fung*” OR “brown rot fung*”) AND (phenol* OR phenoloxidase OR “polyphenol oxidase*”) AND (wastewater OR effluent* OR bioremediation)). The search was carried out every year until November 2023 in the Web of Science Main Collection. 464 publications were retrieved. The studies were read to select only publications that evaluated the use of filamentous fungi in the bioremediation of industrial effluents. During reading, some information was extracted from each selected work, such as the objective of the study, the type of fungus evaluated, the type of effluent studied and the main conclusion of the study. Reviews, duplicates or no access studies were excluded. 109 studies were selected at the end of refinement (Figure 1).

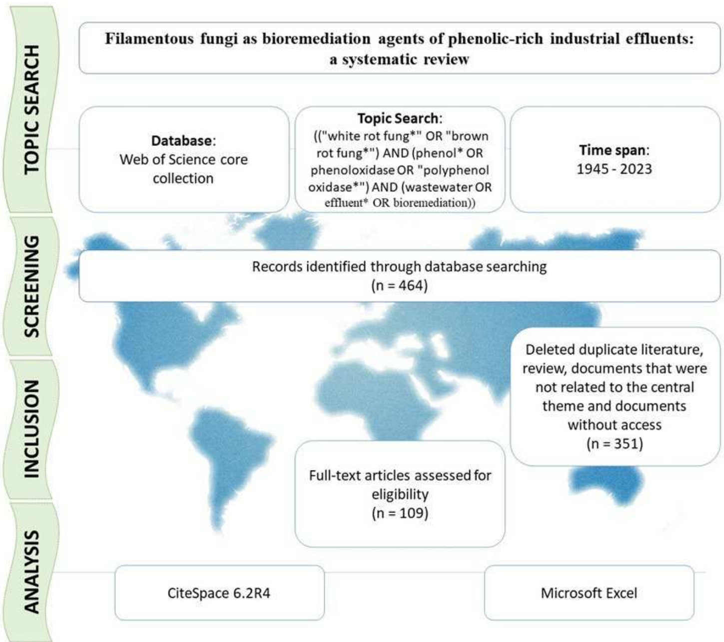

Figure 1. PRISMA flowchart with the refinement strategies for this systematic review on filamentous fungi as bioremediation agents of industrial effluents.

Microsoft Excel and Citespace software were used to analyze the data. In Microsoft Excel, the InOrdinatio index was developed; This measure was carried out considering the journal’s impact factor, the number of citations of the document and the year of publication, defined by equation 1. In the graphs generated by Citespace, the size of the node represents the frequency of occurrence. Centrality, that is, the influence of the research area, is represented by purple rings around the circles. The intensity of the lines between nodes indicates the closeness of cooperation. Red circles show items with citation bursts (Chen, 2020).

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Where:

IF = Impact factor of the journal;

α = Scale from 1 to 10, where: close to 1 favors documents with the highest number of citations; close to 10 favors more recent documents (The value chosen for α was 1, intuiting the classification of documents with the highest number of citations);

Ai = Year of research;

Ap = Year of publication;

Ci = Number of citations;

In CiteSpace Software®, research connections between countries, categories, journals, keywords and citation explosions were analyzed.

3. Publication Analysis

Over the years, the number of publications exploring filamentous fungi as bioremediation agents for industrial effluents has fluctuated, accompanied by corresponding variations in citation numbers. The dataset was initially introduced in 1989. Notably, the years 2006, 2009, and 2015 emerged as the peaks, each recording seven publications (Figure 2). This data set presented an H-index of 40, indicating the topic's relevance. The H index is a reliable and authentic parameter for academic evaluation [31].

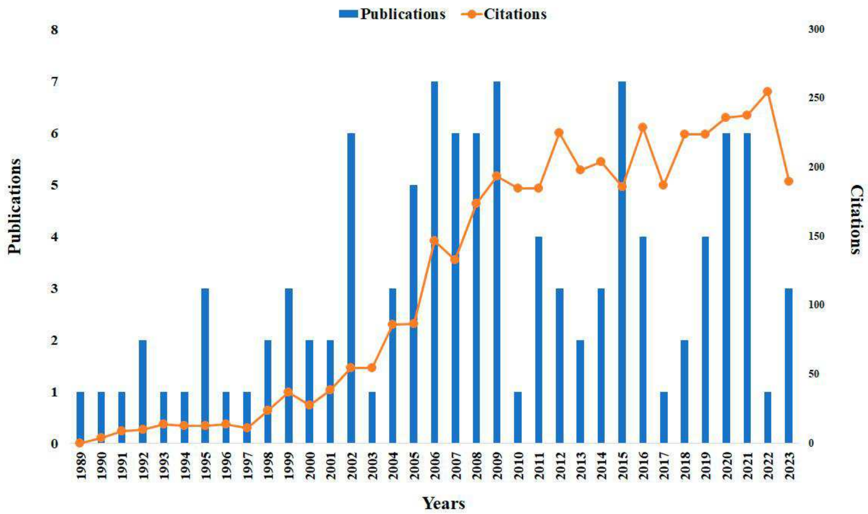

Figure 2. Number of publications and citations between 1989 and 2023 on filamentous fungi as bioremediation agents of industrial effluents.

The most relevant articles over time, based mainly on the number of citations, are presented by *in ordination*. Publications were classified up to the 10th highest value. The time interval between the most relevant publications ranged from 1995 to 2022.

The article with the highest value was "Phenolic removal in a model olive oil mill wastewater using *Pleurotus ostreatus* in bioreactor cultures and biological evaluation of the process" that demonstrated the potencial of the fungus to reduce significantly the phenolic compounds and the toxicity of the olive oil mill wastewater on a bioreactor scale [32]. In the sixth most cited article, forty-nine white-rot strains were tested for the treatment of olive oil wastewater. A good response was obtained with more than a 60% reduction in the total phenolic concentration for almost all fungi [33]. According to the results shown in the tenth most cited article, the composition of wastewater also interferes directly with bioremediation, especially during the process [34].

