

Lignocellulolytic Microbial Systems and its Importance in Dye Decolourization: A Review

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Abstract

Effluents containing dyes from different industrial sectors pose a serious threat to the environment. Different physicochemical strategies are being carried out in industry to reduce the toxicity of dye-containing waste so that dye-mixed wastewater can be further utilized in agriculture or irrigation purposes in water-scarce areas. But those techniques are economically not feasible. There is an alternative mechanism present in biological systems that are biocatalysts which is eco-friendly, low cost, and sustainable. Lignin peroxidase, Laccase, Manganese peroxidase are oxidoreductase classes of enzymes with the ligninolytic ability and are potential biocatalysts for the degradation of environmental toxicants like dyes. Besides ligninolytic enzymes, cellulase, pectinase are also powerful candidates for dye decolourization. Most interestingly these biocatalysts are found in a variety of microbial monoculture as well as in mixed microbial consortia. The consortia are able to reduce the organic load of dye-containing industrial effluent at a higher rate rather than the monoculture. This article critically reviews the efficacy of lignocellulolytic enzymes in dye decolourization by both monoculture and consortia approaches. In addition, this review discusses the genetically and metabolically engineered microbial systems that contribute to dye decolourization as well as put forward some future approaches for the enhancement of dye removal efficacy.

Keywords: Dye Decolourization, Lignocellulolytic Enzymes, Microbial Consortia, Genetic Engineering

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INTRODUCTION

Globally, large-scale synthetic polymers are manufactured from fossil resources. However, our fossil resources are depleting, and their usage has fatal environmental consequences, necessitating a continuous search for alternate and viable sources that can replace fossil fuels while still delivering useful end goods.¹ Lignocellulosic biomass is an alternative and is derived from agricultural crops and forest leftovers, solid municipal wastes, as well as paper-mill sludge, bioenergy crops, animal manures, and forest products. Dyes are coloured chemicals that are commonly used in the printing, textile, rubber, plastics, cosmetics, and leather sectors. In our daily lives, natural and synthetic dyes play an important role. Natural dyes like haematein and hematoxylin are derived from logwood that can be toxic if inhaled, swallowed, or absorbed via the skin. When breathed, bloodroot, the source of another natural dye, can cause irritation and inflammation.² Heavy metals with colours and cancer-causing synthetic dyes have a high impact and are unacceptably harmful. Synthetic dyes can cause an allergic reaction, respiratory trouble, and skin sensitization in industry workers.³ In humans, synthetic cationic dyes can cause hypertension, shock, vomiting, cyanosis, jaundice, quadriplegia, Heinz body development, and tissue necrosis. Therefore, decolourization of the dye is important for lowering its organic load. There are several dye decolourization processes. Among them, the lignocellulolytic enzyme is one of the best sustainable routes. These enzymes are obtained from microorganisms distributed in both prokaryotic and eukaryotic domains including bacteria, fungi, and actinomycetes. Biological pretreatment has various advantages, including minimal energy consumption, the absence of hazardous chemicals, and reduced pollution. Lignocellulolytic enzymes can be characterized as a large group of extracellular proteins, which include hydrolytic activity such as laccase, lignin peroxidase, hemicellulases, cellulases, pectinase, amylase, chitinases, proteases, esterases, mannanases, which are capable of digesting rigid lignocellulose in plant biomass. Lignin and polysaccharides such as cellulose, hemicellulose, pectin, ash, minerals, and salts make up lignocellulosic biomass. Lignin

is an aromatic polymer, unlike cellulose and hemicellulose, which are both carbohydrates. Lignocellulose is a valuable source of renewable carbon that has been largely underutilized. Pretreatment of recalcitrant lignocellulosic biomass for biofuel generation, use in the paper, textile, and food industries, wastewater treatment, bioremediation, organic synthesis, and the cosmetic and pharmaceutical sectors are all examples of lignin-degrading enzyme applications. The peroxidases like lignin peroxidase (LiP), manganese peroxidase (MnP), versatile peroxidase (VP), and dye-decolourizing peroxidase (DyP) are all known for the breakdown of lignin.⁴ DyP enzymes are known originally to oxidize anthraquinone dyes with strong redox potential. DyP has a vast substrate affinity that can act at lower pH levels. Laccases are the second most common type of lignin-degrading enzyme. Lignin-degrading enzymes are already being used in industries including paper and textiles, as well as for wastewater treatment and herbicide breakdown.⁵ In the delignification and bio-bleaching of wood pulp, LiP, MnP, VP, and laccase work to replace chlorine-based delignification. They can also be used to decolourize dye wastewater from the textile industry, as well as decolourize effluent and treat effluent in distilleries and waste treatment facilities. To this end, the aim of this review is to take a look at the role of different types of lignocellulolytic microbial systems used in dye decolourization along with the critical analysis of their efficiencies (Figure) with multiple future prospective mechanisms that can become a perfect workhorse for future research aspirants.

Critical Analysis Of Dye Decolourizing Lignocellulolytic Enzyme Producing Microbial Systems & Its Significance

Here we are going to review some lignocellulolytic enzymes that have dye decolourizing properties.

