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REVIEW ARTICLES

Enhanced decolourisation and degradation of azo dyes using wild versus mutagenic improved bacterial strain: a review

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Abstract

Rapid industrialization over the last few decades has greatly impacted the environment, as various industries release effluent (textile dyeing/printing) which contains copious amount of xenobiotic compound such as azo dyes, salts, organic pollutants, metals, etc. The recalcitrant azo dyes present in textile effluent cause great impact on biota thereby disturbing the integrity of ecosystem. The wastewater discharged into water bodies leads to decline in flora-fauna prevalence due to alteration of the physiochemical characteristics of water (increment in the BOD/COD). Although various physico-chemical methods used for effluent treatment are effective; but are often associated with secondary disposal problem due to the formation of concentrated toxic sludge and presence of toxicants. In recent years, microbial biodegradation has emerged as highly promising approach for textile effluent treatment of xenobiotic toxicants. The indigenous bacteria contain pool of oxidoreductive enzymes (viz. azoreductase, laccase, peroxidase, etc.) that utilize complex xenobiotic compounds of the dyestuffs as substrates and break them into non-toxic by-products. However, limited studies have been reported citing the enhancement in the dye degradation and detoxification efficacy of bacterial species by random mutagenesis approach for the efficient bioremediation of wide spectrum of pigments and dyes. The current review presents the untapped potential of mutagenic approach. It highlights the need by a comprehensive account on the exploration of physical (UV, gamma) and chemical (viz. ethidium bromide, ethyl methane sulfonate) mutagenic agents, respectively. These mutagens alter the genomic profile of the bacteria and facilitate them with higher biodegradation and detoxification efficacies by upregulating the production of oxidoreductive enzymes. The study narrates the prospective for utilization of the wild versus mutant approach consecutively for the mitigation of wide spectrum of azodyes/pigments based on experimental modulations. Review suggests recommendations for the development of novel mutant strains for effective bioremediation strategy as per the permissible compliance limits of pollution board. The approach will thereby able to deal with environmentally noxious pollutants turning one of the most polluted textile industry among the red list to green industry through translational technology.

Keywords Azo dyes · Bioremediation · Mutation · Textile effluent

Introduction

The textile industry is India's oldest and most important economic sector, stretching back for many centuries. It is indeed the second largest sector after agriculture in terms of overall Indian exports, accounting for roughly 11% of total exports. Indian textile sector is predicted to grow more than US$209 billion by 2029 (http://www.ibef.org/industry/textiles.aspx). Since ancient times, various dyes have been used to improve the aesthetic value of objects. According to historical records, dyes were first used 3500 years ago. They were basically derived from various natural resources such as minerals and vegetables found in Middle East Asia viz. *Rubia tinctorum, Reseda luteola* as well as Brazilwood viz. *Caesalpinia* sp. These natural substances contain large amount of biologically active secondary metabolites which impart color to them [for instance, flavonoids (yellow), quinones (brown and red), azaphilones (red)]. On the other hand, some animal based colorants were also available such as cochineal red obtained from *Dactylopiidae coccus* and Tyrian purple from shellfish (Selvaraj et al. 2021; Pinheiro

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et al. 2022). However, by the early twentieth century, natural dyes were nearly swapped by synthetic dyes due to their high demand, wide colour range, low cost, and light-fastness. With the exception of some inorganic pigments, virtually all dyestuffs available in market are synthetic substances. Each year, tons of these similar coloured compounds enter market and are exploited to develop wide range of products (Ventura-Camargo and Marin-Morales 2013).

Dyes are divided into two categories based on their origin as natural dyes, primarily extracted from plant and animal based sources whereas synthetic dyes are manufactured consistently from various organic molecules. These dye compounds have the property of absorbing different wavelength of electromagnetic energy and transmitting the remaining ones (~ 350–700 nm) hence imparting wide range of colours to them. Dye molecules basically have two types of molecules i.e. chromophores i.e. color bearing group (such as –N=N– in case of azo dyes) and auxochromes (such as $–NH_2$, –COOH, $–SO_3H$, etc.) which is responsible for different intensities of colours exhibited by chromophore. This happens because of the alteration in overall energy of the electron cloud around the resonance stabilized molecules (Benkhaya et al. 2020). The dyes are also classified according to chromophore present in their structure as azo (mono-azo, di-azo, tri-azo, poly-azo), phthalein, trimethylmethane, indigo, nitroso, anthraquinone, sulfur, nitro, lactone dyes, etc. (Selvaraj et al. 2021). Among them, azo dyes are the most prevalently used dye group. Brüschweiler and Merlot (2017) reported that they account for 60–70% of all dyestuff used in textile production.