Fungal enzymes played a significant role, as indicated in studies such as the fourth and ninth most cited papers, showcasing the production and activity of laccase in the bioremediation process. Furthermore, these studies reported dephenolization rates exceeding 90% due to the fungi's enzymatic action [35,36]. The eighth most cited publication evaluated the action of lignin peroxidase and manganese peroxidase in decolorization by *Phanerochaete chrysosporium*. A significant level of discoloration (exceeding 70%) was observed in *P. chrysosporium* when cultivated in a medium with low Mn(II) concentration, leading to a pronounced LiP (lignin peroxidase) activity of 0.3 µM. [37].

The second most cited article worked with the bioremediation of pharmaceutical compounds using the fungi *Trametes versicolor* and *Ganoderma lucidum* and its association with advanced oxidative processes, demonstrating a possible correlation between the oxidative mediator used and bioremediation [38]. Some endocrine disruptors also were studied in the fifth most cited paper, being cited by first time the capacity of effective biological degradation of 4-cumylphenol (4-CP), in this case by the non-ligninolytic fungus *Umbelopsis isabellina* [39].

Interesting to note that in the third most cited article, certain fungi, which are less frequently mentioned in the literature, such as *Dichomitus squalens* and *Irpex flavus*, were evaluated for their potential to decolorize certain dyes. These organisms were considered to have significant potential for application in bioremediation when compared to the more commonly studied fungi [40]. The seventh study focused on the fungus *Coriolopsis byrsina*, which reported the degradation of phenanthrene both in vitro and in vivo, achieving rates exceeding 70% [41].

Table 1. Top 10 most relevant publications, according to the InOrdinatio index, considering journal impact factor, number of citations and year of publication.

Ranking	Article	Journal	IF	Citation	Year	InOrdinatio
1	Phenolic removal in a model olive oil mill wastewater using *Pleurotus ostreatus* in bioreactor cultures and biological evaluation of the process	WATER RESEARCH	18	325	2003	308,44612
2	Understanding the role of mediators in the efficiency of advanced oxidation processes using white-rot fungi	CHEMICAL ENGINEERING JOURNAL	19.4	40	2019	268,73684
3	Evaluation of some white-rot fungi for their potential to decolourise industrial dyes	BIORESOURCE TECHNOLOGY	17.4	190	2007	264,71207
4	Mycoremediation of phenols and polycyclic aromatic hydrocarbons from a biorefinery wastewater and concomitant production of lignin modifying enzymes	JOURNAL OF CLEANER PRODUCTION	15.8	41	2020	256,55263
5	Degradation and toxicity reduction of the endocrine disruptors nonylphenol,	BIORESOURCE TECHNOLOGY	17.4	65	2016	246,03947

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	4-tert-octylphenol and 4-cumylphenol by the non-ligninolytic fungus *Umbelopsis isabellina*					
6	Olive mill wastewater biodegradation potential of white-rot fungi - Mode of action of fungal culture extracts and effects of ligninolytic enzymes	BIORESOURCE TECHNOLOGY	17.4	70	2015	241,25146
7	Biodegradation and detoxification of phenanthrene in in vitro and in vivo conditions by a newly isolated ligninolytic fungus *Coriolopsis byrsina* strain APC5 and characterization of their metabolites for environmental safety	ENVIRONMENTAL SCIENCE AND POLLUTION RESEARCH	6.6	33	2022	229,68421
8	Roles of Lignin Peroxidase and Manganese Peroxidase from *Phanerochaete chrysosporium* in the Decolorization of Olive Mill Wastewaters	BIORESOURCE TECHNOLOGY	17.4	246	1995	221,98548
9	Activity and elution profile of laccase during biological decolorization and dephenolization of olive mill wastewater	WATER RESEARCH	18	126	2004	218
10	*Panus tigrinus* efficiently removes phenols, color and organic load from olive-mill wastewater	BIORESOURCE TECHNOLOGY	17.4	134	2004	216

3.1. Characterization of Publications.

Most of the selected studies aimed to assess the use of filamentous fungi in the bioremediation of industrial effluents, primarily focusing on wastewater treatment, substance biodegradation, or discoloration. All studies concluded that fungi were effective in some form of remediation of industrial effluents. The main industrial effluents evaluated are depicted in Figure 3. The most extensively studied type of effluent was wastewater from olive oil mills (39 publications), followed by effluent from the paper industry (8 publications), and wastewater from distilleries (7 publications).

The residues derived from olive oil mills, characterized by a significant concentration of organic matter that contributes significantly to the eutrophication of aquatic ecosystems, also contain substantial quantities of phenolic compounds [42]. This compositional aspect amplifies the complexity associated with the degradation and treatment of these effluents. According to the Environmental Protection Agency, concentrations of phenolic compounds as low as 0.01 mg/mL are deemed toxic in aquatic settings (US Environmental Protection Agency, 1980). Studies investigating olive oil mills' wastewater have reported concentrations as high as 10.8 mg/mL. Consequently, the need for highly efficient treatment methodologies is imperative to facilitate the proper disposal of these effluents into water courses [43,44].

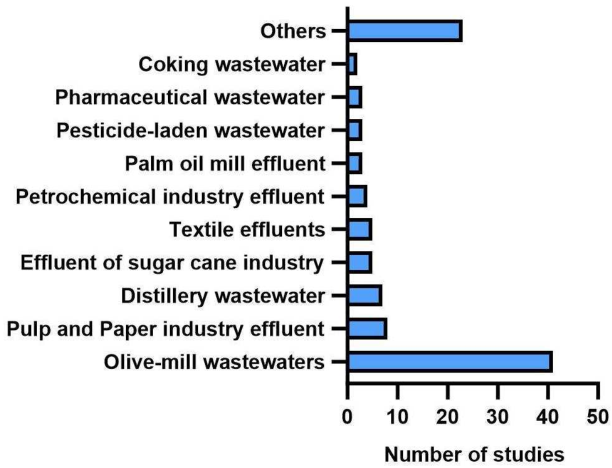

Figure 3. Number of studies per type of effluent evaluated in studies on bioremediation by filamentous fungi.

