Advances in Bioresearch
Adv. Biores., Vol 15 (3) May 2024: 410-423

Print ISSN 0976-4585; Online ISSN 2277-1573
Journal's URL:http://www.soeagra.com/abr.html
CODEN: ABRDC3
DOI: 10.15515/abr.0976-4585.15.3.410423

Advances in Bioresearch

REVIEW ARTICLE

Bioremediation of Indigo dye in textile effluents by Microbes: A Review

Devika P. Vala [1*], Medha Pandya[2], Devayani R. Tipre[1], Shailesh R. Dave[3] Darshna K. Patel[1]
[1]Department of Microbiology and Biotechnology, School of Science, Gujarat University, Ahmedabad, India.
[2]Department of Life Sciences, Maharaja Krishnakumarsinhji Bhavnagar University, Bhavnagar, Gujarat, India.
[3]Xavier's Research Foundation, Loyola Centre for Research and Development, St. Xavier College Campus, Navrangpura, Ahmedabad 380009, Gujarat, India.
Correspondent Author: Devika P. Vala
Email: - vala.devika@gmail.com

ABSTRACT

The release of coloured effluent from industry into the environment has negative effects on the ecosystem and human health. Among the most significant and often utilized synthetic dyes in the denim and textile dyeing industries are indigo dyes. These are compounds getting complex aromatic structures. These colours could be harmful for any living being because most of them are either carcinogenic or mutagenic, or they are toxic to animals and plants as well. Strict regulations regarding the filtration of contaminated water are quite expensive for the indigo dye producing facility. The key objective of this study is to present the most recent data on wastewater treated with indigo dye. Various treatment methods, including chemical, physical, and microbiological degradation, can be used to get rid of the indigo dye. The importance of biological decolourization of textile wastewater is growing due to its low cost. But it takes longer than expected to carry out this process. Although bioremediation techniques for handling indigo textile effluents have evolved over time, the goal of this comprehensive review is to consolidate the information that is now accessible regarding indigo dye, its decolourization by various microbial strains, and its usage in the bio-treatment of dye house effluent.
Keywords: *Indigo Dye, Indigofera tinctoria, Decolourization, Dyehouse effluent, Bioremediation*

Received 24.02.2024 Revised 01.03.2024 Accepted 11.05.2024

How to cite this article:
Devika P. Vala, Medha Pandya, Devayani R. Tipre, Shailesh R. Dave, Darshna K. Patel. Bioremediation of Indigo dye in textile effluents by Microbes: A Review. Adv. Biores., Vol 15 (3) May 2024: 410-423.

INTRODUCTION

Water-insoluble and vat-dyed, indigo dye is regarded as a stubborn material that poses a risk to the environment (1). Indigo dye is often used on cellulosic materials, such as cotton yarn, and is highly popular during production and use, from 10 to 15 percent of these dyes get released into the surrounding environment (2), Aquatic life is poisoned by dyes, which have bright hues. Most of these colours are either oncogenic and mutagenic, or they are harmful to plants and animals (3). So they pose a risk to the health of all living things (4). Textile products need to be considered because they have an impact on both environmental and human health. It is necessary to perform chemical analyses on fabrics and raw materials to produce fabric that is safe and devoid of toxins. World Health Organization (2010). Currently in use is the cleanup of these kinds of pollutants by a variety of physicochemical and biological techniques.

Indigo dye

Indigo dyes are widely used for dyeing and printing protein and cellulose. At present, approximately 13×10^6 kg of indigo, worth around $200 million, is produced every year (5). Natural indigo dye is obtained from the sap of the *Indigofera tinctoria* shrub (6). In 1897, synthetic indigo was first produced for commercial purposes. Because of its aryl ring's displaced electrons, which give it an aromatic structure, it may absorb electromagnetic radiation of various wavelengths. The distinctive colouring of

indigo is caused by this. The molecule absorbs light in the orange region of the spectrum (λ max = 613 nm). These aryl rings, also known as chromophores, are made up of two N-H donors and two C-O acceptors in place of a simple double bond between the carbons. They have NH groups and are made up of a substituted benzene ring. Blue (2,2´-bis-blue), (CI Vat Blue 1) or vat indigo, with a chemical formula of $C_{16}H_{10}O_2N_2$, is a crystalline, dark blue powder whose melting point is between 390°C and 392°C. It forms a blue solution when dissolved in DMSO, chloroform, nitrobenzene, and strong sulfuric acid; it is insoluble in water, alcohol, or ether. The textile sector is a prime use for indigo dyes. Cotton yarn is mostly used to make denim, which is used to make blue and other coloured jeans. Wool and silk are dyed in small quantities. Approximately 20,000 tons of indigo dye are produced annually worldwide, according to estimates. In addition, it is a food colouring agent. Before the turn of the 20th century, the only supply of dye was natural indigo. Synthetic indigo quickly surpassed natural indigo in practically every way; now, nearly all indigo is produced synthetically. Vat textile dyes, like indigo, are often used mainly for cellulosic fibers of cotton (7). Along with its sulfonated derivatives, it functions as a monomer and makes up 31% of the industrial dye market worldwide. Because indigo is water insoluble and unaffine to cellulose fibers, a reduction-oxidation reaction is used as a mechanism to attach it to textiles. Following this intricate series of fabric processing stages, a large volume of wastewater containing several dangerous compounds is produced and needs to be treated. Furthermore, many processes, including the use of hazardous chemicals, are involved in the decolourization of indigo to create various shadings across the cloth.

Types of Indigo Dyes

Natural Indigo blue

Throughout history, various plants have been used to make indigo dye; however, the majority of naturally occurring indigo comes from members of the genus Indigofera, which are found in tropical regions. True indigo, or Indigofera tinctoria, better known as Indigofera sumatrana, was the main species of indigo utilized economically in Asia. Strobilanthes Asia is a frequently utilized substitute source in the somewhat colder subtropical regions, such Taiwan and the Ryukyu Islands in Japan. Two species, *Indigofera suffruticosa* (Añil) and *Indigofera arrecta* (Natal indigo), were also significant in regionals in Central and South America. Woad (*Isatis tinctoria*) and dyer's knotweed (*Polygonum tinctorum*) can also generate indigo in temperate areas, although the Indigofera species yields more (8).

Indigo white

Being non-soluble in water makes indigo the most difficult dye to work with. It requires chemical alterations (reduction) in order to dissolve. "White indigo" (leuco-indigo) is created through reduction of indigo. The brightly coloured, insoluble form of indigo is soon restored when a submerged cloth is taken out of the dye solution due to the white indigo dye's speedy blending with atmospheric oxygen molecules. Due of this unique characteristic, indigo dye proved to be difficult for European dyers and printers to work with when it was first made generally available in the continent in the 16th century. Additionally, there were several chances for worker injuries, several chemical adjustments were necessary, and some of the ingredients used were poisonous (8).

Maya blue

Medieval Mayan and Aztec artists coloured ceramic and paintings with Maya blue, a pigment derived from indigo. It was long believed that the origin of this pre-Columbian azure-coloured pigment was in minerals because it is so stable and lightfast. The process involves raising the temperature of a finely powdered, dried mixture consisting of indigotin and either the mineral or sepiolite clay about 356 degrees Fahrenheit. This temperature causes the indigo to sublimate as a gas, replacing the water that is in the clay and forming a stable mixture. Once chilled Maya blue pigments can be combined with an appropriate binder and utilized for a variety of purposes. Maya blue is better as a pigment than pure botanical indigo since it uses less indigo dye, is lightfast & has a beautiful turquoise hue. Because Maya Blue has chalk in its formulation, it can be used for marbling without the need for a mordant. Prior to the recipe's transformation in recent years, European marblers were unable to obtain this enduring colour (8).