Approximately 1500 azo dyes were used at industrial scale for colouring of fabrics in textile industries, as well as an additive in petroleum-derived products. They also have wide range of applications in pharmaceutical, food, cosmetic, leather and printing industries. Aside from its versatility, there are several other features that makes them superior to the natural dyes such as cost effectiveness, ease of synthesis, great fixative properties, ability to produce variant colours, etc. (Moosvi et al. 2007). Although some azo dyes have been found to be harmless, the vast majority of azo dyes are toxic and not eco-friendly. The wastewater they produce has degrading impact on the ecosystem. Azo dye's presence in water bodies makes them vividly coloured even at very low concentration. Jamee and Siddique (2019) reported that all the synthetic colorants are chemically complex in nature which makes them highly resistant to degradation. Their inappropriate disposal not only challenge aquatic ecosystem aesthetically but also creates stressful conditions for water inhabitants (i.e. flora and fauna) due to low light and oxygen unavailability. Hence, this leads to the inhibition of photosynthesis and eventually degrade the water quality. Aside from their cosmetic impact, azo dyes have a negative influence on BOD, COD of water streams along with increase in turbidity and suspended solids, respectively. Overall, it creates adverse effect on integrity of the ecosystem by affecting the food chain at various trophic levels. Many synthetic azo dyes along with their metabolites are carcinogenic as well as genotoxic, posing human health hazards (Yusuf 2019). Considering the detrimental impact of these chemical dyes on environment, natural dyes can make a comeback in days to follow. Many third world countries are designated as azo free since 1990's, but still do not comply and still use them in industrial scale capacities. In order to maintain the ecological balance, global awareness is being spread among people to encourage the utilization of dyes from natural resources. Looking at the present perspective, promotion of the use of natural dyes are in place but still azodyes are preferred due to its complexation properties with fabric. Although various limitations persists that restricts the use of natural dyes at industrial scale as high cost, lower affinity to fibres, tedious process of dye extraction, use of hazardous mordants, etc. But the increasing interest of mankind in natural products has fascinated the researchers to develop eco-friendly alternatives as a substitute to synthetic dyes and overcome the hindrances for regular usage at industrial scale (Křížová 2015). Bechtold et al. 2003 reported the use of plant sources present in Austrian climate for the production of dyes and their extraction as well as dyeing procedures. Another major challenge associated with use of natural dyes is use of hazardous mordants (rich in heavy metal toxicants), that are essential for binding of dyes with fabric. Elsahida et al. (2019) focussed on the positive environmental, ecological and social impact of use of the natural dyes in textile industries. They have also reported that tannins can be used as an alternative for the toxic mordant that are currently in practice. Similarly, many researchers are working on different aspects of this area in order to promote the sustainability in textile industry.

Furthermore, numerous studies have found that wastewater from textile industries have adverse effects on the plant growth and yield. Azo dyes present in effluent exhibit profound phytotoxic properties such as decreasing seed viability and also affect overall plant health by interfering with the nutrient uptake from environment. Due to above mentioned adversities, vital ecological tasks that are fulfilled by plants are greatly affected such as providing habitat for wildlife, preventing soil erosion and so on (Rawat et al. 2016). Other toxins present in textile effluent include suspended solids, heavy metals, and their conjugates. The most common metals conjugated with dyes are zinc, mercury, copper, lead and arsenic, respectively. Copious amount of these heavy metal conjugates present in the wastewater is a cause of great concern (Saxena and Gupta 2020). As a result, prior to final release into the environment, treatment of industrial effluents containing azo dyes and their metabolites is necessary. Conventionally, various physical and chemical procedures