Laccase

Laccase (1,4-benzenediol) oxidizes several aromatic substances leading to the simultaneous reduction of molecular oxygen to water.⁶ Laccase can also be classified in respect of three different kinds of copper prosthetic groups. Though phenolic and its relevant compounds act as the main

substrate of this enzyme, it is possible to extend the range of substrate by adding specific substances known as mediators. A mediator is a substance that causes the extension of the substrate range of any enzyme.⁷ Laccases have been reported to be produced by several gram-positive and gram-negative bacteria, fungi, and actinomycetes. Both Bacterial and fungal laccase require the mediator system. There are several mediators like ABTS(2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)), HOBt(Hydroxybenzotriazole), and syringaldehyde which lead to the enhancement of redox potential of prokaryotic laccases.⁸ Nowadays, laccase has been the center of attraction in research fields because of its wide range of applications such as textile dyes bleaching, detoxification of effluent and pollutants, bioremediation of phenolic compounds.⁹ Dye effluents cause harmful impacts on the soil and groundwater levels. Laccase can play an important role as a biological tool for several applications such as decolourization of dye effluents of industrial textiles.⁷ At first, the prokaryotic laccase was discovered in the soil bacterium named *Azospirillum lipoferum*. The melanogenic bacteria *Marinomonas mediterranea* also has the potential to produce heterologously expressed laccase. *CotA* gene product, one of the best laccases, is part of the spore coat of *Bacillus subtilis*.¹⁰ Low molecular weight extracellular fungal laccases are produced from *Volvariella volvacea*¹¹ and *Marasmius quercophilus*. Extracellular laccases are also found in the bacteria *B. safensis*, *B. licheniformis*,¹² *B. atrophaeus*, *B. pumilis*,¹³ *B. subtilis*, and *B. tequilensis*. The molecular weight of the laccases from these bacteria ranges from 58kDa to 63kDa. The laccases extracted from fungi exhibit activity in acidic pH. Laccase produced by *B. halodurans* is considered as alkaline bacterial laccase because it is able to show activity in pH optima 7.5-8.0 in presence of substrate i.e., syringaldazine. Some of the bacterial laccases have the potential to demonstrate activity and stability in a wider pH range i.e., 6.0- 9.0 approximately. In comparison to fungal laccase, bacterial laccases can be applied to numerous industrial fields like bio-bleaching and processing of dyestuffs because of their activity over a wider range of pH. Marine *Alteromonas* sp. possesses the ability to produce laccase. Synthetic dyes that are released from textile industries pose a huge

environmental threat to the human race and the environment due to their carcinogenic properties. Microbial decolourization methods render a less costly and eco-friendly alternative to the current physicochemical process such as adsorption, chemical transformation. Decolourization with the help of laccase is performed on numerous types of dyes that include indigoid, azo, triarylmethane, and anthraquinone dyes. As the current methods are unable to degrade dye mixture while treating wastewater mixed with azo dye, the utilization of a laccase mediated system represents a feasible solution to this alarming issue. The production of laccase can be influenced by the presence of several dye-containing media.⁷ The first case of degrading dyes has been reported by the laccase from *Gamma-proteobacterium* JB. The laccase extracted from this microorganism is alkali tolerant and possesses the capability to cause degradation of the dye named indigo carmine at 55°C in pH 9.0. The increase in the rate of breakdown has been found in the presence of syringaldehyde, vanillin, and p-hydroxybenzoic acid. The fungal laccase purified from *Trametes hirsuta* (pH 0.5) and *Sclerotium rolfsii* (pH 5.5) had shown results on this dye. *Ganoderma lucidum* has the potential to cause the production of laccase that contributes to the decolourization of acid orange dye. *Streptomyces ipomoeae* causes the production of laccase SilA that remains active in neutral to alkaline pH in textile wastewaters.¹⁴ It has been checked that the azo dyes are generally degraded by *Streptomyces* species. But *S. psammoticus* demonstrates very limited activity towards decolourization. Double mutant K316N/D500G of the *Bacillus licheniformis* CotA reported in the research by Koschorreck et al. in 2009 exhibits a significant role in the decolourization of dyes such as Remazol Brilliant Blue R (RBBR), Alizarin red S, and indigo carmine (indigo dye).⁷ Extracted laccase from *Streptomyces coelicolor* also contains the ability to decolourize the indigo dye in presence of syringaldehyde that acts as a redox mediator.¹⁵

Lignin Peroxidase

Lignin is basically a complex aromatic polymer and the most important renewable carbon source on Earth just after cellulose, which consists of about 30% non-fossil organic carbon. Around 60% of lignin is wasted by

Table 1. Dye decolorization by lignin peroxidase producing microbes

Microbial System	Origin	Decolourizing Dye	Critical analysis of the Methods and Results	Ref.
<i>Kocuria rosea</i>	Gram-positive bacteria	Azo dye, methyl orange	Purified Lip decolorizes all dyes under long incubational conditions, but the heat-denatured enzyme did not decolorize tested dyes.	[17]
<i>Phanerochaete chrysosporium</i>	Fungus	Synthetic dyes	The aga2 gene of <i>P. chrysosporium</i> has been inserted in the pCTCON2 and generated mutagenesis libraries, which decolorize diverse Azo dyes.	[18]
<i>Nostoc muscorum</i> ,	Blue-green algae	Naphthol Green B	LIP reflects improved stability in partial purified enzymatic extraction (PPE) and degrades Malachite green, Naphthol green B, Brazilian wood, and orange G dyes.	[16, 19]
<i>C. vulgaris</i>	Fungus	Azo dye	High efficiency of decolorization of the dye by <i>Basidiomycetes</i> sp.	[20]
<i>Basidiomycetes</i> sp.	Fungus	Reactive violet	Two Lip isoenzymes that have been purified here are similar to Lip isoenzymes from <i>P. chrysosporium</i> with respect to their catalytic properties and molecular mass. However, the <i>B. adusta</i> Lip isoenzymes are highly acidic than other fungi.	[21]
<i>Genoderma</i> sp., <i>Phanerochaete</i>	Fungi	Direct Violet, Reactive Black 5, Ponceau Xylidin, Diazo dyes, Malachite green	Azo dye degradation by different ligninolytic enzymes.	[22]
<i>Alcaligenes aquatilis</i>	Gram-negative bacteria	Malachite green	<i>Alcaligenes aquatilis</i> DB8 has been selected and identified by 16s rRNA, it has the capacity to decolorize different dyes including malachite green.	[23]
<i>Funaliatrogii</i>	Fungi	Methylene blue	LIP has decolorized Methylene blue	[24]
<i>Phanerochaete chrysosporium</i>	White rot fungi	Crystal violet, Methylene blue	Crystal violet and Methylene blue degradation occur by Lip.	[25]
<i>Phanerochaete</i> sp.	Crust fungi	Melanin	Lignin peroxidase enzyme effectively decolorizes melanin.	[26]
<i>Daedaleopsis confragosa</i>	Fungi	Navy blue HER, Orange HE2R	<i>D. confragosa</i> found to be new fungal isolates that have a role in dye decolorization. It has ligninolytic enzyme-like Lip suggests the possibility of commercialization of the production process.	[27]
<i>Phanerochaete chrysosporium</i> , <i>N. fasciculare</i> , <i>F. rosea</i>	White rot fungi	Congo red, Malachite green.	Lignocellulosic waste contains different ligninolytic enzymes including Lip which plays a vital role in dye decolorization.	[28]
<i>G. lucidum</i> , <i>F. fomentarius</i>	Fungi	Congo Red, malachite green, and Methylene blue.	Production of ligninolytic enzymes and breakdown of three aromatic dyes by using different white-rot fungi.	[29]
<i>Pleurotus ostreatus</i> PLO9, <i>Ganoderma lucidum</i> GRM117	Fungi	RBBR dye	LIP reflects improved stability in partial purified enzymatic extraction (PPE) and breaks RBBR dyes efficiently.	[30]