Indigo carmine

Another colourant used is indigo carmine, often known as indigotin, which is a derivative of indigo. Every year, about 20 million kg of indigo are manufactured, mostly for blue jeans. It is applied as a food colouring as well. Another blue dye called indigo carmine is derived from indigo 5, 5'-disulfonate. Indigo carmine is reactive in water, unlike indigo. The conjugation mechanism is responsible for the vibrant colouring both indigo and indigo carmine, just like it is for many other dyes. Products of breaking down these compounds' conjugation system will be decoloured. Moreover, chemicals that decolourize indigo are anticipated to also decolourize indigo carmine since the chemical reaction mechanism between indigo

& indigo carmine are the same (9), many countries utilize indigo carmine as a synthetic colouring ingredient for food and cosmetics (10). In addition to being a food colouring and textile colouring, indigo carmine is additionally used for healthcare diagnostic tests and as an ingredient in medicinal tablets and capsules (11). Indigo carmine, also known as indigo 5, 5'-disulfonic acid disodium salt, is used in analytical chemistry as a redox indicator and in biology as a microscopic stain (12). Moreover, in the diagnostic, when utilized in alongside acetic acid, the dye makes finding Barret's oesophagus easier (13). Since uniformly stained or unstained areas appear to link with intraepithelial neoplasia, it can also aid biopsies even more (14). I.e. moreover, doctors utilize it as a means of diagnosis to find urethral orifices and do endoscopic examinations for stomach cancer (15), and to modify a catheter's location during hepatic tumours chemotherapy (16). The toxic properties of indigo carmine conversely, several investigations have indicated that indigo carmine resulted in hypotension, severe hypertension, or adverse consequences (17). However, the kidneys can filter indigo carmine instead of easily metabolizing it. The dye has also been shown to cause tumors where it is administered (18). It has also been noted that individuals may experience moderate to severe elevated blood pressure and cardiovascular, and respiratory side effects when given intravenously to assess the effectiveness of the urine collection device (19);(20). Moreover, it could irritate the gastrointestinal tract and result in nausea, vomiting, and diarrhoea (15). The dye's toxicity demonstrated long-term damage in mice (21), and toxicity in pigs over the short term (22). With an LD50 of 5000 mg/kg in mammals, indigo has relatively low oral toxicity.

CHEMICAL SYNTHESIS OF INDIGO DYE

Many techniques have been used to prepare indigo. Although the Baeyer-Drewson indigo synthesis was developed in 1882, it was not practical. Pfleger provided the first workable path in 1901. This procedure involves treating N-phenyl glycine with a molten solution of sodamide, potassium hydroxide, and sodium hydroxide. This process, which yields indoxyl, is extremely sensitive and oxidizes in air to generate indigo. This technique is still in use today in many variations. Heumann is recognized in 1897 for having discovered the most feasible and alternate path to indigo. It entails using sodium hydroxide to heat N-(2-carboxyphenyl) glycine to a temperature of 200 degrees Celsius in an atmosphere of inertia. Although the preparations are more expensive, the procedure is simpler than the Pfleger approach. It produces indoxyl-2-carboxylic acid. This substance decarboxylates easily to produce indoxyl and subsequently oxidizes in the presence of air to generate indigo. College laboratory courses practice the making of indigo dye (23).

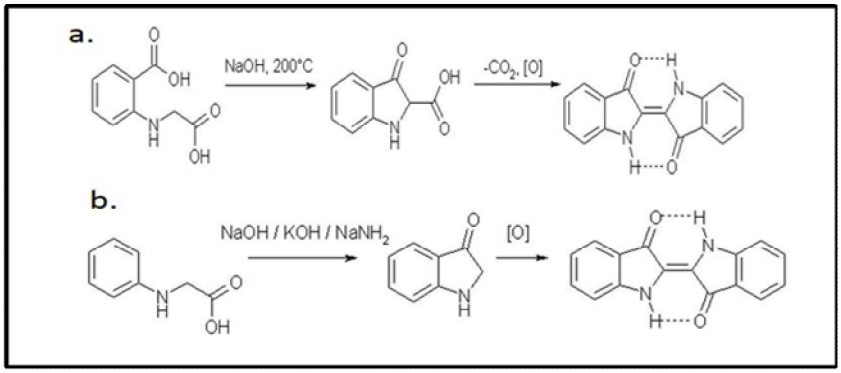

Fig.1: Chemical Synthesis of Indigo Dye (a) The initial Indigo synthesis by Heumann (b) The Indigo synthesis by Pfleger

DECOLOURIZATION OF INDIGO DYE:

One of the most pressing environmental problems related to dye effluents is the improper disposal of wastewater from the dyeing industry (24). Dye results in water and soil bodies that are unacceptably intensely coloured (25). Additionally, they impede light from reaching the lower depths of the water system and reduce the solubility of gases in water bodies. As a result, they interfere with photosynthesis, which kills aquatic life and produces poisonous, foul-smelling water. Because they contaminate the soil and water, these pollutants pose major harm to the ecosystem. Dye contamination of soil alters and deteriorates its physical, chemical, and biological qualities while also restricting plant growth. These substances are primarily to blame for the altered soil fertility (26).

Removal of dyes

Most of the time, physicochemical techniques are used to remove such dye-containing effluents. In general, wastewater from the textile industry is treated using a combination of biological, chemical, and

physical methods. Examples of physical and chemical techniques include the adsorption precipitation by chemicals, occultation, photolysis, chemical reduction and oxidation, electrochemical treatment, as well as ion-pair extraction. These procedures fail to perform well for all dyes, are costly, cause adverse effects, generate a lot of sludge and by-products, and so on (27); (28). Hence, researchers have focused on biological treatment as the best alternative. The operational cost of this method is relatively low when compared with conventional technologies (29);(30);(31). Its goal is to provide an affordable, ecologically friendly treatment for this waste issue. Alkalophilic bacteria were among the many microorganisms that carried out the biodegradation process for indigo pigments (32), Algae, several fungi, anaerobic and thermophilic bacteria (7), Studies have indicated that the best bio sorbents for wastewater treatment facilities are microalgae. There are certain drawbacks to these bio sorbents, nevertheless, namely their poor heat and chemical resistance. Growing curiosity about the field of water treatment has been shown in the encapsulation of microbial cells (33);(34);(35).

Factors affecting Indigo dye decolourization

The industrial effluent generated during fabric processing has a diverse chemical makeup and comprises various harmful chemicals. Numerous physico-chemical parameters like pH, temperature, oxygen that is dissolved, dye structure, dye concentration, donors of electrons, and redox mediators, have an impact on the decolourization and degradation rate of colourants during the biological processing of wastewater. These factors also directly impact the efficacy of microbial dye decolourization (36).

The impact of oxygen on the decolourization of dyes

The impact of oxygen on development and dye decolourization is the most significant factor in dye decolourization process, Oxygen has a vital part in the physiological properties of the cells during growth.

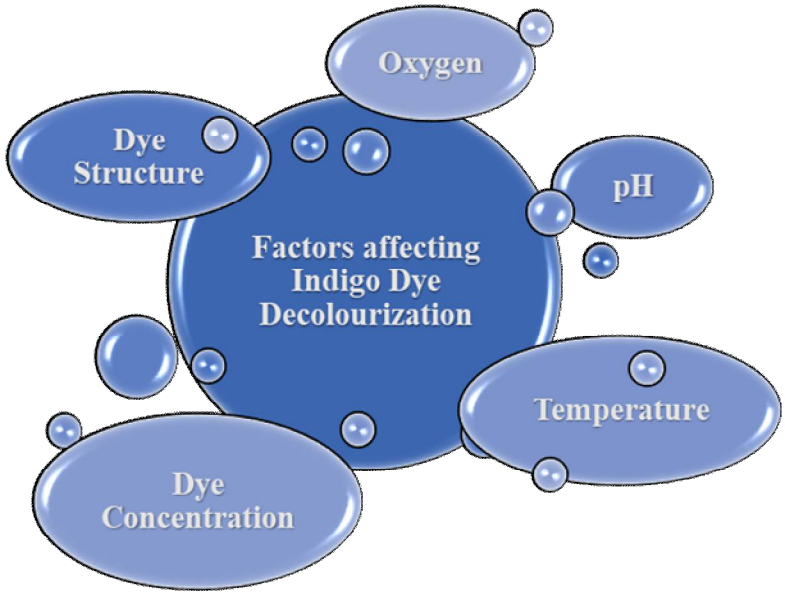

Fig. 2: Factors influencing decolourization of Indigo dye.