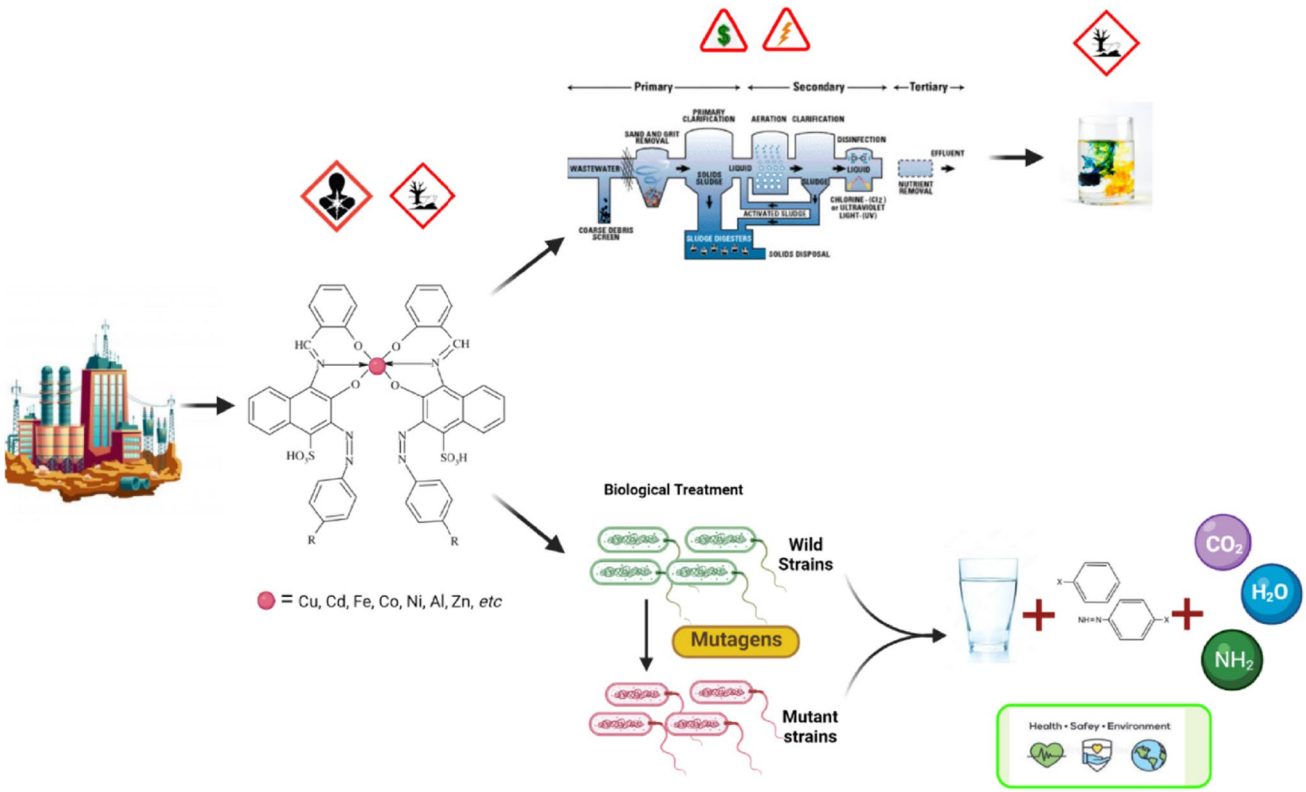

Fig. 1 Exploring the bioremediation potential of genetically modified microorganisms for efficient textile effluent treatment at industrial scale