combustion because of the lack of methods to convert it into valuable end products. For the competitiveness of biofuel vs fossil gasoline, lignin degradation represents the major hindrance. Enzymes like lignin peroxidase (LiP) play an important role in dye – decolourization. Plant cell walls have lignocellulosic biomass as a structural component that mainly comprises cellulose, hemicelluloses, and lignin. In the case of plant lignin-carbohydrate complexes formed with cellulose and hemicelluloses. Wood-decaying fungi, typically classified as white rot-fungi have their extracellular ligninolytic enzymes, basically secreted from fungal class II peroxidase, such as lignin peroxidase (LiP). Lignin has been marked as a complex aromatic structure that is harder to attack and has been considered by most biorefineries to be a low-cost product. Lignin cross-linking is obtained through the oxidative coupling of the various small units such as sinapyl, Coniferyl, and p-coumaryl alcohol. Given the composition of these compounds, lignin is an excellent source of potentially fine chemicals and their evolution through a manufacturing process. Currently, ligninolytic enzymes have many applications like the removal of dye from industrial and bioleaching effluents and the treatment of wastewater. Both phenolic and non-phenolic compounds can be oxidized by lignin peroxidase that can cleave aryl C α bond, C α -C β bond, phenolic oxidation by aromatic ring opening, and finally demethylation.¹⁶ Lignin is a major contaminant in the textile, dye industry and is responsible for its intense unwanted dark-brown colour. Due to recalcitrant composition and corrosion resistance property, the non-hydrolysable bonds of lignin and some non-lignin dyes are resistant to degradation. The potential use of lignin-degrading bacteria and lignin peroxidase has become interesting because they can provide environment-friendly methods for dye-containing wastewater treatment of various industries. Here we will focus on some bacteria, fungi, or other microbes that can produce the lignin peroxidase enzyme and has dye decolourizing efficiency (Table 1).

Manganese Peroxidase

Various unmodified microbial MnPs helps to breakdown approximately 60-99% dye by its decolourizing mechanism. Here are some examples

that come from a different study. Microbial consortium SR can decolourise three different colours like Crystal Violet (63%) Cresol Red(93%), CBB G250(96%) within 6days with a minimum of 20 mg/L, 100 mg/L, 100 mg/L initial concentration of Dyes respectively.³¹ *Trametes pubescens* strain i8 can decolourise Acid Blue 158(95%), Poly R-478 (88%), Remazol Brilliant Violet 5R(76%), Direct Red 5B (66%), Indigo Carmine (64%), Methyl Green (50%), Cibacet Brilliant Blue BG (46%), Remazol Brilliant Blue (48%) with only 50 μ M initial concentration in 24hours.³² *Aspergillus terreus* GS28 decolourise Direct Blue-1(98.4%) with 100 mg/L concentration within 168 hours.³³ In the case of *Bjerkandera adusta* strain CX-9, Acid Blue 158(91%), Poly R-478(80%), Cibacet Brilliant Blue BG (77%), Remazol Brilliant Violet 5R (70%) at 50 μ M initial concentration has been decolourised within 12 hours.³⁴ *Trametes* sp. 48424 can decolourise 100 mg/L of Indigo Carmine(94.6%), Remazol Brilliant Blue R (85%), Remazol Brilliant Violet 5R (88.4%), Methyl Green (93.1%) within 18 hours.³⁵ Microbial consortium ZSY can degrade the colour of the dye Metanil Yellow G(93.39%) at a minimum concentration of 100 mg/L within 48 hours.³⁶ Microbial Consortium ZW1 decolourise the dye Methanil Yellow G(93.3%) with 100 mg/L in 16 hours³⁷. *Trichoderma harzianum* can break the colour of Blue-Black B(92.34%) at 0.03% initial concentration in 14 days.³⁸ *Phanerochaete chrysosporium* CDBB 686 can decolourise with a 50-ppm concentration of Congo Red(41.84%), Poly R-478 (56.86%), Methyl Green (69.79%) within 36 hours³⁹. *Bjerkandera adusta* CCBAS 930 can do the breakdown with a 0.01% concentration of Alizarin Blue Black B(86.5%), Acid Blue 129 (89.22%) by 20 days.⁴⁰ white-rot fungus *Cerrena unicolor* BBP6 helps to break down six colours with 100 mg/L concentration. These are Congo Red (53.9% in 12 hours), Methyl Orange (77.6% in 12hours), Remazol Brilliant Blue R (81% in 5 hours), Bromophenol Blue (62.2% in 12 hours), Crystal Violet (80.9% in 12 hours), Azure Blue (63.1% within 24 hours).⁴¹ *Phanerochaete chrysosporium* breakdown 90.18% of Indigo Carmine with 30 mg/L initial concentration in 6 hrs.⁴² *Ceriporia lacerata* ZISY decolourises 90% of Congo red with 100 mg/L concentration within 48 hrs.⁴³ *Bacillus cohnii* RKS9 helps to break down 99% of Congo red with 100 mg/L concentration in 12 hours.⁴⁴ *Schizophyllum*