Several species of fungi were assessed in industrial effluent bioremediation efforts. In total, 94 different fungal species were studied (see Supplementary Table S1). The most frequently studied species included *Phanerochaete chrysosporium* (32 studies), *Pleurotus ostreatus* (21 studies), *Trametes versicolor* (19 studies), and *Lentinula edodes* (12 studies). Of the 93 studies that exclusively focused on fungi, 69 studies examined only one species of fungus. About 14.6% of the studies investigated fungi in combination with other substances and/or species from different groups. For instance, evaluations involved combinations such as fungi and bacteria, fungi and native soil microorganisms, fungi and aerobic consortium, and fungi and heavy metals.

The white-rot fungus *Phanerochaete chrysosporium* is considered a promising candidate for environmental remediation due to its high potential in degrading recalcitrant compounds, especially those molecularly similar to lignin. These fungi produce extracellular enzymes, such as manganese peroxidase and lignin peroxidase, capable of reducing various compounds [45–47]. Studies utilizing this fungus in the treatment of waste containing phenolic compounds have shown a rapid reduction in compound concentrations, reaching up to 90% within 12 hours of treatment [48]. Furthermore, in samples containing up to 100 mg/L of phenolic compounds, studies have observed an 80% reduction in just 5 days, utilizing an upflow fungal bioreactor featuring the activity of *Phanerochaete chrysosporium* [49].

The fungus *Pleurotus ostreatus*, extensively studied in the dataset, is another highly utilized candidate in environmental remediation, especially for waste containing phenolic compounds [50]. This is attributed to its production of polyphenol oxidase, an enzyme that catalyzes the oxidation of phenols, utilizing oxygen as a hydrogen acceptor [51]. Additionally, the fungus produces a significant amount of extracellular laccase, a multi-copper oxidoreductase facilitating the reduction

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of various compounds. Catalytic processes involving *Pleurotus ostreatus*, as observed in conducted studies, have shown remarkable efficiency in phenol reduction [52]. White rot fungi were utilized in 96.3% of the studies, while 2.7% evaluated brown rot fungi, and 0.9% assessed sour rot fungi. Among the studies, 64.15% evaluated fungus in cultivations with free cells, while the remaining work focused on the immobilized fungus. Additionally, 33.0% of the studies incorporated bioreactors in their evaluations.

3.2. Distribution of Publications by Countries

The most prominent countries in this research are depicted in Figure 4. Italy had the highest number of publications, followed by Spain, Greece, India, and Brazil. Italy and Spain also exhibited the highest centrality, signifying their influence as the leading countries in research on bioremediation conducted by fungi in industrial effluents.

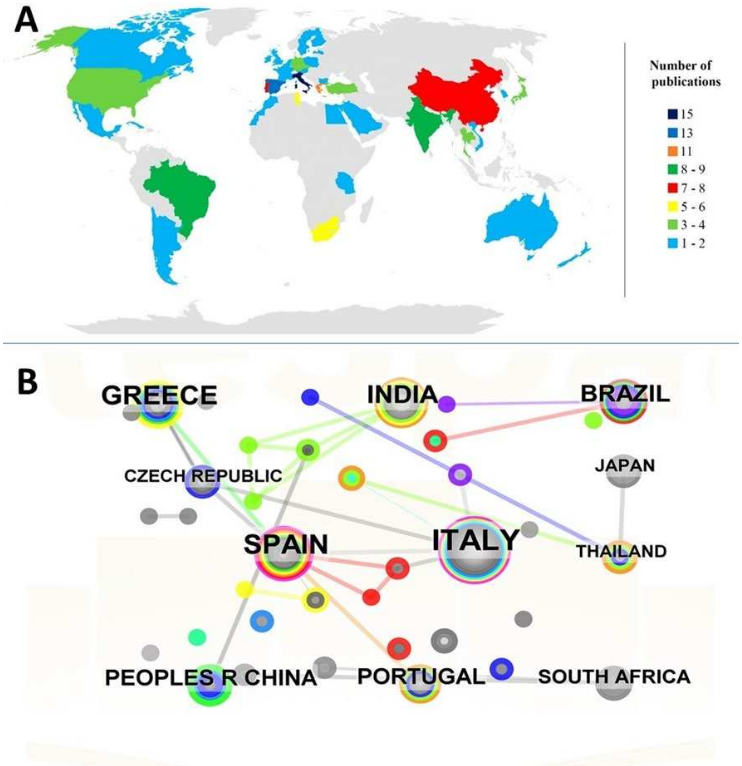

Figure 4. A) Countries with the highest number of publications. B) Collaboration network between countries that publish the most on the use of filamentous fungi for the bioremediation of industrial effluents.

European Union (EU) countries stood out in terms of the number of publications, partly due to strategies managed by the European Commission within this economic bloc. A current example of this is evident in projects supported within the EU, like the Nymphe project initiated in 2023 and coordinated by the University of Bologna (Italy). The project aims to develop bioremediation and revitalization strategies using biological agents [53]. Countries such as Greece, represented by 'CHQ Technologies IKE,' and Spain, represented by "Agencia Estatal Consejo Superior de Investigaciones Cientificas", are also involved in this project. An example of a previously completed project managed by the European Commission is DELAC, in which research focused on bioremediation through the engineering of fungal laccases using directed molecular evolution. This project was coordinated by Spain [54].

About legislation and regulatory bodies, the United States Environmental Protection Agency (USEPA) is one of the leading environmental control bodies globally, allowing a concentration limit

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of 1 μg/L in surface waters. Meanwhile, the EU states that a concentration of 0.5 mg/L of phenol for surface waters and 1 mg/L for the sew-age system is acceptable [55].