that are already in use, such as adsorption, ozonation, ultrafiltration, oxidation, ion-exchange, precipitation, osmosis and radiation treatment, etc. are usually ineffective as they possess large capital investment, limiting their use in terms of scale of operation and inappropriate effluent profile (as per permissible discharge limits). Furthermore, these methods have obvious drawbacks such as requiring more energy and chemicals, being incapable of alleviating hazards related to xenobiotic compound, substantial sludge production (categorized as biohazard) which may further require complicated procedures for its disposal (Saratale et al. 2011). Hence, alternative greener and cheaper methods need to be explored for efficient decolorization and degradation of synthetic dyes. In this review, an attempt is made to comprehend the importance of various biological (mostly bacterial) methods for treatment of textile effluent and subsequently enhancing their potential using mutagenesis (i.e. UV rays, X rays, EtBr, EMS, etc.) with reference to enhanced decolouration, biodegradation and detoxification of their metabolic end products. Mutagens induce alteration in the genetic profile of these bacteria which may lead to multiple fold higher expression of dye degrading genes. Prior review suggest that limited studies along with statistical inferences have been reported exploring the random mutagenic approach to be exploited in the industrial scale capacity. Therefore, the current script may suggest in the overall improvement of efficacy during treatment processes with the intervention of mutagenesis modalities (Fig. 1).

Bioremediation

The exploitation of microbes for bioremediation of toxic pollutants, is a growing field of study in earth sciences. The indigenous microbes become resistant over a period of time and acclimatize to toxic habitat. This leads to the development of new resistant strains that transform various toxic chemicals into simpler non-toxic metabolites. The microbial cells possess various biotransformation enzymes (i.e. oxidoreductases, peroxidases oxygenases, ligninases, hydrolases, and laccases) that are responsible for the degradation and detoxification of xenobiotic compounds (Slama et al. 2021). Degradation may take place by either of these processes i.e., adsorption, alleviation, elimination, biosorption, bioaccumulation, or mineralization of the harmful wastewater molecules. A vast variety of organism has been studied in recent years for the biodegradation of azo dyes such as bacteria, fungus, algae, and yeasts, etc. (Pandey et al. 2007). One of the fascinating biological aspects of wastewater treatment is the identification of those microbial species which

have significant degradation capabilities. Furthermore, azo dyes may undergo mineralization and get converted to non-toxic end products in certain environmental conditions (Chen 2006). This technique drew a lot of attention because it was eco-friendly, safe, easy to use, clean (no sludge), less energy intensive as well as cost-effective due to its retrofittable nature (Singh et al. 2017).

Bio-removal of recalcitrant azo dyes

Several microbes are capable of decolorizing vast range of dyes. Lately, the use of algae has received great interest in the realm of effluent bioremediation. Despite the high toxicity of azo dyes in aquatic environment, there is no significant reduction in algal growth. The sustainable algae in these polluted water bodies perform phycoremediation by adsorption and enzymatic degradation of these recalcitrant dyes. Ergene et al. (2009) reported that *Scenedesmus quadricauda* isolated from lake adsorb significant amount of RBBR dye (48.3 mg dye removed per gram of Biomass) in just 5 h. Also, a literature survey by Pratiwi et al. (2019) states that *Ulva lactuca* is capable of 91.92% degradation of methylene blue in just 110 min through biosorption process. However, in most of the studies it was found that degradation of dyes by algae takes much longer times as compared to bacteria or fungi. Degradation of Reactive black 5 by *Chorella vulgaris* took ten days (Ishchi and Sibi 2020) as compared to *Pseudomonas fluorescens,* which degraded the same amount of dye (200 mg/L) in just 5 days (Sriram et al. 2013).

Alternatively, there have been some studies that shown the use of plants for treatment of dye laden wastewater. As a result, plants growing in the vicinity of dye polluted environment develop specific metabolic mechanism to successfully deal with these synthetic contaminants. Plants transport the contaminants from soil and water and employ their inherent mechanism to metabolize the recalcitrant compounds to release the non-toxic by-products into the ecosystem. According to the study conducted by Aubert and Schwitzguebel (2004), plant species of *Rheum rabarbarum* possess the ability to absorb and metabolize sulphonated anthraquinone dyestuffs. The enzymes present in this plant utilize anthraquinones as substrates to produce non-toxic end products and shows significant dye degradation competency (700–800 mg/L). Some researchers accessed the potential of *Petunia grandiflora Juss.* plant to treat the wastewater saturated with mixture of dyes. The cultured species of *Petunia grandiflora* decolorized 86% of Brilliant Blue G dye and metabolized them into non-toxic compounds, as confirmed by GC–MS and FTIR analysis (Watharkar et al. 2013). Study conducted by Arivoli et al. (2017) involves construction of artificial wet land using textile effluent in lab containing *Alternanthera sessilis*, resulting in significant reduction in various parameters like TDS, TSS, COD, BOD, hardness, etc. Therefore, it was concluded that aquatic plants like *Alternanthera sessilis* also play major role in phytoremediation. In spite of the vast studies done for the development of effective bioremediation techniques, industrial-scale phytoremediation is not currently feasible due to a number of issues such as evaporation as well as transpiration of volatile toxic compounds, relocation of heavy metals, inability of plants to grow in dye saturated environment, lack of detailed metabolic pathways utilized by plants and the requirement of large areas to reclaim.

