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Mycoremediation for Decolourization of Dye in Wastewater Using Fungi Consortium Culture: A Review

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ABSTRACT

Dye is extensively used in industries, such as textile, paper printing, food, and leather. Dye causes significant effects on living organisms and the environment. Current dye treatment methods are inefficient in decolourization as the dye is highly persistent. Efficiency in the decolourization of dye is a challenge for industries as well as for wastewater treatment systems. This paper focuses on the mycoremediation dye treatment method, a sustainable treatment method that leads to green technology. This study explores mycoremediation efficiency and processes for dye decolourization. The gap of study on fungal mixed culture shapes future study direction of dye decolourization. Synergistic or antagonistic effects of mixed culture towards dye decolourization should be further investigated.

Keywords: decolourization, fungi, mixed culture, mycoremediation, wastewater

INTRODUCTION

Dye contaminated water and wastewater are two major issues. World Bank has estimated that nearly 20% of all global industrial water pollution came from textile dye and food industries [1]. Meanwhile, developing countries of South Asia had contributed to 35% dye wastewater out of total generated dye-laden wastewater. Concentrations of dye used in textile processing wastewater are within the range of 10-200 mg/L. Even at a low concentration below 1 mg/L, dye is visible in water and wastewater. Dye tends to accumulate in the environment, affecting the food chain over time [2]. As the dye is designed to be chemically and photolytically stable, it exhibits highly persistent characteristics in a natural environment. Therefore, the discharge of dye contaminated water and wastewater causes ecotoxic hazards and significantly impacts human health. These include diseases such as typhoid, gastroenteritis, diarrhoea, cancer, multiple sclerosis, and Alzheimer's disease. Therefore, treatment methods for dye contaminated water and wastewater are essential even though dye presents at trace levels.

In order to safeguard the environment and human health, dye treatment methods have become the main focus of research. Currently, physico-chemical treatment methods include flocculation with Fe(II), electroflotation, electrochemical destruction, ion exchange, and Katox hybrid treatment method of activated carbon and aeration. However, these technologies are inefficient and expensive in the decolourization of dye at low concentrations and narrow ranges [3]. Activated carbons and resin are the most widely used treatment method in industries. However, these coal and petroleum resin-based materials are expensive and non-biodegradable. Therefore, studies have been conducted to search for low-cost adsorbents, including peat, bentonite, steel-plant slag, fly ash, china clay, maize cob, and wood shavings. These low-cost adsorbents have the disadvantage of low adsorption capacities making physico-chemical treatment methods inefficient [4]. Thus, alternative, economical and efficient dye treatment methods are needed.

In recent years, biological treatment methods studies focusing on *microbes* have been explored as an alternative for dye decolourization in wastewater. Some examples of biological treatment methods include bioremediation, phytoremediation, aerobic oxidation, anaerobic process, and anoxic process based on various *microbes*. Bioremediation, which used algae, bacteria, and fungi, has successfully decolourized a wide range of dyes. Research has shown that fungi decolourized a wide range of dye than bacteria and algae due to their extracellular enzyme production. The enzyme catalyzes the breaking down of complex molecules [5]. The major extracellular enzymes in dye decolourization of mycoremediation are lignin peroxidase (LiP), manganese peroxidase (MnP), and laccase (Lac) [6].

Mixed culture studies show a higher dye decolourization efficiency than individual culture [7]. Mixed culture consists of various constitutions of *microbes* such as bacteria and bacteria, bacteria and algae, and bacteria and fungi. However, dye decolourization by fungal mixed culture is dire of study. Mixed culture may exhibit synergistic or antagonistic effects depending on the types of fungi. Environmental factors such as dye concentration, pH, and temperature also are considered.

DYE TREATMENT METHODS

Several dye treatment methods have been employed to remove dyes from water and wastewaters. The selection of treatment methods for the dye-containing wastewater is vital to ensure effluent complies with the implemented environmental regulations. The selection of treatment method depends on the factors such as types of dye, the concentration of dye, and cost of handling [8]. In general, dye treatment methods are divided into three categories: chemical treatment methods, physical treatment methods, and biological treatment methods (Table 1).