commune IBL-06 decolourise 100% of Solar Brilliant Red 80 with 0.01% concentration within 3 days.⁴⁵ *Irpex lacteus* CD2 decolourise different dye separately, Remazol Brilliant Violet 5R(92.8% in 5 hours), Remazol Brilliant Blue R (87.1% in 5 hours), Indigo Carmine (91.5% in 5 hours) Direct Red 5B (82.4% in 36 hours) with 50mg/L initial concentration.⁴⁶ On the other hand, *Trametes versicolor* can decolourise dye mixture 80.45% of Brilliant Blue FCF and Allura Red AC with an initial concentration of 100 mg/L within 14 days. *Irpex lacteus* can do 86.04% of dye decolourization in 19 days of this same dye mixture and *Bjerkandera adusta* do 82.83% dye degradation within 9 days.⁴⁷

Cellulase

Cellulases are the complex group of enzymes that are secreted by a range of microorganisms which includes fungi, bacteria, and actinomycetes. In a natural environment, the interactions among the cellulolytic microorganisms result in the breakdown of lignocellulosic waste polymeric materials.⁴⁸ Cellulase catalyzes the decomposition of cellulose by cleaving beta-1,4-glycosidic bonds. Complete hydrolysis of cellulose is mediated by the three enzymes. They are endoglucanase, exoglucanase, beta-glucosidase. The exoglucanase attacks the reducing end and non-reducing end of cellulose chains and produces

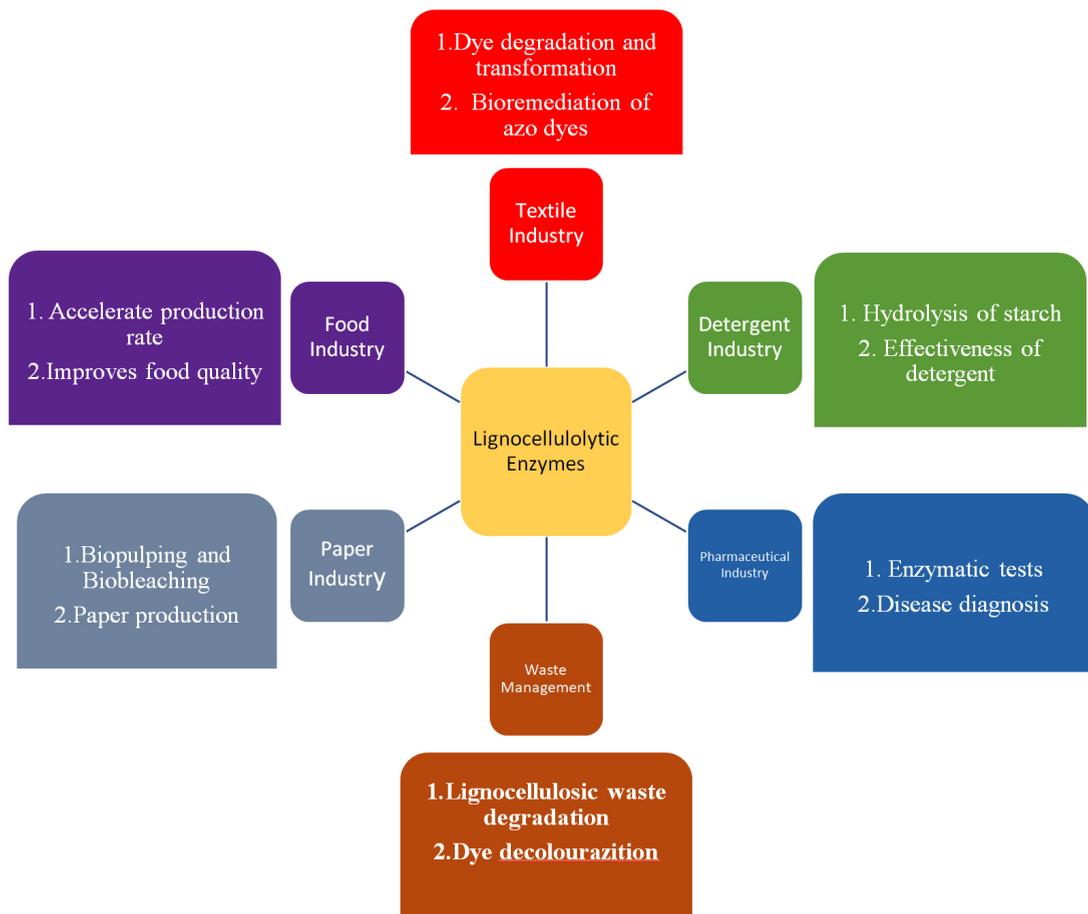


Figure. Multiple functions of lignocellulolytic enzymes
Here, the use and application of lignocellulolytic enzymes in different industries have been illustrated.

Table 2. Dye decolourization by cellulase enzyme

Dyes	The optimum temp. for dye decolourization	Time taken for dye decolourization	Optimum pH for dye decolourization	Cellulases Producing Bacteria/ Fungi	Ref.
Congo red	26°C	2 – 5 days	pH 4.5	<i>Trichoderma reesei</i>	[54]
Malachite green	40°C	1200 min	pH 11	<i>Bacillus cereus</i>	[53]
Grams Iodine	28°C	30 – 40 min	pH 7.0	<i>Bacillus sp.</i> , <i>Pseudomonas sp.</i>	[55]
Drimarene dye	28°C	25 min	pH 2.0	<i>Trichoderma reesei</i> , <i>Streptomyces</i> and <i>Thermobifida</i>	[56]