3.3. Category, Journals, and Keywords Analysis

Articles indexed in WoS belong to one or more thematic categories. The bioremediation of industrial effluents using fungi is an interdisciplinary area that spans numerous disciplinary fields. A co-occurrence analysis was conducted to identify the categories involved in this research area and explore their correlations. Figure 5 presents a collaborative relationship graph among different disciplinary categories. Research in this area is typically published in these categories. "Biotechnology &amp and Applied Microbiology' stood out as the most prominent category, with the highest number of publications (54) and centrality (0.78). The 'Environmental Sciences' category follows closely, with several publications (32) and centrality (0.45). It is expected that many publications will focus on microbiology, as fungi, bacteria, algae, and yeast are central to most biological processes in bioremediation. Additionally, biotechnology plays a key role in detoxifying and destroying environmental pollutants. The prominence of the "Environmental Sciences" category underscores the keen interest in research related to environmental issues.

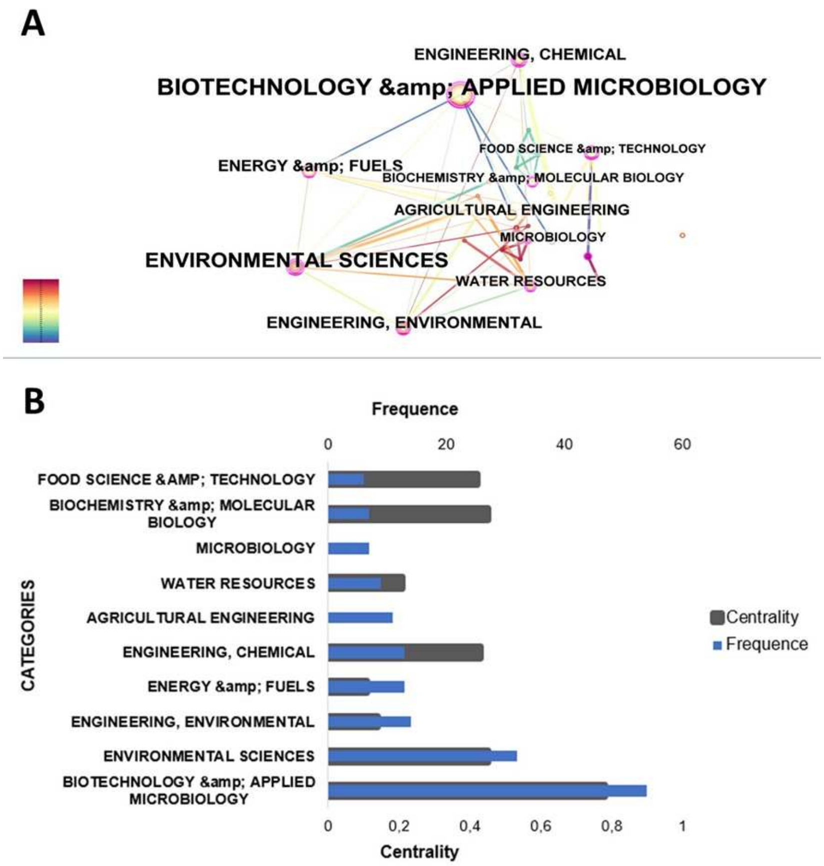

Figure 5. A) Collaboration network between the categories that publish the most on the use of filamentous fungi for the bioremediation of industrial effluents. B) Categories with greater frequency and centrality in publications.

Several journals have published studies on the bioremediation of industrial effluents using filamentous fungi. The co-citation network of top journals is illustrated in Figure 6A. The journal with the highest citation frequency was 'Applied Microbiology and Biotechnology' with 80 publications. "Applied and Environmental Microbiology" exhibited the highest centrality (0.28). The high intellectual impact is evident from the substantial number of citations [56]. The journals depicted in Figure 6B experienced bursts of citations. Notably, the journal with the most significant

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surge in citations was the "Journal of Hazardous Materials". This journal and "'Science of the Total Environment" stand out as having the most recent citation explosions.

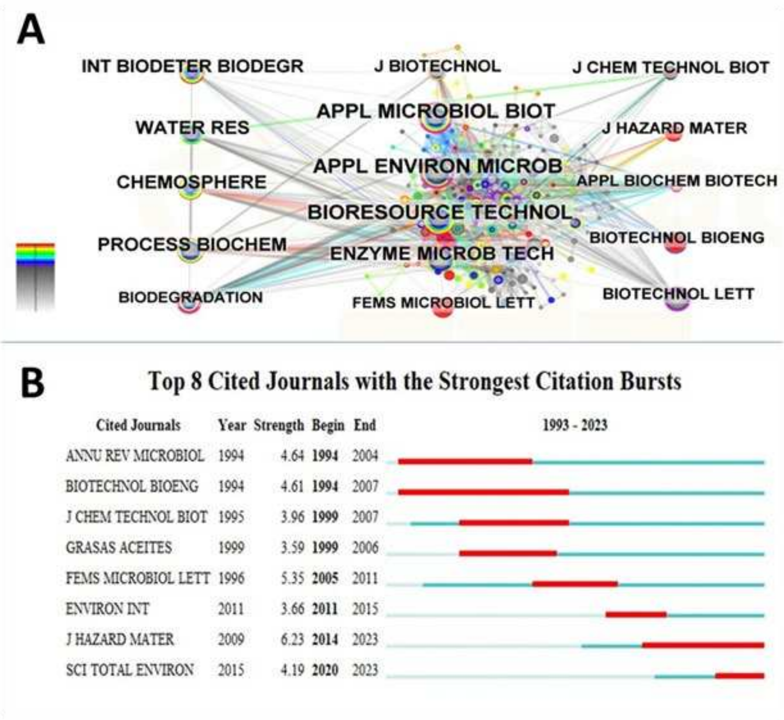

Figure 6. A) Collaboration network between journals that publish the most on the use of filamentous fungi for the bioremediation of industrial effluents. B) Top 8 journals with the most vigorous citation bursts.

The keywords in an article represent its research topic. Keyword analysis reveals the structure of a scientific field, highlighting gaps and trends in research [57]. Research on the bioremediation of industrial effluents using filamentous fungi showed that the most frequent keywords were *degradation*, *white rot fungi*, *decolorization*, and *biodegradation* (Figure 7A). *Degradation* and *decolorization* had the same centrality and were the most influential, followed by *biodegradation*. The terms "*Phanerochaete chrysosporium*" and "*removal*" *biodegradation* (Figure 7B) were the words with the most significant citation explosions, as represented by the red circle.