Based on observations, optimum oxygen is required for better indigo dye decolourization which is provided with Shaking conditions at Optimum RPM generally it is 120 -150 rpm (1).

The impact of Temperature on dye decolourization

Since different types of microorganisms have certain temperature ranges in which they can function, temperature directly affects the decolourization of dye. There is evidence from earlier research that when temperature rises to a certain point, the rate of decolourization increases and the capacity for decolourization decreases. The temperature range (30◦c to 45◦c) is generally optimum for microbial growth which corresponds to maximum colour removal. Furthermore, a drop in the decolourization rate has been reported with rising temperatures; this decrease may be brought on by denaturing dye-decolorizing enzymes or by the loss of cell viability (37). Because the microenvironment inside the support medium protects the cells, immobilized cells cause a change in the optimal temperature towards high temperatures. When examined against other temperature ranges, the decolourization rates of the

indigo dye and its waste products were higher between 28 and 33°C (24). This demonstrated that *P. aeruginosa's* dye decolourization activity is independent of temperature fluctuations. At a temperature of 20 °C, *Paenibacillus larvae* were shown to have limited indigo dye decolourization activity, according to (38). This strain can effectively decolourize indigo carmine under a wide range of the conditions of the temperature, indicating that it could be employed in dye decolourization. In order to determine the highest decolourization (39), evaluated a number of reaction parameters, including pH (5.0–10.0), incubation time (24–96 hours), and temperature of incubation (20, 33, & 37 °C) a 100 mg/L of indigo carmine concentration employing Bacillus sp. In the 96th hour, the maximum decolourization rate was found to be approximately 67 percent at pH 6.0, 37 °C, and a speed of 120 rpm of agitation (40).

The impacts of PH on dye Decolourization

The effectiveness of indigo dye decolourization is significantly impacted by pH. The ideal pH for dye decolourization is often around a neutral or slightly alkaline pH, according to past research. The rate of dye decolourization was higher at the ideal pH, but it tends to drop off quickly at pH values that are too acidic or too alkaline. Several researches have shown that for several dyes, decolourization peaked in the pH value range of 5.0 to 8.0. Since it allows them to be used in the biological processing of textile effluents containing dyes, pH tolerance across a broad range is crucial (25). In a previous study, it was shown that when the pH was elevated from 5.0 to 7.0, the dye's reduction rate nearly doubled, but in a range of 7.0 to 9.5, the rate remained insensitive to pH (41). The Highest Indigo dye decolourization rate was noted in the pH range of 4.0 to 5.0 (8). The pH of the medium may affect the ability the bacteria to decolourize colours (20). Therefore, the effect of pH on this bacteria's capacity to decolourize dye was also investigated at different initial pH levels (150 rpm and 30 °C). One desired feature of dyes is their capacity to decolourize throughout a broad pH range. According to (38), Larvae of Paenibacillus showed low levels of indigo dye decolourization (6–15 %) at pH 3.0, but decolourization activity maximized around pH 7.0 and 8.0 (40).

The impact of dye Concentration on dye decolourization

As dye concentration rises, the rate at which the dye decolourizes gradually decreases. This might occur from the dye's harmful effects on specific bacteria or bacterial consortia, which would result in an insufficient cell-to-dye ratio. Several factors, such as the dye's toxicity (as well as that of co-contaminants) at greater concentrations and the enzyme's capacity for identifying the substrate effectively at very low concentrations, which might be found in some waste waters, can affect the quantity of dye substrate and the potential for dye removal (42). The amount of time needed to remove the colour increases with the dye concentration. Above dosages of 1–10 μM, dyes were easily decoloured; however, above 30 μM, colour removal is reduced (43). However, there are no evidence that the concentration of dye had a negative effect on the pace of decolourization. This could be because non-enzymatic reduction mechanisms are regulated by processes that are not affected by dye concentration. According to earlier research, the decolourization rate steadily decreases as the amount of the dye increases because of its hazardous effects upon the growth of organisms (44).

The impact of agitation on bio decolourization

High decolourization activity is largely dependent on agitation. Therefore, the impact of agitation on *P aeruginosa's* capacity to decolourize dye was also examined. To do this, the bacterium's capacity for dye decolourization was examined in both static and variously agitated environments. After 4 hours of incubation, this bacterium successfully discoloured this dye at all agitation rates (Fig. 3). When compared to shaken cultures (38), found that *Paenibacillus larvae* grown in non-shaken cultures had lower indigo dye decolourization activity. Similar to this, *Pseudomonas luteola* free cells have been shown to be extremely sensitive to dissolved oxygen during reactive red 22 dye decolourization (37). This variation may result from the utilisation of various species. Over the period of 6 hours, the dye decolourization ability of this bacteria was tested in both static and agitated state. At a pH 6.0, a dye concentration of 50 mg/L, and two different temperatures (30 and 40 °C), the dye decolourization activity was examined. In both static and agitated conditions, this bacterium's dye decolourization activity was shown to be fairly strong (40).

The impact of dye structure on dye decolourization

While the rate of colour removal is lower for dyes replaced moreover, simple-structured dyes, low molecular weight dyes, and dyes having an electron-withdrawing group, like $-SO_3H$ or $-SO_2NH_2$, at the para position of the phenyl ring are more likely to undergo decolourization than high molecular weight dyes, (43) revealed that the phenyl ring's electron-withdrawing groups speed up colour removal. They came to the following conclusions: (a) acid dyes have low colour removal because of the number of sulphonate groups in the dye; (b) direct dyes have high colour removal levels that are unaffected by the

number of sulphonate groups in the dye; and (c) reactive dyes have low colour removal levels (20);(44), The intricate structure of indigo dyes, which was previously discussed in depth, adds to their resistant nature.

Table 1: Optimum condition for Indigo dye decolourization using microbes and enzyme

Decolourization	PH	Temperature	Aeration (Rpm)	Reference
Bacterial	7-8	30-40 ◦C	120	(38)
Fungal	4-5	28-33 ◦C	160	(13)
Algal	6.5-7.5	28 ◦C	120	(36)
Enzyme (Laccase)	5	30 ◦C	200	(45)