Similarly, several studies are performed to investigate the oxidation potential of filamentous fungi. Specific oxidoreductases i.e. laccases and peroxidases (LiP and MnP), present in most basidiomycetes play a vital role in degrading aromatic compounds (Madhavi et al. 2007). Similarly, *Phanerochaete chrysosporium* can mineralize and degrade ABR 5 dye with up to 92% efficacy (Enayatizamir et al. 2011). Although the fungal treatment of textile effluent is very effective but it has some limitations such as the prolonged retention of fungal mycelium in water in order to achieve successful decolorization whereas another cause of concern is safe disposal of fungal culture after completion of the reaction.

On the other hand, there has been very little research done for yeast's decolorization ability and it has primarily been studied regarding biosorption. Some yeast species of ascomycetes like *Pseudozyma rugulosa* Y-48, *Issatchenkia occidentalis* and *Candida krusei* G-1 have been screened to study the degradation and detoxification of azo dyes and it was concluded that yeast act as a promising dye adsorbent or bio-accumulators (Ramalho et al. 2004; Yu and Wen 2005). Dye molecules bind to the surface of cell via biosorption or accumulate in the cell via bio-accumulation. These process don't degrade the organic toxicants rather get them accumulated in the ecosystem but biodegradation has the potential to degrade the pollutants through metabolization and mineralization. Hence, algae and yeast are not considered favourable for bioremediation at industrial scale for azo dyes treatment. However, extensive studies have been done on bacteria as they are considered principal contributors to wastewater treatment due to their high degradation and decolourization potential. Additionally, they are cost-effective, applicable to broad spectrum of disseminated dyes and produces very less/no sludge (Khehra et al. 2006) (Table 1).

Bacteria as potential candidates for bioremediation

Amongst all the bio-based treatments found to alleviate these recalcitrant dyes, indigenous bacterial species found at contaminated sites are showcased as versatile organisms as they can be applied in either of these conditions i.e., single organism cultures, in bioreactors, microbial consortia, for detoxification of azo dye and its hazardous metabolites