Bioremediation and biodegradation are corresponding processes, as both involve the conversion of pollutants by living organisms. However, bioremediation is a technology, whereas biodegradation is a natural process. In bioremediation, microorganisms are employed to degrade toxic compounds to a minimum level [58]. Therefore, the words *degradation*, *removal*, and *biodegradation* are extensively used in the evaluated studies. The keywords "*white rot fungi*" appeared in various forms in the publications (*white rot fungi*, *white-rot fungi*, and *white rot fungus*), aligning with previous findings that indicate a predominant focus on these types of fungi. Less than 4% of studies evaluated other types of rot fungi, emphasizing the need for more research efforts in this area. As previously reported, *Phanerochaete chrysosporium* was the most studied species, receiving significant prominence among the keywords.

Synthetic dyes are considered highly toxic and are widely used in textile and other industries [59]. As wastewater discoloration remains a concern due to the inefficiency of conventional treatments, there has been extensive research on the ability of filamentous fungi to decolorize synthetic dyes. Many strains have demonstrated a high capacity to decolorize various dyes. Consequently, fungi show excellent potential in addressing the discoloration of industrial effluents [60].

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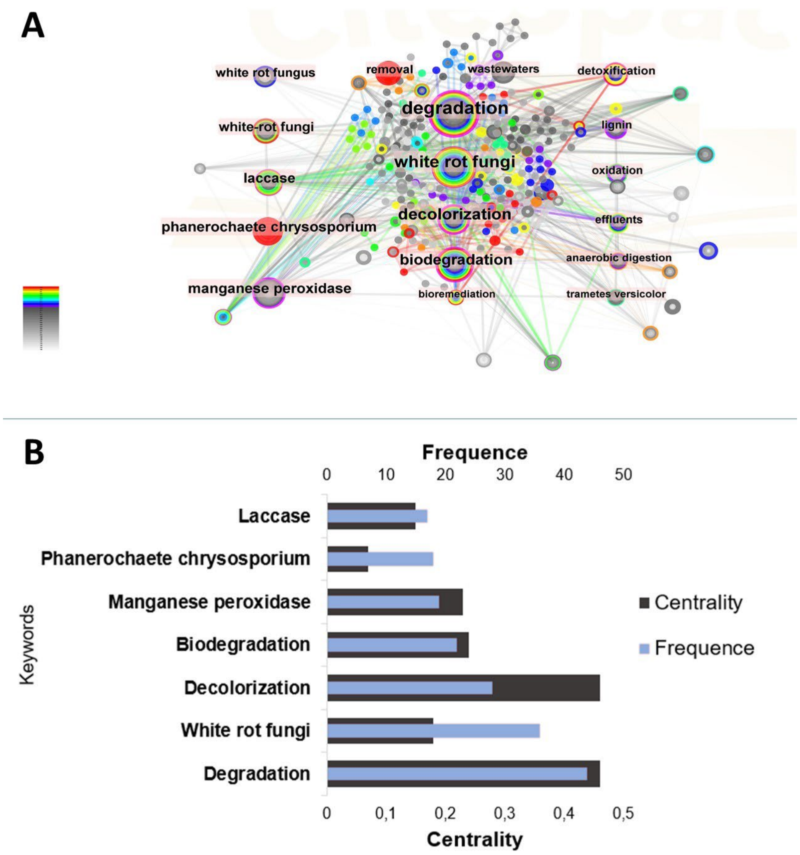

Figure 7. A) Collaboration network between keywords most used in studies on the use of filamentous fungi for the bioremediation of industrial effluents. B) Frequency and centrality of the most used keywords.

Cluster analysis conducted in Citespace reveals groupings among similar publications, highlighting the most prominent areas in the research field. The identified keywords were classified into 12 clusters (Figure 8): #0 peroxidase; #1 mushroom; #2 polyphenols; #3 phanerochaete chrysosporium; #4 bisphenol a; #5 effluent treatment; #6 phytotoxicity; #7 laccases; #8 ligninolytic enzymes; #9 textile waste water; #10 decolourisation; #11 pycnoporus cinnabarinus; #12 fungal treatment.

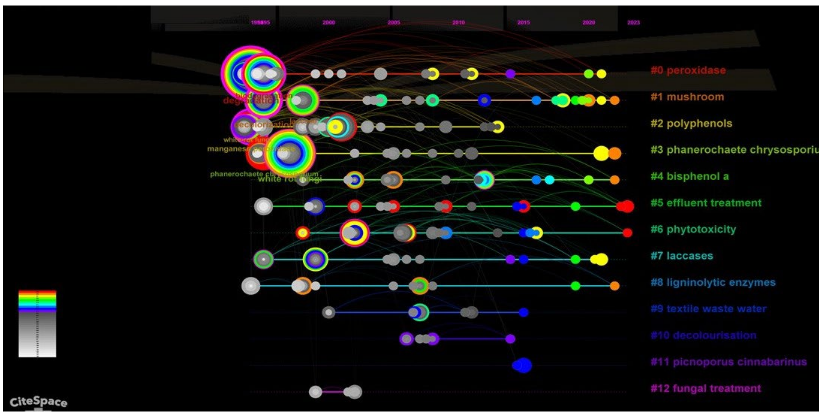

Figure 8. Timeline of keyword clusters.

The keyword cluster timeline visualization shows the research hotspots and frontiers of bioremediation of industrial effluents by filamentous fungi [61]. The most extensive area of research, indicated by the highest number of references (represented in red), was peroxidase, which has been

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a focus from the initial research phase until 2021. It is worth mentioning that white rot fungi degrade lignin through the synergistic action of peroxidase, laccase, and other auxiliary enzymes [62]. The second-largest clusters were mushrooms (1995 to 2022), followed by polyphenols (1989 to 2013). Effluent treatment and phytotoxicity represent the most recent areas of research, extending until 2023.