Mechanism of Dye Degradation

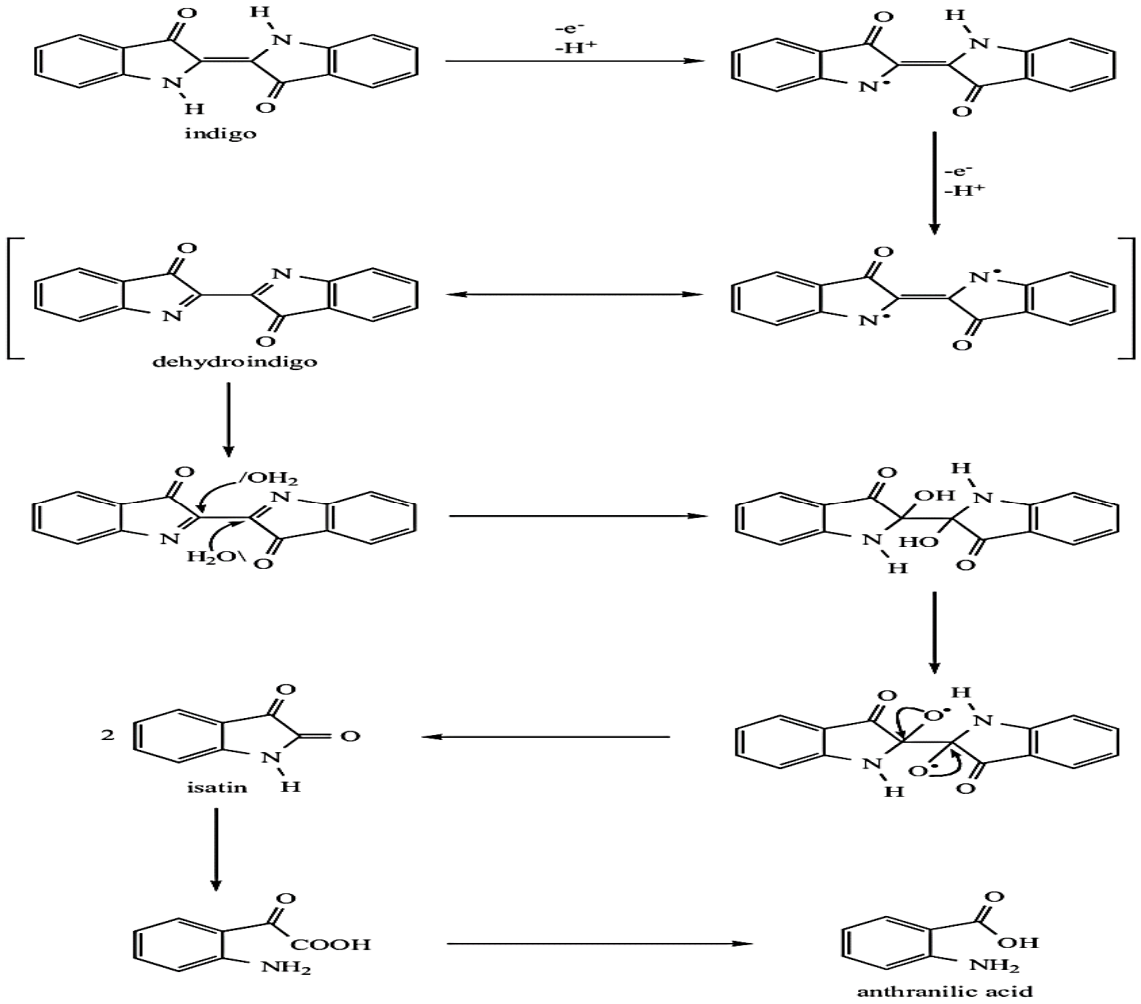

Fig.3: A possible mechanism for laccase-catalyzed degradation of indigo dye (Figure re-printed from (46).

DECOLOURIZATION ASSAY

The decolourization of the dye effluent is detected by UV-Visible spectrophotometer (Beckman Coulter DU® 800, USA) at the λmax of indigo dye (620 nm) using the supernatant of the liquid culture medium. The supernatant is obtained by centrifugation at 10,000 rpm for 10 min in a refrigerated centrifuge (Beckman Coulter Microfuge® 1, USA) followed by decantation. The removal of colour is reported as % decolourization (% = 100(Aini – Aobs) / Aini), where Aini and Aobs are the absorbance of the dye solution initially and at cultivation time (t), respectively. Each decolourization value is the mean of three replicated experiments. Abiotic controls (without the microorganism) were always included in. Thin layer chromatography, Liquid chromatography-Mass spectrometric analyses (LC-MS) were also used for the degradation study.

Table 2: Decolourization/degradation studies for the treatment of indigo using various Microorganisms

Organisms	Dye	Remarks	Optimum Condition	References
Fungi				
Trametes hirsuta	Indigo dye	Pure Enzyme used for degradation of Indigo. Dye Removal from Fabric. Results based on Particle size reduction.	pH 4-5 Temp: 30 ◦C Aeration:160 RPM	(46)
Myceliophthora thermophila	Indigo carmine	31 % decolourization in 16 hrs with 20mg/L dye concentration with crude fungal laccase.	pH 5.5, Temp: 30 ◦C Aeration:160 RPM	(47)
Funalia(Trametes) trogii ATCC 200800	Indigo carmine	57% decolourization in 5 min with 100mg/L dye concentration with crude fungal laccase.	pH 3, Temp: 30 ◦C Aeration:150 RPM	(41)
Sclerotium rolfsii	Indigo dye	Pure Enzyme used for degradation of Indigo. Dye Removal From Fabric.Results based on Particle size reduction	pH 4-5 Temp: 30 ◦C Aeration:160 RPM	(46)
Pleurotus ostreatus fungi	Indigo	Decolourization 10–22 d, plate assay 100%	pH:7 Temp: 25 $_o$C Aeration: 150 RPM	(48)
Diutina rugosa KR262715 (Yeast)	Indigo	Decolourization 99.97%after 5 d, 2-g cell biomass	pH:7 Temp: 35 ◦C Aeration: 150 RPM	(49)
Aspergillus alliaceus strain 121C	Indigo	Decolourization9 d, 98.6%	pH:7 Temp: 30 ◦C Aeration: 150 RPM	(50)
Bacteria				
Pseudomonas aeruginosa	Indigo carmine	93% decolourization was observed in 24 hours.	pH:6-8 Temp: 30 ◦C Aeration: 150 RPM	(41)
Bacillus sp. MZS10	Indigo carmine	87% decolourization in 15 hrs.	pH:7 Temp: 30 ◦C Aeration: 150 RPM	(51)
Citrobacter amalonaticus Y19	Indigo carmine	12.5 % decolourization in 48 Hrs.	pH -6-7 Temp: 35 ◦C Aeration: 150 RPM	(52)
Bacillus sp.	Indigo carmine	66.6% decolourization in 96 Hrs.	pH -6 Temp: 37 ◦C Aeration: 150 RPM	(39)
Bacillus aryabhattai DC100	Indigo carmine	Decolourization 72 h, 250-mL flask 98.31%	pH -6 Temp: 37 $_o$C Aeration: 150 RPM	(53)
Paenibacillus larvae	Indigo carmine	Decolourization 100% after 8 Hrs. Yeast Extract and Peptone 1% Medium	pH:7-8 Temp:30-40 ◦C Aeration:120 RPM	(38)
Aeromonas hydrophila DEC1	Indigo carmine	60% decolourization in 24 Hrs.	pH:7 Temp: 30 ◦C Aeration: 150 RPM	(54)
Algae				
Scenedesmus quadricauda ABU12	Indigo blue	Immobilized Algal cells giv>50% decolourization.	pH:7 Temp: 25 ◦C Aeration: 150 RPM	(36)
Anabaena flos-aquae UTCC64	Indigo	Decolourization 71.92% after 14 d, 50-mL flask	pH:7 Temp: 25 $_o$C Aeration: 150 RPM	(55)
Phormidium autumnale UTEX1580	Indigo	Decolourization 91.22% after 14 d, 50-mL flask	pH:7 Temp: 25 $_o$C Aeration: 150 RPM	(55)

BIOREMEDIATION OF INDIGO DYE

Bioremediation of indigo dye in textile effluents by microbes is a process of using microorganisms to break down or transform indigo dye compounds into less harmful substances, ultimately reducing the environmental impact of textile dyeing. This approach can be effective and sustainable, but its success depends on a variety of factors, including the specific microbes used, environmental conditions, and the characteristics of the dye compounds themselves (56). Bioremediation refers to a wide range of procedures that employ natural resources to mitigate pollution produced by xenobiotics. Xenobiotics are substances that are alien to certain ecological systems, are typically of anthropogenic origin, and have a high persistence in the environment. They can be aromatic ring systems replaced by electron-drawing groups such as azo, nitro, or halogens (57). Several remediation techniques have been used to reduce toxicity levels caused by xenobiotics, including "microbial degradation" using microorganisms such as bacteria and fungi; "phytoremediation" by plants, which involves several biological mechanisms; and "enzyme remediation" using specific enzymes to degrade pollutants (58). Many microorganisms, such as fungus, and yeasts, bacteria, have been identified to be capable of discolouring textile colour (59); (60). Reactors comprising mixed cultures, isolated organisms, or isolated enzymes can be utilised for decolourization (27). In mixed cultures, one species may be implicated in chromophoric group cleavage. Some species may bio transform the changed dye further, while others may not be involved in bioremediation at all but may help to balance the entire ecosystem (61). Relative to isolated organisms, only a small portion of the produced enzymes are directly related to colour biological conversion. In enzyme remediation, they can be employed after being separated from the biomass. Their activity may be influenced by the presence of other chemicals such as co-factors, co-substrates, or mediators. Table 2 summarises role of several fungi and bacteria for decolourization of indigo dye.