Table 1 Degradation of various azo dyes using various biological agents

Remediation approach	Specification		Culture conditions	References
	Strain	Dye		
Algae (phycoremediation)	*Chlorella pyrenoidosa*, *C. vulgaris*, *Oscillateria tenuis*	C.I red, acid red, acid yellow, direct blue, etc.	Algae culture broth, 96 h, 25 ± 1 °c, ph 7.5, photoperiod (16 h light; 8 h dark)	Jinqi and Houtian (1992)
	Scenedesmus quadricauda (immobilized and heat inactivated strain)	Remazol brilliant blue R	BG11 medium, pH 7, 21 °C, shaken at 150 rpm, Photoperiod (16 h light; 8 h dark)	Ergene et al. (2009)
	Ulva lactuca	Methylene blue	25 mg/l (dye conc.), pH 8, 110 min, Photoperiod (16 h light; 8 h dark)	Pratiwi et al. (2019)
	Chlorella vulgaris	Reactive black 5, direct blue 71, and disperse red 1	MSM media, pH 5–8, 30 °C, 12 days, static condition, Photoperiod (16 h light; 8 h dark)	Ishchi and Sibi (2020)
Plants (phytoremediation)	*Apium graveolens*, *Rheum rabarbarum*, *Rumex hydrolapatum*, *Rumex acetosa*	Sulphonated anthraquinone dyes (AQ-1-S; AQ-2-S; AQ-1,5-SS; AQ-2,6-SS; AQ-1,8-SS)	Luwasa as plant nutrient in effluent filled tanks, 20–25 °C, light exposure of about 15 h/day,	Aubert and Schwitzguebel (2004)
	Petunia grandiflora Juss (wild and tissue cultured plantlets)	Brilliant blue G and effluent	Dye containing water, 36–72 h	Watharkar et al. (2013)
	Alternanthera sessilis	Raw effluent (10L)	Arificial Wet land (gravel and sand layer), diluted effluent as nutrient source	Arivoli et al. (2017)
Fungi (mycoremediation)	*Phanerochaete chrysosporium*	Diazo dye reactive black 5 (RB5)	Potato dextrose agar (PDA), 72 h, 37 °C, immobilized on nylon sponge and sunflower seed shells, veratryl alcohol used as supplement	Enayatizamir et al. (2011)
	Ganoderma sp.	Amaranth dye	optimized medium containing yeast extract and starch, pH 5.5, 28 °C, shaken at 150 rpm, 8 h	Madhavi et al. (2007)
Yeast bioremediation	*Pseudozyma rugulosa* Y-48 and *Candida krusei* G-1	Reactive brilliant red, weak acid brilliant red, reactive black, acid mordant yellow, etc	Yeast extract peptone dextrose (YEPD), 28 °C, 24 h, shaken at 200 rpm, pH 5.0–6.0	Yu and Wen (2005)
	Issatchenkia occidentalis	Methyl orange, orange II with heavy metal conjugates	Mineral salt base supplemented with glucose, 26 °C, shaken at 120 rpm, 20 h	Ramalho et al. (2004)
Bacterial bioremediation	*R. paucimobilis,R. radiobacter, B. subtilis* (immobilized and free cells)	Congo red and methyl orange conjugated with heavy metals	Cultured at 37 °C for 2–4 days at 120 rpm	Allam (2017)
	Pseudomonas putida, Bacillus cereus, Pseudomonas fluorescens, Bacillus subtilis, Alcaligens sp. and *Staphylococcus aureus*	Acid orange 10	Optimized nutrient medium, static condition, 37 °C, pH7, 24 h	Tripathi and Srivastava (2011)
	Lysinibacillus fusiformis	Methyl red	Minimal salt Medium, 120r/min, 30 ± 2 °C, pH 7.5, 2 h	Sari and Simarani (2019)
	Escherichia coli	C.I. reactive red 22	LB broth, static conditions, 30 ± 2 °C, pH 7- 9	Chang et al. (2000)
	Marinobacterium sp.	Metanil yellow G	Minimal salt Medium, static conditions, 5% salinity, 30 °C	Tian et al. (2021)
	Rhodococcus strain UCC 0010	Congo red	pH 2.8, 0.75%v/v carbon source, at 35 °C, coconut waste as substrate) within 4 h	Sundarajoo et al. (2022)
	Pseudoarthrobacter, Gordonia, Stenotrophomonas, Sphingomonas PsGo consortia	Reactive Black 5	MSM media, 25–30 °C, pH 9, and using glucose as C-source and NH4H2PO4 N-source	Eskandari et al. (2019)
	Pseudoarthrobacter, Gordonia, Stenotrophomonas, Sphingomonas StSp consortia		MSM media, 37 °C, pH 11, and with lactose as C-source and $NH_4H_2PO_4$ used N-source	