4. Microorganisms that Degrade Phenolic Compounds, and Bioremediation with Filamentous Fungi

Phenol and its derivatives are widespread environmental pollutants, commonly present in the effluents of various industrial processes such as oil refineries, petrochemical plants, pulp and paper industries, textile manufacturing, chemical, rubber, ceramic, and steel plants, as well as in pharmaceutical, food and beverage, metallurgical, electronic, and pesticide industries, among others. Wastewater containing phenols and other toxic compounds requires careful treatment before discharge into aquatic bodies [63,64]. One strategy to enhance the degradation of these compounds involves inoculating the environment with either a single microorganism or a combination of microorganisms recognized for their phenol-degrading capabilities [30,65]. Various microbial species commonly demonstrate notable adaptive mechanisms enabling the transformation of xenobiotics into compounds integrable into natural biogeochemical cycles [65]. The utilization of diverse microorganisms, including bacteria, fungi, yeasts, and algae, for this purpose is recognized as the bioremediation process. Consequently, the initial assessment of contaminated areas before bioremediation typically involves a thorough investigation, including the identification, quantification, and evaluation of the activity of microorganisms specialized in xenobiotic degradation [66]. Fungi also possess advantages over bacteria, as fungal hyphae can penetrate contaminated soil, accessing not only heavy metals but also xenobiotic compounds [67]. The pathway typically involves enzymatic reactions (extracellular and intracellular enzymatic processes) that modify the chemical structure of the xenobiotic, making it more amenable to microbial metabolism [68].

While enzymatic activities are the most well-known processes among microorganisms, there are several alternative strategies that can be employed. These include co-metabolism, plasmid-mediated degradation, evolution of catabolic pathways, adaptation to environmental conditions, bio-adsorption, biosurfactant production, bio-mineralization, and bio-precipitation [30,68,69].

Because industrial wastewaters have a multicomponent composition, various fungi known for their lignin-degrading capabilities, such as *Aspergillus niger*, *Cunninghamella elegans*, *Fusarium oxysporum*, *Ganoderma lucidum*, *Mucor* spp., *Penicillium chrysogenum*, *Phanerochaete chrysosporium*, *Pleurotus ostreatus*, *Rhizopus oryzae*, and *Trametes versicolor*, have been studied [68,70,71]. According to some authors, different species of *Penicillium* as *P. simplicissimum*, *P. chrysogenum*, and *P. frequentans* exhibit the capability to convert phenol and its toxic derivatives into less mutagenic products [72,73].

Fungal strains were isolated from a stainless-steel industry in Minas Gerais, Brazil. Fifteen strains, including *Fusarium sp.*, *Aspergillus sp.*, *Penicillium sp.*, and *Graphium sp.*, were selected based on their ability to degrade phenol. Among them, strains FIB4, LEA5, AE2 (*Graphium sp.*), and FE11 (*Fusarium sp.*) exhibited the highest percentage of degradation, with FIB4 achieving a remarkable 75% degradation of 10 mM phenol in 168 hours [63]. This study suggests the potential of these fungal strains in mitigating phenol pollution and protecting the environment.

Karas et al. [74] further demonstrated the potential of *Trametes versicolor* and *Pleurotus ostreatus* to degrade pesticides in agro-industrial effluents. These findings are supported by Ghosh et al. [68], who highlight the diverse cellular mechanisms and species diversity of filamentous fungi in pollutant removal. Ryan et al. [75] specifically investigated the use of *Trametes pubescens* in an airlift reactor for the bioremediation of phenolic wastewaters, successfully achieving a high rate of phenol removal. According to this study, a removal rate of 0.033 g phenol/g biomass/day was achieved, representing one of the highest reported rates for white rot fungi in the degradation of phenolic compounds from water.

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Khalil et al. [71] isolated thirty-one strains of endophytic fungi from different parts of Hibiscus sabdariffa. These strains were subsequently studied for their ability to environmentally and efficiently degrade synthetic phenol waste, specifically catechol and resorcinol at concentrations of 0.4%, 0.6%, and 0.8%. Table 2 shows the best phenolic compound degradation results with the respective fungi employed in the bioremediation study conducted in an orbital shaker at 28°C for 8 days.

Table 2. Percentage of catechol and resorcinol degradation by various filamentous fungi.

Microorganisms	Phenolic / Concentration	Degradation (%)
Aspergillus niger 13r7	Cathecol/0.6%	92.48
	Resorcinol/0.6%	97.41
Aspergillus japonicus 4r2	Cathecol/0.6%	92.24
	Resorcinol/0.8%	85.55
Alternaria chlamydospora 6l4	Cathecol/0.6%	94.58
	Resorcinol/0.6%	97.06
Cochliobolus australiensis 5l7	Cathecol/0.8%	83.45
	Resorcinol/0.8%	99.20
Emericella quadrilenata 1f7	Cathecol/0.6%	98.50
	Resorcinol/0.6%	89.74
Fusarium poae 11r7	Cathecol/0.6%	83.99
	Resorcinol/0.8%	98.92

According to the same authors [71], these six fungi were evaluated for 5 days to identify the most effective ones in reducing the percentage of phenol in samples from the paper and pulp industry. On the 2nd day, *Fusarium poae* 11r7 was considered the most effective, reducing phenol by 37.4%, followed by *Aspergillus japonicus* 4r2 (42.34%), while the lowest phenol percentage (71.82%) was observed in *Cochliobolus australiensis*. Over the subsequent days, phenol concentration gradually decreased until it was entirely absent in all six species, confirming the biodegradation of phenol.

Various groundbreaking advanced molecular methodologies, including genomics, metagenomics, proteomics, transcriptomics, and metabolomics, offer comprehensive insights into microbial activities, revealing information about their genetic makeup, proteins, mRNA expression levels, enzymes, and metabolic pathways in response to changing environmental conditions [65].