glucose and cellobiose. The endoglucanase works against crystalline cellulose substrates such as cello-oligosaccharides, and beta-glucosidase hydrolyses cellobiose to glucose from the non-reducing ends.⁴⁹ In fungi, the fungal Cellulases are secreted by *Trichoderma reesei*. In the case of actinomycetes, the genera produce cellulases are *Streptomyces* and *Thermobifida*, and for bacteria, it is *Pseudomonas* and *Sphinomonas* that produce cellulases. These are some important sources of enzymes that are used for textile dyes remediation. In the textile industry, cellulases are used as dye decolourization enzymes. Currently, in the textile industry cellulases are best applicable in the bio-stoning and biopolishing process.⁵⁰ Microbial cellulases are an alternative to the traditional method of bio-stoning. Cellulases act on cotton of denim fabric. The indigo dye is used for the colouration of the denim fabric. The dye is trapped inside the cellulose fibre in the cotton material. The dye is mostly attached to the surface of the yarn and most exterior of short cotton fibres. Cellulases hydrolyze and breaks the small fibres of fabric by breaking the beta-1,4- linkages of the cellulose. This hydrolysis process removes the fibres which trap indigo dye. The dye is easily removed from the fabric after this process.^{48,51} *Trichoderma reesei* endoglucanase II is the best suitable candidate for bio-stoning. Cellulases have several advantages and disadvantages over the conventional approach which is a stonewash with a pumice stone. The advantages of using cellulases are: it gives high productivity, less work intensiveness, are safer for the environment, it takes short time than the conventional method. There are several disadvantages also. The major disadvantage of cellulase is back staining which is

the redeposition of dye on the fabric and losses of the shade look given by the treatment.⁵¹ The latest trend of bio stone washing is using an enzyme mixture composed of amylase, cellulases, lactase.⁵² Cellulases also play a critical role in biopolishing where they remove excess stain from the denim fabric. Apart from indigo dye, cellulases are involved in various dye decolourizations (Table 2). Dye like methylene blue, malachite green, Congo red, methyl orange dye, grams iodine dye is decolourized by Cellulase enzymes under optimum conditions like temperature, pH, and time.⁵³

Pectinase

Pectinase enzymes act on pectin by cleaving the glycosidic bond of galacturonic acid.⁵⁷ Pectins are the chain molecules with a rhamnogalacturonan backbone associated with other polymers and carbohydrates.⁵⁸ Pectins are heteropolysaccharide structures made up of alpha (1,4) linked D-galacturonic acid residues.⁵⁹ Pectinase enzymes depolymerize pectin through hydrolysis, trans-elimination, and de-esterification mechanisms. These reactions hydrolyze the ester bond of pectin.⁵⁸ Pectinase enzymes are classified according to their mode of action like 1: Methylsterases, remove methoxy groups from esterified galacturonan. 2: Polygalacturonases, that is subdivided into endopolygalacturonase (catalyzes the hydrolysis of the glycosidic bond randomly), and exopolygalacturonase, which releases galacturonic acid residues from the non-reducing ends of homogalacturonan.⁵⁹ Pectinase has an important role in the food industry and is commercially used for juice extraction, wine clarification, and decolourization

Table 3. Dye decolorization by Microbial Consortia

Dye	Microbial Source	Microbes Name	Consortia	Decolorizing (%)	Time	Enzyme	Ref.
Acid Red 88	Local textile industry effluent in Punjab, India	<i>Bacillus cereus</i> (BN-7), <i>Pseudomonas fluorescense</i> (BN-5), <i>Stenotrophomonas acidaminiphila</i> (BN-3)	Bacterial consortia	78-94%	24 hrs	Azo-reductase	[69]
Mono azo, DI-azo, tri-azo	Textile effluent	<i>Klebsiella</i> sp.1, <i>Klebsiella</i> sp.2, <i>Staphylococcus aureus</i> , <i>Bacillus cereus</i> , <i>Pseudomonas fluorescense</i>	Bacterial consortia	Yellow dye 63%, green dye 92%, orange dye 40%, blue dye 65%, black dye 96%	15 days	catalase	[70]
Azo dyes (DB151 and DR31)	Textile effluent	<i>Bacteroides</i> spp., <i>Eubacterium</i> spp., <i>Clostridium</i> spp., <i>Proteus vulgaris</i> , <i>Streptococcus faecalis</i>	Bacterial consortia	95.25%-98%	5 days	Catalase, oxidase	[71, 72]
Azo dyes	Industrial effluent	<i>Acinetobacter</i> sp., <i>Klebsiella</i> sp., <i>Pseudomonas</i> sp.	Bacterial consortia	80%	72hrs	Peroxidase	[71, 73]
Reactive Green -19	Textile wastewater	<i>Zobellella iwanensis</i> , <i>Bacillus pumilus</i>	Bacterial consortia	86-93%	24 hrs	laccase	[71, 74]
Disperse Red	Textile effluent	<i>Microbacterium</i> sp., <i>Leucobacter albus</i> , <i>Klebsiella</i> sp., <i>Staphylococcus arlettae</i>	Bacterial consortia	80%	72 hrs	Azo reductase	[71, 75]
Reactive black 5	Textile effluent	<i>Anoxybacillus flavithermus</i> , <i>Anoxybacillus kamchatkensis</i> , <i>Anoxybacillus pushchinoensis</i>	Bacterial consortia	70%	24hrs	Azo reductase	[76]
RB-5	Textile wastewater	<i>Pseudoarthrobacter</i> , <i>Gordonia</i> , <i>Stenotrophomonas</i> , <i>Sphingomonas</i>	Bacterial consortia	54-34%	7 days	Azo reductase	[76]
Azo blue and Red dye	Polluted water	<i>Enterobacter aerogenes</i> , <i>Proteus rettgeri</i> , <i>Pseudomonas fluorescens</i>	Bacterial consortia	80-90%	96 hrs	Azo reductase	[77]
Red, green, yellow, black Azo dyes	Effluent from Hudaira drain near Nishat mills ltd, Lahore	<i>Bacillus subtilis</i> , <i>Bacillus areus</i> , <i>Bacillus mycoides</i> , <i>Bacillus</i> sp., <i>Micrococcus</i> sp., <i>Pseudomonas</i> sp.	Bacterial consortia	84%	24hrs	reductase	[68]
Safranin, congo red and CBBG-250	Municipal sewage system waste water	<i>Bacillus subtilis</i> , <i>Aeromonas hydrophila</i> , <i>Proteus mirabilis</i> , <i>Pseudomonas</i> sp., <i>Shewanella</i> sp.	Bacterial consortia	90%	36hrs	reductase	[78]
Azo dye	Textile effluent Bangladesh	<i>Bacillus thuringiensis</i> , <i>Bacillus pumilus</i> , <i>Enterococcus faecium</i>	Bacterial consortia	77.1%	72hrs	Azo reductase	[79]
Reductive red dye	Textile effluent	<i>Bacillus subtilis</i> , <i>A. niger</i>	Bacterial-fungal consortium	90%	72hrs	Laccase, azo reductase, NADH-DCIP reductase	[80]