(Pinheiro et al. 2022). Also, various strains have the ability to act under distinct physico-chemical parameters, such as pH, salinity, and temperature, with good performance under industrial and environmental relevant conditions. According to study conducted by Sarkar et al. 2017, several bacterial species and their degradation mechanism have been explored throughout these years for remediation of dye laden sites (Fig. 2). Sari and Simarani (2019) reported that *Lysinibacillus fusiformis* W1B6 decolorize 96% of methyl red (upto 100 mg/L) within span of two hours under the optimized conditions i.e., static condition, pH 7.5, inoculum 10% and at 28–32 °C through biosorption and action of oxidoreductive enzymes. However, Chang et al. (2000) used *Escherichia coli* NO_3 to study the kinetics of bacterial dye decolourization under optimized conditions. The study revealed that the strain is highly potent for wastewater treatment as it can degrade up to 2000 mg/L of C.I. Reactive Red 22 over a wide range of temperature (20–45 °C) at around 7–9 pH. Also, the dye degradation potential decrease sharply with increase in the dissolved oxygen (DO) level of water. Some halophilic strains of bacteria are also capable of dye degradation at salinity level of 1–15%. They have decolorized metanil yellow G (MYG) at concentration of 100-400 mg/L in static conditions. It was found that *Marinobacterium* was the primary genus that was responsible for dye degradation at such high salt concentration (Tian et al. 2021). Allam (2017) demonstrated that the consortia of bacterium containing *S. paucimobilis, R. radiobacter,* and *B. subtilis,* shows better bioremediation efficacy when immobilized on Ca-alginate beads as compared to consortium of free cells. The higher efficiency may be ascribed to collaboration of multiple metabolites generated by bacteria and further shielding of cells by hard matrix of calcium alginate, thereby promoting efficient bio-treatment of industrial effluents containing dyes and heavy metals. Mahmood et al. (2016) suggested that the dye degradation potential of bacterial species is attributed to the presence of various oxidative and reductive enzymes in them. Molecular analysis revealed that these enzymes carry out the mineralization of azo dyes by azoreductase and oxidative enzymes, respectively. Few years back, a new insight has been provided by Kumar et al. (2015) on combined in vitro and in silico methods for degradation of azo dyes. Bioinformatics aided approach has been used to dock the dye molecules with the enzymes (laccase and azoreductase) present in bacteria. This data is then compared with the wet lab degradation analysis