Table 1: Advantages and Disadvantages of Different Dye Treatment

Process	Advantages	Disadvantages	References
Chemical methods			
Electrokinetic coagulation	Economically feasible	High sludge formation	[9]
Electrochemical destruction	Breakdown compounds are non-hazardous	High cost of electricity	[10]

H_2O_2-Fe (II) (Fenton's reagent)	Effective for both soluble and insoluble dye	Sludge formation poisonous	[11]
Ion exchange	The resin contains a high concentration of acid sites, making them an effective catalyst for the facile reaction.	Not applicable for wide range of dye High operating cost	[12]
Photochemical with H_2O_2	No sludge formation	Formation of by-products	[13]
Ozonation	Able to convert bio-refractory dye in wastewater into biodegradable species	Short half-life	[14]
Sodium Hypochlorite (NaOCl)	Imitates and accelerates azo-bond cleavage	Release of aromatic amines	[15]
Physical methods			
Activated carbon	Good removal of a wide variety of dye	Very expensive	[16]
Cucurbituril	Presence of organic substances does not interfere with the formation of complexes.	High cost	[17]
Irradiation	Simple and efficient in eliminating a wide variety of organic contaminants and disinfect harmful microorganisms	High cost and requires high maintenance	[18]
Membrane filtration,sand filtration and screen filtration.	Able to removes all types of dyes	Concentrated sludge formation	[19]

Wood chips	Good removal of organics Good sorbent for dye and colour causing polar organics	Longer contact times are needed due to their hardness Large amount needed for treatment	[20]
Biological methods			
Aerobic	Cheap and applied to a wide range of contaminants	Heavy metals in dye molecules will inactivate the microorganisms	[21]
Anaerobic	Production of biogas which is reused to provide heat and power	Longer acclimatisation phase	[22]
Enzymatic treatment	Able to remove compounds that interfere with the downstream treatment process	A tedious method during enzyme isolation and purification	[23]

Biological treatment methods are the most sustainable economic method [24] than chemical and physical treatment methods due to minimal impact on the environment and cost-effectiveness. This is due to the fact that the bioremediation process produces non-toxic end products and does not require intensive monitoring. The disadvantage of biological treatment methods is that the performance of *microbes* is affected by environmental factors. In line with the global aim to achieve a sustainable environment, concepts on minimisation of by-product generation and clean technology development are highlighted. Therefore, biological treatment methods become the focus of current studies as they lead to green technology and a sustainable environment. Moreover, biological treatment methods are suitable for industrial wastewater treatment as many stabilised modeling studies are used liquid-based, using groundwater, broth culture, and wastewater as a media [25].

Chemical treatment methods include coagulation/ flocculation, electrokinetic coagulation, oxidation, and ion exchange (Table 1). Chemical treatments effectively degrade highly concentrated dye contaminants.

Meanwhile, high-cost chemicals, narrow range of dye decolourization, and the generation of sludge are the limitations of these treatment methods. Physical treatment methods typically consist of screens filtration, sand filtration, membrane filtration, activated carbon, and irradiation. Physical methods are resistant to temperature and adverse chemical environments. However, the drawbacks of high capital cost, high clogging rate, and narrow range dye decolourization limit the applicability of physical treatment methods.

Biological treatment methods such as aerobic, anaerobic, and enzymatic treatment are shown in Table 1. Bacteria, algae, and fungi are the *microbes* that are most widely studied in the removal of dye-laden wastewater. Biological treatment methods are classified according to the microorganism or plant involved, such as bioremediation, phytoremediation, and mycoremediation. Bioremediation is the process of using bacteria to remove toxic and hazardous contaminants. Phytoremediation involves natural or genetically engineered plants to absorb, filter, and remove contaminants. Meanwhile, mycoremediation involves fungi in the degradation and conversion of complex contaminants to simple substances.