Indigo dye degradation by fungi

In particular, filamentous fungus have been found to breakdown dye more efficiently because ligninolytic enzymes, like as laccase and peroxidase, are present and have a role in the breakdown of dyes, making them more bioavailable than other microbes. One of the most effective biological processes for treating undesirable industrial wastes and effluents containing dyes is the use of fungi. The white-rot fungus *Trametes hirsuta* has been shown to be able to break down indigo dye, which is frequently utilized in the textile sector. Indigo dye is notoriously difficult to remove from wastewater, and its release into water bodies can have negative environmental impacts. Therefore, finding ways to degrade indigo dye is important for mitigating these impacts. *Trametes hirsuta* has been demonstrated in studies to be capable of degrading indigo colour by the synthesis of enzymes such as laccase, manganese peroxidase, and lignin peroxidase. These enzymes are able to break down the chemical structure of the dye, ultimately resulting in its degradation. *Myceliophthora thermophila* is a thermophilic filamentous fungus that has also been found to be capable of degrading indigo dye. Like *Trametes hirsuta, Myceliophthora thermophila* produces a variety of enzymes that are able to break down the chemical structure of indigo dye, including laccase, manganese peroxidase, and lignin peroxidase. Several research revealed that *Myceliophthora thermophila* can degrade indigo dye at high temperatures, making it a potential candidate for use in industrial applications where high temperatures are used to treat wastewater. Additionally, the fungus has been shown to be effective at degrading a variety of other dyes and pollutants, indicating that it may be useful in a range of environmental remediation applications. Funalia (Trametes) trogii is another type of white-rot fungus that has been studied for its potential in decolourizing indigo dye. This fungus produces ligninolytic enzymes, such as laccase, manganese peroxidase, and lignin peroxidase, which are able to break down the complex structure of indigo dye and remove its colour. Studies have shown that *Funalia trogii* is effective in decolourizing indigo dye in both liquid and solid media. The decolourization process is dependent on factors such as pH, temperature, and the concentration of the dye in the medium. In addition to its potential for indigo dye decolourization, *Funalia trogii* has also been found to have applications in the treatment of other environmental pollutants, such as polycyclic aromatic hydrocarbons and phenolic compounds *Sclerotium rolfsii* is a soil-borne plant pathogen that belongs to the group of fungi known as Ascomycota. While this fungus is primarily known for its ability to cause disease in plants, it has also been studied for its potential in the decolourization of indigo dye. Research has shown that *Sclerotium rolfsii* is capable of decolourizing indigo dye through the production of enzymes such as laccase and peroxidase. These enzymes are able to break down the chemical bonds that give the dye its colour, resulting in a reduction in the intensity of the dye's colour. *Pleurotus ostreatus* is a type of mushroom that has been found to have the ability to degrade a wide range of environmental pollutants. Studies have shown that *Pleurotus ostreatus* can effectively degrade indigo dye through a process called enzymatic oxidation. The mushroom produces an enzyme called laccase, which breaks down the dye molecules into smaller, less harmful compounds that can be easily assimilated by

microorganisms in the environment. The use of *Pleurotus ostreatus* for indigo dye degradation has several advantages over conventional chemical methods. It is a natural and sustainable approach that does not require harsh chemicals or energy-intensive processes. *Diutina rugosa* KR262715 is a type of yeast that has been found to have the ability to decolourize textile dyes. The use of this yeast as a bioremediation tool for dye removal is an eco-friendly and sustainable approach that offers several advantages over conventional chemical methods. Studies have shown that *Diutina rugosa* KR262715 can effectively remove various textile dyes from wastewater through a process called Biosorption. Biosorption involves the binding of dye molecules to the cell wall of the yeast, where they are adsorbed and subsequently metabolized by the organism. Khelifi and co-workers demonstrated that *Aspergillus alliaceus* strain 121C was able to effectively decolourize several types of textile dyes, including indigo. The decolourization process was found to be dependent on several parameters, including pH, temperature, dye concentration, and agitation speed.

Indigo dye degradation by bacteria

Fungi are reportedly superior to other microbes in terms of discolouration and degrading activities. Nevertheless, their wide-scale application has been constrained by the drawbacks of the sensitive growing conditions, such as pH, nutrient load, and extended lag times, as well as the production of poisonous by-products. A number of bacteria, cyanobacteria, and actinomycetes have also been shown to play a significant part in the breakdown of indigo, and it is considered crucial to secure active bacterial strains by screening. Fast-growing bacteria with high degrading potential have drawn the attention of researchers due to their high efficiency and cost savings. The Bacillus species are among them and are great candidates for dye degradation. They have been shown to breakdown a number of commercial dyes very effectively. Bacillus sp. MZS10 is a bacterial strain that has been reported to have the ability to decolourize indigo dye in textile wastewater. Indigo dye is commonly used in the textile industry and is known to be resistant to biodegradation. One study by (62) investigated the ability of Bacillus sp. MZS10 to decolourize indigo dye. The results showed that the strain was able to decolourize up to 85% of the indigo dye within 72 hours. The study also reported that the strain was able to decolourize a range of other textile dyes as well. *Paenibacillus larvae* is a spore-forming bacterium that is known to be a causative agent of American foulbrood disease in honeybees. However, studies have also shown that this bacterium has the ability to degrade indigo dye, which is commonly used in the textile industry. Indigo dye is known to be resistant to biodegradation, but certain microorganisms, including *Paenibacillus larvae*, have been reported to be capable of breaking it down. (44) Investigated the ability of *Paenibacillus larvae* to degrade indigo dye. The researchers found that the bacterium was able to degrade up to 99% of the indigo dye within 48 hours. The study also reported that the optimal temperature for indigo dye degradation by *Paenibacillus larvae* was 35°C. Research by the researchers found that the bacterium was able to degrade up to 70% of the indigo dye within 24 hours. The study also reported that the optimal pH for indigo dye degradation by *Paenibacillus larvae* was 7.0. *Pseudomonas aeruginosa* is a gram-negative, rod-shaped bacterium that is known for its ability to degrade a wide range of organic compounds. This bacterium has been studied for its potential in the decolourization of indigo caramine, which is a synthetic dye used in the textile industry. Research has shown that *Pseudomonas aeruginosa* is capable of decolourizing indigo caramine through the production of enzymes such as laccase and azoreductase. These enzymes are able to break down the complex structure of the dye and remove its colour. In addition to its potential for indigo caramine decolourization, *Pseudomonas aeruginosa* has also been found to have applications in the treatment of other environmental pollutants, such as polycyclic aromatic hydrocarbons and phenolic compounds. *Aeromonas hydrophila* DEC1 is a Gram-negative bacterium that has been reported to have the ability to degrade various pollutants. One study by (13) investigated the ability of *Aeromonas hydrophila* DEC1 to degrade indigo dye. The results showed that the bacterium was able to decolourize up to 97% of the indigo dye within 12 hours. The researchers also found that the optimal temperature for indigo dye degradation by *Aeromonas hydrophila* DEC1 was 30°C. Another study by (13) also reported the ability of *Aeromonas hydrophila* DEC1 to degrade indigo dye. In their study, the researchers found that the bacterium was able to degrade up to 95% of indigo dye within 16 hours. The study also reported that the strain was able to degrade a range of other textile dyes as well.