Table 3. Cont...

Dye	Microbial Source	Microbes Name	Consortia	Decolorizing (%)	Time	Enzyme	Ref.
Orange, G-red	Textile effluent	<i>Chlorella vulgaris</i> , <i>Lyngbya</i> sp., <i>Nostoc lincolni</i> , <i>Elnatothrixviridis</i> , <i>Volvox</i> sp.,	Bacterial-algal consortium	47-59%	7 days	Laccase, peroxidase	[81]
Methyl red	Textile effluent	<i>Klebsiella</i> sp., <i>Bacillus</i> sp., <i>Clostridium</i> sp.,	Bacterial consortium	100%	16hrs	Azo reductase	[82]
Yellow EXF, Red EXF	Textile effluent	<i>Proteus mirabilis</i> , <i>Morganella morganii</i> and <i>Enterobacter cloacae</i>	Bacterial consortium	94%	72hrs	Laccase, azo reductase, oxidase	[83]
Acid blue	Textile effluent	<i>Citrobacter freundii</i> , <i>Moraxella osloensis</i> , <i>Pseudomonas aeruginosa</i> , <i>Citrobacter</i> sp.,	Bacterial consortium	97%	48hrs	Azo reductase	[84]
Azo dyes	Wastewater	<i>Bacillus subtilis</i> , <i>Achromobacter xyloxidans</i>	Bacterial consortia	70%	7 days	Laccase	[85]
Direct Red 28	Wastewater	<i>Bacillus pumilus</i> , <i>B. cereus</i> , <i>B. subtilis</i> and <i>B. megaterium</i> .	Bacterial consortia	72-92%	10 days	Azo reductase	[86]
Reactive blue 160	Textile effluent	<i>Alcaligenes</i> sp., <i>Bacillus</i> sp. BAB2731, <i>Escherichia</i> sp. BAB2734, <i>Pseudomonas</i> sp. BAB3054, <i>Providencia</i> sp. BAB2749, <i>Acinetobacter</i> sp. BAB2750, <i>Bacillus</i> sp. BAB2751, <i>Bacillus</i> sp. BAB3055	Bacterial consortium	100%	4hrs	Azo reductase, lignin peroxidase	[87]
Acid Red 88	Local textile industry effluent in Punjab, India	<i>Bacillus cereus</i> (BN-7), <i>Pseudomonas fluorescens</i> (BN-5), <i>Stenotrophomonas acidaminiphila</i> (BN-3)	Bacterial consortia	78-94%	24 hrs	Azo-reductase	[69]
Mono azo, DI-azo, tri-azo	Textile effluent	<i>Klebsiella</i> sp.1, <i>Klebsiella</i> sp.2, <i>Staphylococcus aureus</i> , <i>Bacillus cereus</i> , <i>Pseudomonas fluorescens</i>	Bacterial consortia	Yellow dye 63%, green dye 92%, orange dye 40%, blue dye 65%, black dye 96%	15 days	catalase	[70]
Azo dyes (DB151 and DR31)	Textile effluent	<i>Bacteroides</i> sp., <i>Eubacterium</i> spp., <i>Clostridium</i> spp., <i>Proteus vulgaris</i> , <i>Streptococcus faecalis</i>	Bacterial consortia	95.25%-98%	5 days	Catalase, oxidase	[71, 72]
Azo dyes	Industrial effluent	<i>Acinetobacter</i> sp., <i>Klebsiella</i> sp., <i>Pseudomonas</i> sp	Bacterial consortia	80%	72hrs	Peroxidase	[71,73]
Reactive Green -19	Textile wastewater	<i>Zobellella iwanensis</i> , <i>Bacillus pumilus</i>	Bacterial consortia	86-93%	24 hrs	laccase	[71,74]

of both.⁶⁰ The microbial sources of pectinase enzymes are *Aspergillus niger*, *Aspergillus oryzae*, *Penicillium restrictum*, *Trichoderma viridae*, *Bacillus subtilis*, and *Bacillus cereus*.⁶¹ Pectins and various polysaccharides are the substances present in fruit, they lead to colloid formation and fouling, also reduce the commercial value of juices.⁶² Pectinase degrades the pectin by cleaving beta-1,4-glycosidic bonds present in pectin. It reduces viscosity and cluster formation in juices that enhance the clarity of juices.⁶³ The decolourization process is important to give a pleasant colour of fruit juices.⁶⁴ Pectinase enzyme helps in the decolourization of Congo red dye. This decolourization occurs under certain conditions like at pH 6 – 6.5, temperature 28°C, and it takes 5 days to decolourize the congoed by the microorganism *Aspergillus oryzae* by forming a zone on congo red-agar medium.⁶⁵