BIOREMEDIATION OF DYE

Bioremediation for dye treatment is based on the biotransformation of dye by microorganisms. Table 2 shows the types of microorganisms used in dye decolourization. In general, the microorganisms involve in dye decolourization are bacteria, algae, and fungi. Prokaryotic and unicellular bacteria decolourize dye through various mechanisms, including enzymatic and non-enzymatic processes [26]. The decolourization of azo-metal complex dye by Halobactillus sp. recorded a low decolourization percentage at 66% in 48 hours duration [27]. Abdullah [28] also reported that the sulfonated reactive red 195 dye was decolourized by Rhodopseudomonas palustri at 70% in 72 hours. Table 2 shows different types of species used, their decolourization efficiency, and various types of dyes they act on.

Table 2: Individual Cultures Used in Dye Decolourization

Microorganism	Species	Dye (type)	Decolourization	Reference
Bacteria	Cosmarium sp.	Malachite Green (Triphenylmethane dye)	92	[29]
	Rhodopseud-omonas palustris	Reactive Blue GL (Reactive dye)	11	[30]
	Kurthia sp.	Ethyl Violet (Azo dye)	8	[31]
	Trichosporan porosum	Vilmafix Yellow 4R-HE (Reactive dye)	67	[31]
Algae	Chlorella vulgaris	Orange II (Azo dye)	43	[32]
	Chlorella vulgaris	Remazol Golden Yellow RNL (Anthraquinone)	52	[33]
	Chlorella sp.	Malachite Green (Triphenylmethane dye)	33.67	[34]
	Lyngbya lagerlerimi	Methyl Red (Azo dye)	33.61	[32]
	Nostoc linckia	Orange II (Azo dye)	25.17	[32]
Fungi	Aspergillus lentulus	Acid Blue 120 (Azo dye)	99	[35]
	Fomes lividus	Amido Black 10B (Azo dye)	98.9	[36]
	Trichophyton rubrum	Remazol Blue RR (Anthraquinone dye)	86	[37]
	Trametes versicolour	Acid Orange 7 (Azo dye)	81	[38]
	Penicillium lanosum	Reactive Blue 21 (Reactive dye)	82	[39]

As a eukaryotic and photosynthetic microbe, algae have been reported in dye wastewater removal [40]. Biosorption, bioconversion, and biocoagulation processes are the mechanisms of algae dye decolourization [40]. Chlorella vulgaris and Volvox aureus have been reported to decolourize basic cationic and fuschin dyes with performance ranging from 86% to 91.20% in seven days [32]. Meanwhile, in the same study, C. vulgaris and V. aureus showed a low decolourization percentage within the range of 3.25% to 59.12% on azo dye orange II and anthraquinone dye G – Red. This study concludes that algae have low efficiency and narrow range types of dye it can decolourize. Likewise, the bacteria also have been reported to be inefficient in dye decolourization. Bacteria recorded 8-92% in dye decolourization, as shown in Table 2. This may be attributed to the secreted enzymes involved in the dye decolourization mechanism and pathway. Table 2 presents consistent results where both bacteria and algae showed low efficiency in dye decolourization for azo, reactive, and anthraquinone dye types, primarily used in the industries. Therefore, bacteria and algae are not suitable for further dye decolourization study in pilot and large-scale applications.

Decolourization by fungi also has been investigated intensively. Fungi have been reported to be the most suitable microbe for dye wastewater treatment [41]. Aspergillus flavus was used to decolourize reactive dye reactive red 198 and achieved complete decolourization within 24 hours [42]. In another study, Pleurotus ostreatus decolorized 99.32% of azo dye direct blue 14 in 18 hours [43]. Table 2 supports the findings where fungi have high efficiency in a wide range of dye decolourization. This is due to the extracellular enzymes synthesized in the cell but secreted out to the external environment.

On the other hand, intracellular enzymes are synthesized and retained in the cell. The extracellular enzymes degrade or convert dye from complex to simple substances before being ingested by fungi. Moreover, extracellular fungi enzymes are an advantage in tolerating high concentrations of contaminants [44]. Thus, fungi are the most potent agent in dye decolourization.