Indigo dye degradation by Algae

Algae are photosynthetic organisms that can utilize sunlight and carbon dioxide to synthesize organic compounds. Studies have shown that certain algae species, such as Anabaena, Phormidium and Scenedesmus can effectively degrade indigo dye through a process called biosorption. Biosorption involves the adsorption of the dye molecules onto the surface of the algae cell wall, where they are subsequently metabolized by the organism (63). *Scenedesmus quadricauda* ABU12 is a green microalga that has been reported to have the ability to degrade various pollutants, including indigo dye. One study

by (36), investigated the ability of *Scenedesmus quadricauda* ABU12 to degrade indigo dye. The results showed that the microalga was able to remove up to 89% of the indigo dye within 96 hours. The study also reported that the optimal pH for indigo dye degradation by *Scenedesmus quadricauda* ABU12 was 8.0. (6), also reported the ability of *Scenedesmus quadricauda* ABU12 to degrade indigo dye. In their study, the researchers found that the microalga was able to degrade up to 94% of indigo dye within 120 hours. The study also reported that the strain was able to degrade a range of other textile dyes as well. However, further research is needed to fully understand the potential of microorganisms for large-scale indigo dye decolourization. Factors such as the optimal conditions for growth and enzyme production, the effectiveness of the organism in different types of wastewaters, and the potential for scale-up will need to be investigated in order to determine whether microbes can be used effectively in industrial applications. Additionally, the potential risks associated with the use of bacteria and fungi in environmental applications will need to be carefully considered.

Enzymes for indigo dye degradation

A potential candidate for dye degradation is any microorganism that generates the enzymes peroxidase, laccase, monooxygenase, and dioxygenase. The active enzymes should ideally be excreted into the medium by the appropriate organisms. Alternatively, bio elimination could be limited by transit inside the cells. The ability of an organism to resist the harmful effects of dyes and other compounds contained in the effluent is another important criterion. Consequently, isolated enzyme systems may be favoured in situations where the target molecule or additives hinder growth. This may occur particularly at high dye concentrations since several studies have found that when dye concentrations rise over a certain point, microorganism decolourizing rates decline. In a nutshell, it must address the following significant issues: Depending on the production charge, Dyehouse effluents can substantially vary and are complicated combinations with high loads of additives (salts, detergents, dispersants and metals). Dyehouse effluents frequently exhibit high temperatures and severe pH. Due to their numerous structural variations, dyes do have a wide range of chemical and physical characteristics. They are designed to cope with extremely hard circumstances. Thus, it is necessary to create biological systems that can function in such environments and nevertheless successfully not just decolourize but ideally entirely destroy dyestuff. Owing of these limitations, using a bioremediation technology alone is not yet a straightforward answer. And hence, isolated enzyme systems may be recommended in situations where the target molecule or additives inhibit growth. The oxidation of many different organic substrates, including lignin and aromatic compounds, can be catalysed by the laccase enzyme. Fabrics have been coloured blue for millennia using indigo, an organic dye. Yet, indigo is not easily degraded and may last a very long period in the environment. It has been demonstrated that laccase works well to oxidise indigo and cause its degradation. The process by which indigo dye is broken down by laccase involves the oxidation of indigo to isatin, which is then broken down to produce colourless by products such oxindole and anthranilic acid. Indigo is oxidised to isatin by producing a radical intermediate, which is subsequently attacked by oxygen to produce isatin. The carbonyl group in isatin undergoes further oxidation, resulting in the oxidation of isatin to oxindole and anthranilic acid. Many research have looked at the usage of laccase for the deterioration of indigo dye. Table 2 summarizes instances of the laccase enzyme used by various microorganisms to break down indigo dye. Research by (51) for instance, demonstrated that the laccase from Trametes sp. could degrade indigo in a pH-dependent manner, with maximal degradation happening at pH 5. According to the study, adding a mediator such 1-hydroxybenzotriazole could enhance the efficiency of indigo degradation. The utilisation of laccase from Cerrena sp. for the degradation of indigo dye was the subject of another investigation by (28). The study discovered that a pH of 6.0 and a temperature of 30°C were ideal for indigo breakdown. Also, it was discovered that the inclusion of a mediator, such as violuric acid, might considerably improve the effectiveness of indigo degradation.

Enzyme	Origin	T (°C)	pH	DC (mg/L)	DT	DR (%)	Reference
Laccase	*Trametes trogii* BAFC 463	30	4.5	23	30 min	94	(64)
Laccase	*Myceliophthora thermophila*	30	5.5	20	16 h	31	(65)
Laccase	*Trametes versicolor* DSM 11269	50	5.5	62.5	6 h	10	(65)
Laccase	*Trametes modesta*	50	4.5	250	6 h	58	(3)
Laccase	*Trametes* sp. SYBC-L4	30	4.5	100	36 h	99with2.5 mM HBT)	(60)
Laccase	*Pleurotus sajor-caju*	32	5.0	46.6	3 h	≥ 90	(38)
Laccase	*Ceriporiopsis subvermispora* CZ-3	30	7.0	100	24 h	95	(66)
Laccase	*Trametes* sp. SYBC-L4	30	7.0	100	0.5 h	≤ 100 (with 6 mM HBT)	(60)
Laccase	*Funalia* (*Trametes*) *trogii* ATCC 200800	30	-	100	72 h	87	(67)
Laccase	*Funalia* (*Trametes*) *trogii* ATCC 200800	30	3.0	100	5 min	57	(40)
Laccase	*Trametes orientalis*	50	4.0	50	48 h	42.74	(68)
Laccase	*Panus rudis*	45	4.0	40	30 min	81	(69)

Table3: Decolourization/degradation studies for the treatment of Indigo with use of purified Laccase

Abbreviations*: Dye concentration (DC), temperature (T), decolourization time (DT), decolourization rat DR)*

CONCLUSION

Indigo is one of the most frequently used dyes in the world and throughout human history, and because of its physical and chemical characteristics, it has been exposed to the environment on a large scale, endangering natural ecosystems. Precipitation, filtering, and advanced oxidation are only a few examples of physical and chemical removal techniques that are feasible, but their utilization is currently constrained by issues with operational expenses and the production of chemical sludge. In this review, we can see various decolourization rates of isolated strains, demonstrating that fungal strains exhibit the greatest decolourization. Which, while costly at the industrial level, takes more time and a sophisticated media for its Growth maintenance. In order to address these limitations, biological treatment methods have drawn more and more attention. One such technique is the ongoing study of indigo breakdown and discolouration utilising laccase enzymes. As well as being employed in ligninolytic reactions and the breakdown of indigo, laccase enzymes have been used to break down a variety of colours. Due to their high activity and stability, these enzymes have the tremendous benefit of being usable at the industrial level. The requirement for high-yield enzyme synthesis and separation while retaining a suitable degree of enzyme activity and stability, however, poses a serious obstacle to the widespread use of laccase enzymes. The synthesis of diverse biological laccase enzymes coming from fungus, bacteria, and plants in order to get around these restrictions was thoroughly evaluated in indigo discolouration and degradation tests.

There are benefits and drawbacks to indigo discolouration and breakdown caused by bacterial whole cells or enzymatic conversion. The full cell system can be applied directly to a large scale in activated sludge, but it must be adjusted for cell cultivation and intracellular enzyme synthesis. Fungi, in particular, need a long the fermentation process cycle to produce the ideal cell mass and enzyme output. Laccase can oxidize a range of phenolic molecules, producing randomly cross-linked or oxidized by-products; if not, it may inhibit the whole cell reaction and reduce the efficiency of degradation.