Dye Decolourization By Microbial Consortia

Microbial consortia meaning is when two or more microbial groups live together symbiotically. Microbial consortia can be ectosymbiotic or endosymbiotic or sometimes maybe both. The evolution of land plants and their transition from algal communities in the sea to land microbial consortia suggest symbiotic evidence between their necessary precursors. Microbial consortia can decolourize dyes (Table 3); mostly the synthetic dye(azo dyes) that are present in the industrial effluents. Azo dyes contain one or more –N=N- groups which are commonly found in synthetic group release in nature. Azo bond is metabolized by reductive cleavage while the consequent aromatic amines are metabolized under gaseous conditions. Hence, the microbial population of the treatment system should work under both anaerobic/anoxic and gaseous conditions to gain complete mineralization of dye molecules. By using microbial consortia, the azo dye decolourization occurs faster. Dye contaminated soil has been isolated from textile wastewater of Orissa, India, that contains the pure culture of bacterial consortium-BP of *Bacillus flexus* TS8(BF), *Proteus mirabilis* PMS(PM), and *Pseudomonas aeruginosa* NCH (PA). Physico-chemical parameters have been optimized to gain maximum discoloration efficiency. The formation of metabolites by degradation

of Indanthrene Blue RS has been confirmed through UV-Vis spectroscopy, FT-IR, and GC-MS analysis. When the agricultural residual wastes have been supplemented, it shows an enhanced decolourization efficiency of consortium-BP. Mineralization of Indanthrene Blue RS has determined the higher reduction in TOC(Total Organic Carbon). COD(Chemical Oxygen Demand) has been removed by consortium-BP. *Bacillus flexus*, *Proteus mirabilis*, and *Pseudomonas aeruginosa* show a positive result in the catalase test. Bacterial consortia of *Pseudomonas aeruginosa*, *Rhodobacter sphaeroides*, *Proteus mirabilis*, *Bacillus circulans* have the reaction in anoxic-oxic condition to decolourize Renazol Black B. They incubate the consortia to observe the colour reduction, and they found that 90% of colour reduction and a COD reduction of 80% occur by using synthetic wastewater with a dye concentration of 100mgL⁻¹. For decolourization of golden yellow HER dye under aerobic and microaerophilic conditions, Microbial consortia GG-BL consisting of *Galactomyces geotricium* and *Brevibacillus laterosporus* NCIM 2298 has been developed. They have catalase, reductase enzymes. Bacterial consortia *Zobellelata iwanensis* ATI-3 and *Bacillus pumilus* HKG212 are used under static conditions to decolourize Reactive green -19 dyes. Yeast extract has been added as co-substrate, the decolourization efficiency of 97% with initial dye concentration has been observed. It is difficult to determine the impact of the experimental condition and decolourization process together.⁶⁶ Textile Acid Orange dye from textile effluent contaminated soil of Tanda, Uttar Pradesh (India) has been isolated. This dye is decolourized by a bacterial strain RMLRT03. Bushnell and Haas medium (BHM) amended with Acid Orange dye has been used for decolourization studies. 16s rRNA sequence of the bacterial strain identifies it as *Staphylococcus hominis*.⁶⁷ This bacterial strain has good decolourization ability with glucose and yeast extract supplements as co-substrate in static conditions. The optimal conditions of Acid Orange dye decolourization are at pH 7.0 and 35°C in 60 hours incubation by *Staphylococcus hominis*. The textile dyes can be absorbed or degraded by many bacterial and fungal species. Anthraquinone dye can be decolourized either by aerobic or anaerobic conditions. They are

Table 4. Genetically engineered microorganisms and their impact on dye decolourization

Gene name	Extracted from	Expressed in	Vector	Dye	Ref.
azoreductase	<i>Rhodobacter sphaeroides</i> AS 1.1737	<i>Escherichia coli</i> JM109	pGEX4T-1	Acid red GR	[93]
azoreductase	<i>Rhodobacter sphaeroides</i> AS 1.1737	<i>Escherichia coli</i> JM109	pGEX4T-1	C.I. Direct blue 71	[94]
azoreductase	<i>Rhodobacter sphaeroides</i> AS1.1737	<i>Escherichia coli</i> JM109	pGEX4T-1	Acid red B	[95]
azoreductase	<i>Halomonas elongata</i>	<i>Escherichia coli</i> DH5	pET21a	Methyl red and Remazol Black B	[96]
azrS	<i>Bacillus</i> sp. MR-1/2	<i>Escherichia coli</i> DH5a	-	Congo red And reactive black 5	[97]
sodC	<i>Synechococcus</i> sp. PCC 9311	<i>Synechococcus elongatus</i> PCC 7942	pSyn_6	Acid black 1	[98]
azoreductase	<i>Bacillus latrosporus</i> RRK1	<i>Escherichia coli</i> DH5a	pAZR-SS125	Remazol red	[99]
laccase	<i>Bacillus subtilis</i> CotA	<i>Synechococcus elongatus</i> PCC 7942	pCV0062	Indigo carmine, Reactive blue 19, Reactive black 5, Reactive blue 4	[100]
add	<i>Rhodococcus</i> sp.	<i>Escherichia coli</i> DH5a	pAZRS1	Reactive red 22	[101]
azoG	<i>Halomonas</i> sp. Strain GT	<i>Escherichia coli</i> DH5a	pET30a(+)	Azo dye wastewater	[102]
cotA	<i>Bacillus subtilis</i> 168	<i>Escherichia coli</i>	pET-28a(+)	Malachite green, Acid blue 62 and Methyl orange	[103]
-	<i>Pseudomonas</i> sp. SUK1	<i>Escherichia coli</i>	-	Red BLI, Navy blue-HER and Golden yellow-HER	[104]
azoA	<i>Enterococcus</i> sp. L2	<i>Escherichia coli</i> <i>Pseudomonas fluorescens</i>	pBBR1-MCS2fdh-azoA pBBR1-MCS2 azoA	Reactive red 97, acid red 119, reactive black B5, like azo dyes	[105]