FUNGI AS DECOLOURIZATION AGENT

The use of white-rot fungi *Phanerochaete chrysosporium* and *Tinctoporia* sp. to decolourize the lignin-containing pulp and dye wastewater was reported as early as in 1980 [45]. In general, dye decolourization involves two classes of fungi based on their life states; living and dead states, as shown in Table 3. The living state involves the mechanisms of biodegradation and biosorption, while the dead state, the only mechanism, is biosorption.

Table 3: Individual Cultures Used in Dye Decolourization

Fungi	Dye	Decolourization (%)	Mechanism	References
Living state				
Alternaria alternate CMERI F6	Congo Red	99.9	Biosorption, Biodegradation	[41]
Aspergillus niger	Procion Red MX-5B	98	Biorsorption, Biodegradation	[46]
Umbelopsos isabellina	Reactive Black 5	99	Biosorption Biodegradation (Maganase peroxidase)	[47]
Funalia trogii	Reactive Blue 19 Reactive Blue 49 Acid Violet 43 Reactive Black 5 Reactive Orange 16 Acid Black 52	100	Biodegradation (Laccase & Manganase peroxidase)	[48]
Pleurotus ostreatus	Remazol Brilliant Blue R	100	Biosorption Biodegradation (Laccase)	[49]
Pleurotus florida	Blue CA	93	Biodegradation (Laccase)	[50]
Dead state				
Aspergillus niger	Basic Blue 9	41	Biosorption	[51]
Thuja orientalis	Acid Blue 40	48	Biosorption	[52]

Penicillium chrysogenum	Reactive Orange 16	67	Biosorption	[53]
Funalia trogii	Astrazone Blue FGRL	48	Biosorption	[54]
Rhizopus arrhizus	Gryfalan Black RL	59	Biosorption	[55]

For the living state, the major mechanism is biodegradation as fungi secrete the lignin modifying enzymes such as LiP, MnP, and Lac to degrade dye [56-58]. The relative contribution of LiP, MnP, and Lac in dye decolourization is different for each fungus. LiP from P. chrysosporium is responsible for decolourizing azo, triphenylmethane, heterocyclic, and polymeric dyes [59]. In another study by Zhang [60], MnP of unidentified white-rot fungus played a vital role in the decolourization of cotton bleaching dye. Meanwhile, T. versicolor released Lac as its major extracellular enzyme in decolourizing anthraquinone, azo and indigo dyes [61]. Table 3 also shows that different type of fungal species has a different function in dye decolourization. The biosorption mechanism also plays a vital role in dye decolourization for living state fungi. However, biosorption performance is always limited and generally less than 50% of efficiency [62]. Yesilada [63] reported that biosorption by Funalia trogii mycelium accounted for only 24% of decolourization for basic dye astrazon red FBL.

Dead state fungi only involve biosorption mechanisms in dye decolourization. Physico-chemical interactions, such as adsorption, deposition, and ion exchange, are examples of biosorption processes. Zhou and Banks [64] revealed that the adsorption of dead state Rhizopus arrhizus showed no chemical reaction between the cell wall and dye. Meanwhile, only physical adsorption was observed according to the findings from infrared spectra. Dead state Aspergillus niger decolourized Basic Blue 9 and achieved maximum decolourization at 37% in two days [51]. In another study by Gallagher [65], dead state Rhizopus oryzae decolourized 41% of Reactive Brilliant Red in four weeks. Table 3 also summarises that the decolourization of dye by fungi in the dead state was low compared to the living state.

In conclusion, living state fungi have high decolourization efficiency due to their variety of decolourization mechanisms. In contrast, low decolourization efficiency was recorded by dead state fungi as only a biosorption mechanism is involved. Thus, the living state fungi are the potential state for dye decolourization.