REFERENCES

1. Spadaro JT, Isabelle L, Renganathan V. (1994). Hydroxyl radical mediated degradation of azo dyes: Evidence for benzene generation. Environ Sci Technol 28(7):1389-93.
2. Li HX, Xu B, Tang L, Zhang JH, Mao ZG. (2015). Reductive decolorization of indigo carmine dye with Bacillus sp. MZS10. Int Biodeterior Biodegrad 103:30-7. doi: 10.1016/j.ibiod.2015.04.007.
3. Bankole PO, Adekunle AA, Obidi OF, Olukanni OD, Govindwar SP. (2017). Degradation of indigo dye by a newly isolated yeast, *Diutina rugosa*, from dye wastewater polluted soil. J Environ Chem Eng 5(5):4639–48.

4. Kurade MB, Waghmode TR, Patil SM, Jeon BH, Govindwar SP. (2017). Monitoring the gradual biodegradation of dyes in a simulated textile effluent and development of a novel triple-layered fixed-bed reactor using a bacterium-yeast consortium. Chem Eng J 307:1026–36. doi: 10.1016/j.cej.2016.09.028.
5. Jeffords DL, Lance PH, Dewolf WC. (1977). Severe hypertensive reaction to indigo carmine. Urology 9(2):180–1. doi: 10.1016/0090-4295(77)90192-3.
6. Chia MA, Odoh OA, Ladan Z. (2014). The indigo blue dye decolorization potential of immobilized *Scenedesmus quadricauda*. Water Air Soil Pollut 225(4):1–9. doi: 10.1007/s11270-014-1920-2.
7. Papadopoulou K, Kalagona IM, Philippoussis A, Rigas F. (2013). Optimization of fungal decolorization of azo and anthraquinone dyes via Box-Behnken design. Int Biodeterior Biodegrad 77:31–8. doi: 10.1016/j.ibiod.2012.09.011.
8. Velmurugan G, Ramprasath T, Swaminathan K, Mithieux G, Rajendhran J, Dhivakar M, et al. (2017). Gut microbial degradation of organophosphate insecticides induces glucose intolerance via gluconeogenesis. Genome Biol 18(1):1–17. doi: 10.1186/s13059-016-1134-6.
9. Balan DSL, Monteiro RTR. (2001). Decolorization of textile indigo dye by ligninolytic fungi. J Biotechnol 141:141-5. doi: 10.1016/S0168-1656(01)00304-2.
10. Chang JS, Chou C, Chen SY. (2001). Decolorization of azo dyes with immobilized *Pseudomonas luteola*. Process Biochem 36:757–63. doi: 10.1016/S0032-9592(00)00274-0.
11. Boran F, Birhanli E, Yesilada Ö. (2019). Comparison of indigo carmine decolorization by *Pseudomonas aeruginosa* and crude laccase enzyme from Funalia trogii. Turk J Biol 43(1):37–46.
12. Hsueh CC, Chen BY. (2007). Comparative study on reaction selectivity of azo dye decolorization by *Pseudomonas luteola*. J Hazard Mater 141(3):842–9. doi: 10.1016/j.jhazmat.2006.07.034.
13. Chen KC, Wu JY, Liou DJ, Hwang SC. (2003). Decolorization of the textile dyes by newly isolated bacterial strains. J Biotechnol 101(1):57-68. doi: 10.1016/S0168-1656(02)00303-6.
14. Fang H, Wenrong H, Yuezhong L. (2004). Investigation of isolation and immobilization of a microbial consortium for decoloring of azo dye 4BS. Water Res 38(16):3596–604.
15. Arutchelvan V, Albino DJMV. (2003). Decolourization of textile mill effluent by *Sporotrichum pulverulentum*. Indian J Environ Ecoplaning 7:59–62.
16. Hao OJ, Kim H, Chiang PC. (2000). Decolorization of wastewater. Crit Rev Environ Sci Technol 30(4):449–505.
17. Deshmukh R, Khardenavis AA, Purohit HJ. (2016). Diverse metabolic capacities of fungi for bioremediation. Indian J Microbiol 56(3):247–58. doi: 10.1007/s12088-016-0584-6.
18. Yavuz M, Kaya G, Aytekin Ç. (2014). Using *Ceriporiopsis subvermispora* CZ-3 laccase for indigo carmine decolourization and denim bleaching. Int Biodeterior Biodegrad 88:199–205. doi: 10.1016/j.ibiod.2013.10.014.
19. Chia MA, Odoh OA, Ladan Z. (2014). The indigo blue dye decolorization potential of immobilized *Scenedesmus quadricauda*. J Water Air Soil Pollut 225:1-9. doi: 10.1007/s11270-014-1920-2.
20. Verma P, Madamwar D. (2003). Decolorization of synthetic dyes by a newly isolated strain of Serratia marcescens. World J Microbiol Biotechnol 19:615–8.
21. Curr E. (2002). Encyclopedia of microscopic stain. Leonard Hall (Books) Ltd.
22. Aksu Z. (2003). Reactive dye bioaccumulation by *Saccharomyces cerevisiae*. Process Biochem 38:1437–44.
23. Knackmuss HJ. (1996). Basic knowledge and perspectives of bioelimination of xenobiotic compounds. J Biotechnol 51:287–95.
24. Christopher J, Owen PW, Ajay S. (2002). Biodegradation of dimethyl phthalate with high removal rates in a packed bed reactor. World J Microbiol Biotechnol 18:7–10.
25. Nilsson R, Nordlinder R, Wass U, Meding B, Belin L. (1993). Asthma, rhinitis, and dermatitis in workers exposed to reactive dyes. Br J Ind Med 50:65–70.
26. Yang J, Lin Q, Ng TB, Ye X, Lin J. (2014). Purification and characterization of a novel laccase from *Cerrena* sp. HYB07 with dye decolorizing ability. PLoS One 9:1–14. doi: 10.1371/journal.pone.0110834.
27. Ramya M, Anusha B, Kalavathy S. (2008). Decolorization and biodegradation of indigo carmine by a textile soil isolate *Paenibacillus larvae*. Biodegradation 19:283–91. Available from: https://pubmed.ncbi. nlm.nih.gov /17554499.
28. Theerachat M. (2012). Comparison of synthetic dye decolorization by whole cells and a laccase enriched extract from *Trametes versicolor* DSM11269. Afr J Biotechnol 11:1921–31. Available from: https://www.ajol.info/index.php/ajb/article/view/100368.
29. Sharma MK, Sobti RC. (2000). Rec effect of certain textile dyes in *Bacillus subtilis*. Mutat Res Genet Toxicol Environ Mutagen 465:27–38.
30. Rafi F, Fraeankalin WCC. (1990). Optimization of cultural condition for decolorization of textile effluent. Appl Environ Microbiol 56:2146.
31. Jadhav JP, Govindwar SP. (2006). Biotransformation of malachite green by *Saccharomyces cerevisiae* MTCC 463. Yeast 23:315–23.
32. Claus H, Faber G, König H. (2003). Redox-mediated decolorization of synthetic dyes by fungal laccases. Appl Microbiol Biotechnol 59:672–78. doi: 10.1007/s00253-002-1047-z.
33. Li HX, Zhang RJ, Tang L, Xu B, Zhang JH, Guo Y, Mao ZG. (2015). Biodegradation of indigo carmine by a newly isolated strain of *Bacillus* sp. MZS10. J Environ Chem Eng 3:1250–59. doi: 10.1016/j.jece.2015.03.022.
34. Huang C, Yu X, Zheng B, Liu Y, Liu X, Yan L. (2008). Optimization of laccase-catalyzed decolorization of reactive black 5 using response surface methodology. J Biotechnol 136:54–62. doi: 10.1016/j.jbiotec.2008.01.024.