The degradation of phenol is influenced by various factors, including temperature, pH, agitation, and the physical properties of contaminants [71,76]. White-rot fungi, particularly *Trametes versicolor*, have been found to be effective in degrading phenol, with an optimal pH of 5-6 and temperature of 25°C [77]. A mixed microbial culture comprising *Candida tropicalis*, *Aspergillus fumigatus*, *Candida albicans*, *Candida haemulonis*, and *Streptomyces alboflavus* has been found to degrade phenol, with the highest degradation occurring at an initial concentration of 1000 mg/L, a temperature of 35°C, and a pH of 7.0 [78].

The white rot fungus *Phanerochaete chrysosporium* was immobilized with Italian poplar wood chips to demonstrate efficient phenolic compound degradation in coking wastewater [79]. According to these authors, the immobilization process of the fungus maintained high activity for 9 months, achieving removal rates of 87.05% for phenolic compounds and 72.09% for COD (chemical oxygen demand) within 6 days, surpassing the cultured system with free cells. Optimal biodegradation conditions were identified at pH 5.0 and 35 °C, making it a highly effective method for coking wastewater treatment.

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5. Biochemical Mechanisms Involved in Bioremediation Processes with Filamentous Fungi

Filamentous fungi exhibit numerous mechanisms associated with the bioremediation of phenolic compounds, which may be enzymatic or non-enzymatic. Among the primary non-enzymatic mechanisms, adsorption and biosurfactant production are among the best-characterized. Enzymatic mechanisms are linked to fungal metabolism, occurring either intracellularly or extracellularly [68] (Figure 9).

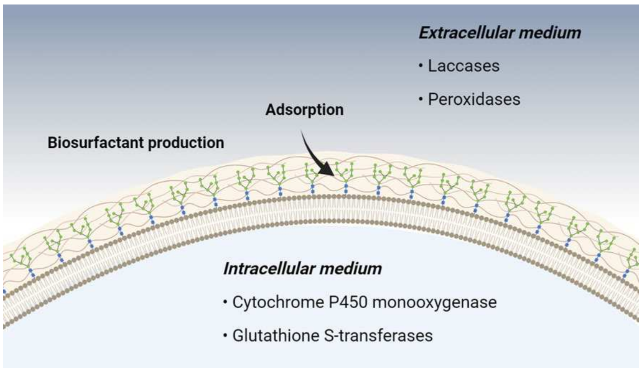

Figure 9. Main metabolic pathways used by filamentous fungi in bioremediation.

Aminopolysaccharide molecules present in the fungal cell wall act as adsorbents of toxic phenolic compounds, reducing the bioavailability of these substances in the extracellular environment [80]. Some fungi are capable of producing biosurfactants, substances that reduce the surface tension of liquids, increasing the degradation of poorly soluble and high molecular weight compounds, such as petroleum-derived hydrocarbons [81].

Currently half of the enzymes used in different industrial sectors come from fungal metabolic processes [82]. These enzymes can be of intracellular or extracellular origin and are capable of promoting biotransformation [68]. In this process, several reactions involving hydroxylation, aromatic ring fission, ether cleavage, oxidative coupling products, among others, result in the biotransformation of toxic products into smaller molecules, facilitating bioremediation [83,84].

Intracellular cytochrome P450 enzymes are presented in the form of monooxygenases containing a heme group bound to the cell membrane. These enzymes, in the presence of molecular oxygen and the cofactors NADH or NADPH, are capable of adding an oxygen atom to the substrate. In this process, sequential reactions of molecular oxygen activation, heterolytic cleavage and formation of a hydroxylated product occur [85]. The ability of cytochrome P450 monooxygenase to catalyze a wide variety of reactions, such as aromatic hydroxyl, dealkylation, epoxidation, and dehalogenation, makes this intracellular enzyme promising in the cleavage of polluting phenolic compounds [86].

Glutathione S-transferase is an intracellular enzyme located in different compartments of the cell, this enzyme is capable of catalyzing the nucleophilic attack of an electrophilic C, N or S atom in nonpolar compounds using a molecule of glutathione in the reduced form (GSH) [87]. Due to its broad substrate specificity, this enzyme has been studied as an adjuvant in the degradation of xenobiotic compounds [88].

Filamentous fungi, due to their degradative metabolism (heterotrophic), produce extracellular enzymes that are fundamental for the bioconversion of numerous complex substrates [89]. The extracellular enzymes laccases and peroxidases are considered the two most important subclasses of fungal enzymes used in the degradation of xenobiotic compounds, as well as the removal of dangerous phenolic substances of industrial origin [90].

Laccases are multi-copper oxidases with broad-spectrum action on substrates capable of degrading phenolic compounds via one-electron oxidation. This enzyme is considered environmentally friendly as it requires molecular oxygen as a co-substrate during catalysis and resulting in water as the only by-product [91]. Structurally, the enzyme can present a homodimeric,

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heterodimeric and even multimeric form with molecular weight varying between 50 and 110 kDa depending on the microorganism. Fungi produce laccases weighing around 70 to 70 kDa with an isoelectric point at pH 4.0, and redox potential of +0.79V [92–94]. The high redox power of fungal laccase enables the oxidation of various substrates; for example, phenol has a redox potential ranging between +0.5 to +1.0 V versus a standard hydrogen electrode. In some instances, its oxidation is made possible by transferring electrons to the enzyme's type 1 (T1) copper [95].

The oxidation reaction of phenolic compounds using a fungal laccase occurs through four copper atoms organized in three active sites (T1, T2 and T3). During enzymatic catalysis, the copper active site in T1 accepts electrons from the substrate, which are then transferred by a cluster composed of the active sites T2 and T3. The electrons in T2 and T3 now reduce O2, leading to the intermediate and transient formation of a peroxide molecule, which soon converts into water, a harmless byproduct of the reaction (Figure 10). The oxidized products can follow different pathways, forming new substrates or even having their toxicity reduced/annulled by the reaction [96]. In addition to phenol, laccases can degrade compounds such as polyphenols, benzenethiols, polyamines, hydroxyindole and aryldiamines [97,98].