LIVING STATE FUNGI IN MYCOREMEDIATION FOR DYE DECOLOURIZATION

Fungi in the living state are divided into two classes; non-ligninolytic and ligninolytic fungi. Table 4 shows the comparison between ligninolytic and non-ligninolytic fungi and their dye decolourization efficiency. Non-ligninolytic fungi can degrade azo and triphenylmethane dyes. For example, Tricophyton rubrum successfully decolourized 80% of azo dye supranol turquoise GGL [38]. In another study, non-ligninolytic fungus, Fusarirum solani successfully decolourized 100% of Glycoconjugate Azo Dye [66]. Meanwhile, ligninolytic fungi were reported to completely degrade reactive, basic, and anthraquinone dyes, which are hard to be degraded [67-69]. This is because ligninolytic fungi excrete one or more extracellular enzymes of LiP, MnP, and Lac. Table 4 is consistent with the observation that ligninolytic fungi are a better decolourization agent than non-ligninolytic fungi. Thus, the study focuses on ligninolytic fungi-based mycoremediation for dye decolourization.

Table 4: Individual Cultures Used in Dye Decolourization

Fungi	Dye	Decolourization (%)	References
Ligninolytic fungi			
Aspergillus ochraceus	Reactive blue – 25	100	[67]
Aspergillus sp.	Reactive red - 120	70	[70]
Lenzites elegans WDP2	Brilliant Green, Malachite Green, Congo Red	21 - 99	[71]

Penicilium sp.	Cotton Blue, Poly R-428, Poly S-119, Reactive Red – 198, Reactive - Blue 214, and Reactive Blue – 21	80 – 100	[68-69]
Phanerochaete chrysosporium	Sulfonated azo and azotriphenylmethane	60 – 70	[72-73]
Pleurotus sp.	Crystal Violet, Reactive Blue – CA, Reactive Black – 133	92 – 100	[50, 74]
Non-ligninolytic fungi			
Pycnoporus sanguineus	Azo, Orange G and Amaranth partially and triphenylmethane, Bromophenol blue and Malachite green	30 - 60	[75]
Pyricularia oryzae	Phenolic azo	44	[76]
Saccharomyces cerevisiae	Malachite green and Methyl red	52	[77]

MIXED CULTURE STUDY IN LIVING STATE MYCOREMEDIATION FOR DYE DECOLOURIZATION

Mixed culture is the constitution of several individual *microbes*. Individual culture of algae, bacteria, and fungi is applied in industries such as dye decolourization enzyme production, antibiotic production, and pigment production. Individual culture for dye decolourization study has several advantages, such as ensures the reproducibility of data, eases in the interpretation of experimental results and leads to the elucidation of detailed mechanisms. However, there are limitations in individual culture in which the isolated individual culture is selective to only specific dye and not practical and unsuitable as wastewater treatment plant using mixed culture system [78].

In some cases, individual culture decolourizes the dye due to alteration in the chromophore group of dye compounds. Sometimes, the compounds are not completely degraded [79]. Complete decolourization of dye can be accomplished when synergistic effects occur when enzymes present in mixed cultures working together [80]. This is because a specific species of fungus secreted different enzymes that show excellent performance in biodegradation mechanisms of dye decolourization, as showed in Table 3. To overcome these limitations, the study focuses on mixed culture to increase the efficiency of dye decolourization.

Bacterial mixed culture has been reported to perform well in various mechanisms of dye decolourization. It has a higher decolourization percentage compared to individual bacteria cultures. Table 5 shows constitutions of mixed culture used in dye decolourization. Bacterial mixed culture has been reported to successfully decolourized 100% acid (methyl orange), azo (remazol navy blue GG, remazol golden yellow RNL, and remazol red RB), disperse (Disperse Navy D2GR), reactive (cibacron red C 2G, cibacron orange CG, reactive violet 5R, remazol blue B and remazol black B) and phthalocyanine (remazol turquoise blue G) dye [19, 79-80]. Thus, the decolourization performance of mixed culture is better than individual culture. Such conditions may be due to the synergistic effect of bacterial mixed culture.