35. Yoshida O, Harada T, Miyagawa M, Kato T. (1971). Bladder cancer in workers of the dyeing industry. Igaku No Ayumi:421–2. Available from: https://hero.epa.gov/hero/index.cfm/reference/details/reference_id/377145.
36. Wang W, Zhang Z, Ni H, Yang X, Li Q, Li L. (2012). Decolorization of industrial synthetic dyes using engineered *Pseudomonas putida* cells with surface-immobilized bacterial laccase. Microb Cell Fact 11:75. doi: 10.1186/1475-2859-11-75.
37. Fujita M, Kuroda C, Hosomi N, Inoue E, Kuriyama K, Ohhigashi H, et al. (1995). Dye-injection method for placement of an infusion catheter in regional hepatic chemotherapy. J Vasc Interv Radiol 6(1):119–23.
38. Jabs CFI, Drutz HP. (2001). The role of intraoperative cystoscopy in prolapse and incontinence surgery. Am J Obstet Gynecol 185(6):1368–73. doi: 10.1067/mob.2001.119078.
39. Held C, Kandelbauer A, Schroeder M, Cavaco-Paulo A, Guebitz GM. (2005). Biotransformation of phenolics with laccase containing bacterial spores. Environ Chem Lett 3(2):74–7. Available from: https://www.researchgate.net/publication/225652682.
40. Nakajima K, Hirota K, Nodasaka Y, Yumoto I. (2005). *Alkalibacterium burdens* sp. nov: An obligate alkaliphile that reduces an indigo dye. Int J Syst Evol Microbiol 55:1525–30.
41. Nicholson SK, John P. (2004). Bacterial indigo reduction. Biocatal Biotransformation 22(5–6):397–400.
42. Jayarajan M, Arunachalam R, Annadurai G. (2011). Agricultural wastes of jackfruit peel nano-porous adsorbent for removal of rhodamine dye. Asian J Appl Sci:263–70.
43. Banat IM, Nigam P, Singh D, Marchant R. (1996). Microbial decolorization of textile-dye-containing effluents: A review. Bioresour Technol 58(3):217–27. doi: 10.1016/S0960-8524(96)00113-7.
44. Click RB, Pasternak JJ. (2002). Molecular biotechnology: Principles and applications of recombinant DNA. 2nd ed. ASM Press.
45. Jamee R, Siddique R. (2019). Biodegradation of synthetic dyes of textile effluent by microorganisms: An environmentally and economically sustainable approach. Eur J Microbiol Immunol 9(4):114. doi: 10.1556/1886.2019.00018.
46. Gaunt IK, Kiss IS, Grasso P, Gangolli SD. (1969). Short term toxicity study of indigo carmine in the pig. Food Cosmet Toxicol 7(1):17–24.
47. Stolte M, Vieth M. (2001). Pathological basis of mucosal changes in the esophagus. What the endoscopist can (and must) do. Atta Endoscopica 31(2):125–30.
48. Kennedy WF, Wirjoatmadja K, Akamatsu TJ, Bonica JJ. (1968). Cardiovascular and respiratory effects of indigo carmine. J Urol 100(6):775–8.
49. Zollinger H. (2003). Colour chemistry: Synthesis, properties and applications of organic dyes and pigments. 3rd ed. WILEY-VCH Publishers.
50. Ahn DH, Chang WS, Yoon TI. (1999). Dyestuff wastewater treatment using chemical oxidation, physical adsorption and fixed bed biofilm process. Process Biochem 34(5):429–39.
51. Azmi W, Sani RK, Banerjee UC. (1998). Biodegradation of triphenylmethane dyes. Enzyme Microb Technol 22(3):185–91.
52. Paz A, Carballo J, Pérez MJ, Domínguez JM. (2017). Biological treatment of model dyes and textile wastewaters. Chemosphere 181:168–77. Available from: https://europepmc.org/article/med/28437742.
53. Dellamatrice PM, Silva-Stenico ME, Moraes LAB, Fiore MF, Monteiro RTR.. (2017). Degradation of textile dyes by cyanobacteria. Braz J Microbiol 48(1):25–31.
54. Ikeda K, Sannohe Y, Araki S, Inutsuka S. (1980). A new trial in endoscopic diagnosis for stomach cancer: Intra-arterial dye (IAD) method. Gastrointest Endosc 26(1):1–4. doi: 10.1016/S0016-5107(80)73247-9.
55. Ng TY, Datta TD, Kirimli BI. (1976). Reaction to indigo carmine. J Urol 116(1):132–3. doi: 10.1016/S0022-5347(17)58715-7.
56. Yang PY, Nitisoravut S, Wu JS. (1995). Nitrate removal using a mixed-culture entrapped microbial cell immobilization process under high salt conditions. Water Res 29(6):1525–32.
57. Tian C, Zhou Y, Chen Q, Cheng H. (2013). Decolorization of indigo dye and indigo dye-containing textile effluent by *Ganoderma weberianum*. Afr J Microbiol Res 7:941–7.
58. Krull R, Hemmi M, Otto P, Hempel DC. (1998). Combined biological and chemical treatment of highly concentrated residual dyehouse liquors. Water Sci Technol:339–46.
59. Ismail T. Dyes, (2008). colors & pigments. Book Dyes.
60. Pearce CI, Lloyd JR, Guthrie JT. (2003). The removal of colour from textile wastewater using whole bacterial cells: A review. Dyes Pigments 58:179–96.
61. Butterworth KR, Hoosen J, Gaunt IK, Kiss IS, Grasso P. (1975). Long term toxicity of indigo carmine in mice. Food Cosmet Toxicol 13(2):167–76.
62. Birhanli E, Yesilada Ö. (2006). Increased production of laccase by pellets of *Funalia trogii* ATCC 200800 and *Trametes versicolor* ATCC 200801 in repeated-batch mode. Enzyme Microb Technol 39(6):1286–93. doi: 10.1016/j.enzmictec.2006.03.015.
63. Li S, Tang B, Liu Y, Chen A, Tang W, Wei S. (2016). High-level production and characterization of laccase from a newly isolated fungus *Trametes* sp. LS-10C. Biocatal Agric Biotechnol 8:278–85. doi: 10.1016/j.bcab.2016.10.008.
64. Jaiswal S, Gomashe AV, Agrawal S. (2014). Decolorization potential of *Bacillus* sp. for removal of synthetic textile dyes. J Curr Microbiol Appl Sci 3(12):83–88. Available from: http://www.ijcmas.com.

65. Mora Muñoz E, Pineda Insuasti J, Duarte Trujillo S, Del Río CA, Soto Arroyave C, Huaca Pinchao MJ. (2016). Technology for denim finishing using laccase enzymes: A review. IOSR J Polym Text Eng (IOSR-JPTE). Available from: https://www.iosrjournals.org/iosr-jpte/papers/Vol3-Issue6/C03061519.pdf.
66. Nyanhongo GS, Gomes J, Guebitz GM, Zvauya R, Read J, Steiner W. (2002). Decolorization of textile dyes by laccases from a newly isolated strain of *Trametes modesta*. Water Res 36(6):1449–56. doi: 10.1016/S0043-1354(01)00365-7.
67. Kitamura S, Kohno Y, Yamada T, Sugihara K, Ohta S. (2006). Production of superoxide radical in reductive metabolism of a synthetic food coloring agent. Indigo carmine and related compounds. Life Sci 11:60–6.
68. Levin L, Melignani E, Ramos AM. (2010). Effect of nitrogen sources and vitamins on ligninolytic enzyme production by some white-rot fungi. Dye decolorization by selected culture filtrates. Bioresour Technol 101(12):4554–63. doi: 10.1016/j.biortech.2010.01.102.
69. Sarnthima R, Khammuang S. (2008). Evaluation of dyes decolourisation by the crude enzyme from Pleurotus sajor-caju grown on sorghum seed media. Pak J Biol Sci 11(14):1859–64. doi: 10.3923/pjbs.2008.1859.1864