resistant to corrosion due to their mixed aromatic structure. It lasts for a long time and causes considerable concern for environmental pollution and waste retention. Bioremediation techniques have attracted a lot of attention, making microbial discoloration and degradation economical and environmentally friendly compared to various conventional methods. Some potential bacteria can fade synthetic/ commercial dyes are used in textile dyeing. Here firstly five bacterial consortia have been isolated (*Bacillus* sp.1, *Bacillus* sp.2, *Acinetobacter* sp., *Citrobacter* sp., and *Klebsiella* sp.). Now they are divided into three groups; (a) *Bacillus* sp.1, *Bacillus* sp.2, *Acinetobacter* sp., *Citrobacter* sp. (b) *Acinetobacter* sp., *Citrobacter* sp., *Bacillus* sp.1., (c) five bacterial consortia. The results show that a mixture of

five bacterial consortia has more efficiency to decolourize the dye.⁶⁸ *Bacillus cohnii*, *Aspergillus terreus* HTCC, *Penicillium citrinum* are able to decolourize Basic violet dye. Various parameters like initial dye density, dye to inoculum ratio, and incubation time duration has been studied for dye discoloration. The evolving fungal bacterial association exhibits the highest percentage of discoloration (92%) ability compared to dye treatment by the monoculture approach. Fungal –Bacterial (*Penicillium citrinum* and *Bacillus cohnii*) consortia are more efficiently decolourized Basic violet dye. An integrated degradation and detoxification of textile dyes may be possible by the combination of fungi and bacteria that provide a good alternative technology for contaminant removal of water. To degrade the textile effluent

dyes basically the Acid dyes by bacterial and fungal consortia, these isolates have been used to form a mixed microbial consortium cell factory that could quickly fade and biodegrade the organic load on the waste material and be used to develop a continuous process for the treatment of a variety of textile dyed textile wastes, including reactive dyes.

Genetically Engineered Microorganisms And Their Impact On Dye Decolourization

Dye is a natural or chemical ingredient that imparts colour when applied to something. It is of two types, natural and synthetic. Synthetic dyes are broadly used in different industries like textile, paper, leather, food, pharmaceutical industries. These dyes have replaced natural dyes over the past few years due to their wide variety of colours, low cost, and capacity to withstand damage by sunlight, water, and chemicals.⁸⁸ Dyes are categorized into 14 types based on their structure. These are Acid dyes, direct dyes, and azo dyes, disperse dyes, sulfur dyes, fibre reactive dyes, basic dyes, oxidation dyes, mordant dyes, developed dyes, vat dyes, pigments, fluorescence or optical brighteners, and solvent dyes. Azo dyes are the largest group of synthetic dyes with more than 2000 different types. These are substantially used in the textile industry due to their bright colour, water fastness, and simple application technique. Although dyes are greatly used in industries, the intense usage of synthetic dyes has augmented water pollution. Dyes have a great solubilizing capability in water, which makes it so difficult to be removed from water.⁸⁹ According to WHO, dyeing treatment in the textile industry causes 17-20% of the industrial water pollution and among these dyes, 80% is azo dyes.⁹⁰ Synthetic nitrogen-based dyes are so toxic that they are banned in European Union, China, Japan, India, and Vietnam. The toxic effect of the dyes causes damage to the flora and fauna including humans. Therefore, the degradation and decolourization of these dyes are very important. The traditional dye decolourization technology involves physical (flocculation, coagulation, adsorption etc.), chemical (precipitation, oxidation), and biological (microbes, enzymes, microbial fuel cells etc.) methods.⁹¹ Biological methods involve the use of microorganisms and their pathways to perform the

decolourization of dyes. Averse to the physical and chemical methods, biological methods are more efficient, eco-friendly, and versatile. Biological methods using microorganisms are advantageous over the others because it is inexpensive, low cost and completely mineralize the organic pollutants. Microorganisms like bacteria, fungi, yeast, algae possess the ability to decolourize dyes. Genetic engineering plays a significant role in dye decolourization (Table 4). The recent advancement in molecular biology and genetic engineering has opened a new way to fight the pollution problem caused by these dyes. Each microorganism has a different capability for dye degradation and bioremediation.⁹² GMOs can be made by transferring a specific gene from one species to another or by gene modification. Genetically engineered microorganisms possess enhanced dye decolourization or bioremediation capacity. Insertion of various naturally occurring genes in a suitable host linked to several enzymatic activities resulting in the expression of designed pathways leading to the degradation of these dyes could be a useful tool for reducing pollution.

CONCLUSION AND FUTURE PERSPECTIVE

Enzymes are essential in many biological processes, the role of the lignocellulolytic enzyme in dye decolourization is very important. Dye decolourization is necessary because the dye is toxic in nature. The removal of dye contaminants from waste effluents using microorganism-derived lignocellulolytic enzymes has shown promising results with maximum efficiency because they show outstanding decolourization capabilities for various classes of dyes and could be used in place of synthetic dye decolourizing agents. Decolourization of the dyes will reduce their biological load. In the future, scientists can develop a genetically modified consortium that will contain all the lignocellulolytic enzymes which will help in dye decolourization. This will make the job easier and less time-consuming. Bio-decolourization has gained importance as an alternative, eco-friendly, low-cost, and efficient technology for industrial dye removal treatment. In addition, genetic, metabolic engineering technologies, Omics strategies, and synthetic biology approaches have significantly enhanced

the stability and capacity of biocatalysts that reduce the reaction time. Still, many challenges are limiting large-scale commercial production. Future research should be carried out to determine (1) dye-degradation mechanisms, process parameter optimization for microbial growth; (2) Response Surface Methodology (RSM) generation for consortia parameters to reduce enzyme loss and improve enzymes durability; (3) improve catalytic domain for enhancement of catalytic performance by *in-silico* protein-ligand interaction model generation in the dry laboratory and to achieve this, mutagenesis study in the wet laboratory.

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CONFLICTS OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTION

DG, SD conceptualized the study. SD, AG, AC, SM, SR wrote the manuscript. DG reviewed and edited the manuscript. All authors read and approved the final manuscript for publication.

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All datasets generated or analyzed during this study are included in the manuscript.

ETHICS STATEMENT

Not applicable.

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