Table 5. Mixed Cultures Used in Dye Decolourization

Constitution on Mixed Cultures	Dye	Decolourization (%)	Time (h)	References
Bacteria + bacteria				
Alcaligenes faecalis and *commamonas acidovorans*	Cibacron Red C 2G, Remazol Red RB, Remazol navy Blue GG, Cibacron Orange CG, Remazol Golden Yellow RNL, Disperse Navy D2GR, Remazol Blue B, Remazol Turquoise Blue G Remazol Black B	80-100	48	[20]

Bacillus sp. V1DMK, *Lysinbacillus sp.* V3DMK, *Bacillus sp.* V5DMK, *Bactillus sp*. V7DMK, *Ochrobacterium sp*. V10DMK and *Bactillus sp*. V12DMK.	Reactive Violet 5R	100	48	[81]
Bacillus vallismortis, Bacillus pumilus, Bacillus cereus, Bacillus subtilis, and *Bacillus megaterium*	Congo red, Bordeux , Ranocid Fast Blue, Blue BCC, Lavenderred, and Remazol Golden Yellow RNL	70 - 100	120	[83]
Paenibacillus polymyxa, Micrococcus luteus, and *Micrococcus sp*.	Reactive Violet 5R	100	36	[84]
Pseudomonas putida, Psuedomonas aeruginosa, pseudomonas mendocina	Remazol Black B	98	18	[85]
Pseudomonas luteola and *Escherichia coli*	Reactive Black B	90	36	[86]
Aeromonas hydrophilla CCRCV1170T, Comamonas Testosterone CCRC 17105, Acinetobacter baumannii CCRC 17105	Red RBN	98	24	[87]

Escherichia coli, Enterobacter dissolvens, Pseudomonas citronellois	Congo Red	94 – 96	24	[88]
Bacillus firmus and *Bacillus laterosporus*	Pigmented Red 208	93	24	[89]
P.fluorescence and *Acinetobacter baumannii*	Methyl Orange	100	61	[82]
Bacteria + Fungi				
Galactomyces geotrichum and Bacillus sp.	Disperse Brown 3REL	100	2	[80]

There is only one report on bacteria and fungi mixed culture study. Jadhav [90] reported that a mixture of Bacillus sp. and Galactomyces geotrichum decolourized 100% disperse dye disperses brown 3REL with an initial concentration of 50 mg/L in two hours. This is because fungi have an advantage on extracellular enzyme secretion [91]. Bacillus sp. is an excellent extracellular enzyme producer and a candidate in dye decolourization [81,83,89]. There is little study regarding fungal-based mixed culture. In solid-state fermentation technology, Daldinia concentrica and Xylaria polymorpha fungal consortium showed higher dye removal efficiency than the individual strains within five days [92]. To the authors' best knowledge, there is only one fungal mixed culture study reported where the mixed fungal culture of Dichotomomyces cejpii MRCH1-2 and Phoma tropica MRCH 1-3 successfully decolourized 97% congo red and 91% Reactive Blue [93]. This indicates that the synergistic effect of mixed fungal culture enhances the decolourization performance. In conclusion, the bacterial and fungal mixed culture is a better decolourization agent than the bacterial mixed culture. However, fungal mixed culture study in dye decolourization is still lacking, and further investigations are needed to elucidate and confirm the synergistic or antagonistic effects towards decolourization performance.

CONCLUSION

Opting for solutions to overcome the increasing amount of dye released into wastewater is vital for water sustainability. An economical, environmental-friendly method must be employed instead of the current conventional methods that are expensive and produce hazardous by-products. Fungi are the most potential microbe for dye decolourization. Decolourization by fungi in the living state is highly efficient due to complex mechanisms such as biodegradation and biosorption. In contrast, the dead state showed low dedolorisation efficiency as only the biosorption mechanism involved. Mixed culture in dye decolourization is a promising alternative method compared to the individual culture. Catabolic activities lead to complete decolourization of dye in a short period of time. Fungi-based mixed culture is the potential decolourization agent for dye decolourization. However, fungal mixed culture study is still in lack. The outcome of this review provides a clear understanding of the potential agent in the decolourization of dye by bioremediation that highlighted mycoremediation treatment and processes. It also insights gaps of study, thus leading to further dye decolourization studies.

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