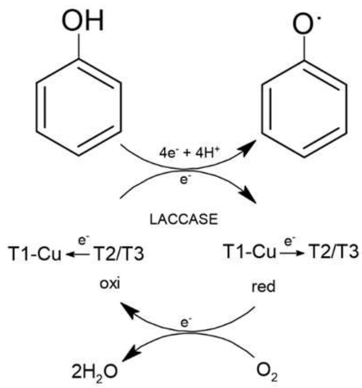

Figure 10. Main chemical reactions of laccase.

Peroxidases and oxidases are extracellular enzymes produced by fungal cells generally in response to the presence of H_2O_2 and Reactive Oxygen Species (ROS) in periods of oxidative stress. Peroxidase acts by inactivating H_2O_2 through the transfer of ions and charged radicals, which consequently affects the substrate resulting in oxidation and hydrolysis [99]. Recent studies have supported the role of glutathione peroxidase and catalase peroxidase in decomposing plastics, such as polyethylene and polyvinyl chloride, through oxidation reactions [100–102].

The main fungal peroxidases are Manganese Peroxidase (MnP), Lignin Peroxidase (LiP), Versatile Peroxidases, and Dye Decolorizing Peroxidases, with each type depending on the substrate as the reducing agent [103]. Manganese Peroxidase is capable of oxidizing aromatic amines and phenolic compounds through the oxidation of an electron and producing more reactive free radicals. This enzyme has been employed in the degradation of recalcitrant aromatic contaminants (industrial dyes), polycyclic aromatic hydrocarbons, chlorophenols and antibiotics [104].

Lignin Peroxidase is capable of cleaving β-O-4′ ether bonds and C – C bonds in lignin, enabling the depolymerization of the molecule, making it susceptible to new biological/chemical actions [105]. The enzyme can also degrade lignin oligomers including non-phenolic and phenolic compounds [84].

Versatile Peroxidase is known for its hybrid activity, combining catalytic functions of MnP and LiP. It oxidizes Mn^{2+} and non-phenolic compounds with high redox potential. Additionally, it can oxidize phenolic, non-phenolic, and lignin derivatives without the presence of manganese and without requiring a mediator for oxidation [106,107].

Finally, Dye Decolorizing Peroxidases (DyPs) are extracellular enzymes that present in their structure a heme group that catalyzes the reduction of hydrogen peroxide in water with simultaneous oxidation of several other substrates, including anthraquinone dyes and phenolic

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compounds and not phenolic [108]. Although the peroxidase family is one of the most studied, little is known about the physiological role and catalytic mechanism of DyPs. Studies have highlighted their importance mainly in the treatment of textile effluents [108,109].

6. Summary, Perspectives, and Final Considerations

The coexistence of humans and healthy ecosystems is a cornerstone of sustainable human development and a fundamental principle for a nation's economic growth. Industrial expansion contributes to human well-being through income generation and consumer goods supply. Globally, industries have increased investments in research and new technologies to address effluent treatment, driven by stringent regulations and heightened global awareness of the importance of preserving natural resources. The growth of industrial complexes is directly linked to the rising demand for food, medicines, energy, and consumer goods, resulting in an increased generation of industrial waste and effluents. This situation challenges humanity to adopt new technologies for environmental preservation and improved quality of life. Microbial strategies and technologies have been developed and utilized in the industrial sector to treat various wastes and effluents. Notably, advances in bioremediation have played a significant role in detoxifying environments and treating effluents effectively. While the use of microorganisms in detoxification processes is not new, recent years have seen substantial growth in knowledge, allowing for the selection, identification, and enhancement of the remediation potential of these microorganisms. Understanding the biochemical mechanisms involved in the remediation of toxic compounds, metabolic pathways for microbial enzyme production, and advances in molecular biology and genetics have potentiated the effectiveness and sustainability of biological treatments. Among the main microbial groups employed in this field are algae, bacteria, and filamentous fungi. Despite fluctuations in scientific studies on filamentous fungi as agents of effluent bioremediation, they remain the primary microbial group studied for treating industrial effluents. Filamentous fungi have demonstrated excellent remediation capacity for various toxic compounds, including phenolic compounds. Their efficiency is closely associated with the production of specific enzymes, such as Manganese Peroxidase (MnP), Lignin Peroxidase (LiP), Versatile Peroxidases, and Dye Decolorizing Peroxidases, particularly in the remediation of phenolic pollutants. Additionally, mycelial biomass exhibits detoxification activity through pollutant adsorption. The findings highlight the predominance of white rot fungi in industrial effluent remediation processes, comprising 96.3% of the studies reviewed, with brown rot fungi representing a smaller proportion at 2.7%. Noteworthy distinctions include the prevalence of cultures with free cells (64.15%) over immobilized cells and cultures using isolated fungi surpassing those involving microbial consortia. Various species of filamentous fungi, including *Phanerochaete chrysosporium*, *Pleurotus ostreatus*, *Trametes versicolor*, and *Lentinula edodes*, have been extensively studied. Most investigations focus on the isolated use of fungi in effluent treatment. Still, recent studies explore combinations with other microbial groups, such as fungi and bacteria, fungi and native soil microorganisms, and fungi and aerobic consortium. White rot fungi, especially *Phanerochaete chrysosporium*, stand out in effluent treatment studies. Countries that stand out in publishing studies on fungi in effluent bioremediation include Italy, Spain, Greece, India, and Brazil. European countries contribute significantly, supported by the European Union (EU) and national agencies. In contrast, emerging countries like Brazil and India show increasing contributions, potentially due to their growing industrial parks, internal environmental policies, and external product export requirements. Future perspectives in the biological treatment of industrial effluents involve addressing bottlenecks and specific demands, making the economical use of fungi in effluent treatment viable and advantageous. This includes scaling up fungal treatment systems, exploring combined use with other microbial groups, new studies using species of filamentous fungi that are rarely studied, but which have potential in the bioremediation of industrial effluents, and intensively employing recombinant DNA technology to enhance fungal potential.

Supplementary Materials: The following supporting information can be downloaded at the website of this paper posted on Preprints.org, Table S1: Species of fungi evaluated in studies on bioremediation in industrial effluents.

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