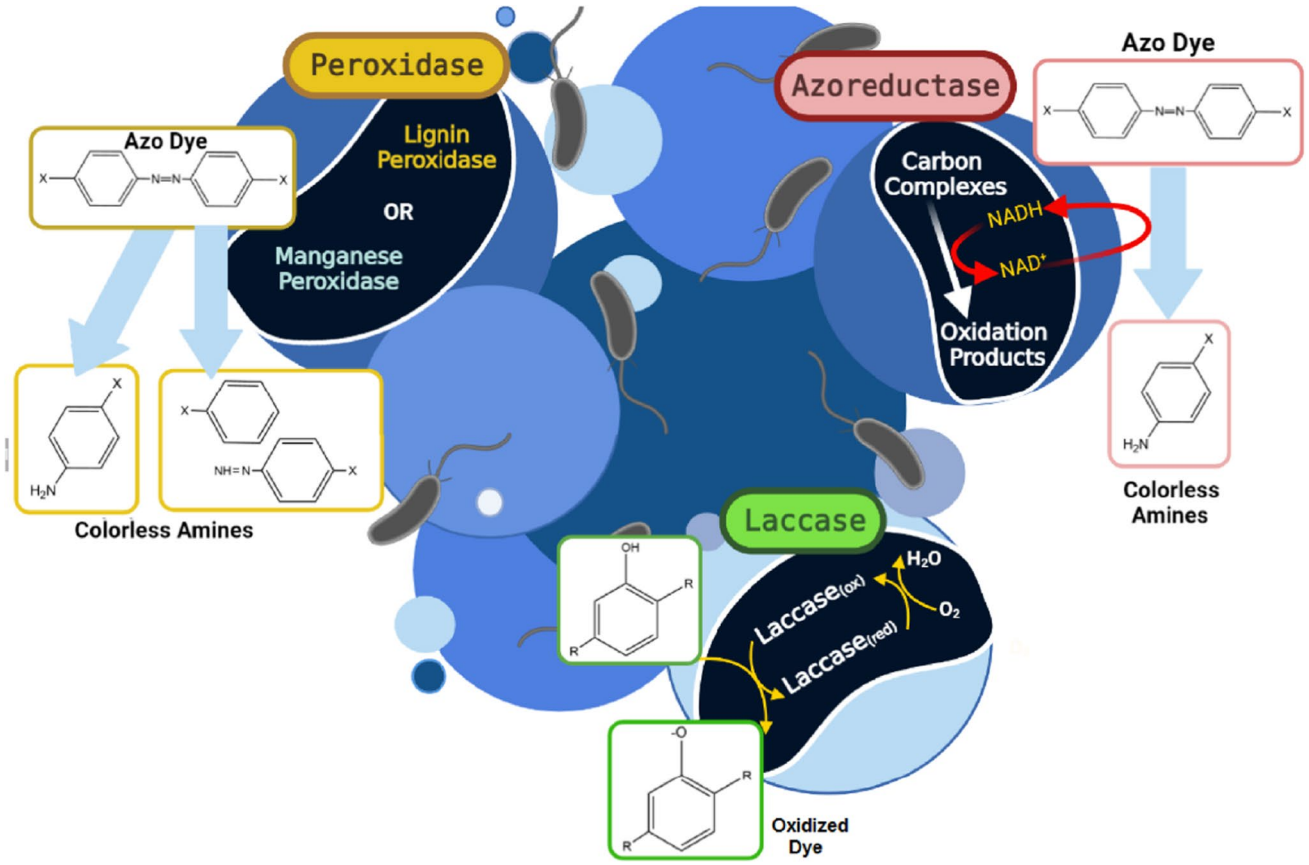

Fig. 2 Schematic diagram showing proposed degradation mechanism by three bacterial enzymes i.e. azoreductase, laccase and peroxidases respectively

and it was found that two novel bacterial strains *i.e., Lysinibacillus sphaericus* (KF032717) and *Aeromonas hydrophila* (KF032718) were capable of decolorizing Joyfix Red efficiently. The proposed in silico approach can eliminate the time-consuming preliminary screening procedures that are performed in vitro. Similar study that used response surface methodology to save the incubation time and improve the decolourization efficiency, was reported by Sundarajoo et al. (2022). They have used *Rhodococcus strain* UCC 0010 to decolorize congo red that is discharged by textile industries in rivers of Malaysia. It has reported 100% decolorization (0.2 g/L) at optimized parameters (i.e. pH 2.8, 0.75%v/v carbon source, at 35 °C, coconut waste as substrate) within 4 h. Also, phytotoxicity studies are also done on *Vigna radiata* and *Vigna unguiculata* which resulted in 100% germination rate. Maniyam et al. (2020) also used *Rhodococcus strain* UCC0016 to study bioremediation of methyl red. They have encapsulated the bacteria in gellan gum beads. Their immobilization has significantly increased methyl red disappearance and it was also found that the end products have not impacted the growth of test plants (*Triticum aestivum* and *Vigna radiata*) during toxicity analysis therefore indicating the complete breakdown of toxic dye. Bacteria have ability to easily adapt to the surrounding environment. Some mesophilic bacterial strains were isolated from cold region to study their biodegradation potential. The consortia PsGo and StSp were used to study dye degradation at 20, 50 and 100 mg/L od RB-5 azo dye and it was also reported that optimization of condition for these two consortia leads to 100% decolourization at 50 mg/L concentration of dye. Carbon and nitrogen supplements are provided such as lactose, glucose and $NH_4H_2PO_4$ (Eskandari et al. 2019) to improve their efficacy. Above reported studies indicate that bacteria are the versatile organisms that can be used for bioremediation of textile effluent due to their quality of easy adaptation to wide range of environmental parameters without compromising their efficacy.

Advantages over conventional methods

Pollution Control Board (PCB) has put the industrial sector under intense pressure due to increasing deterioration of environmental conditions. Textile industries are bound to limit the use of hazardous dyestuffs that can cause mutations and poses serious health issues. Nowadays, a variety of techniques have been evolved to lessen the impact of toxic dyes in wastewater but removal of these xenobiotic compounds is still a challenging issue as physical methods, such as use of adsorbents, ion exchange technique, coagulation and filtration, etc., just transfer the dye moieties from one phase to another rather than degrading. Also each method has its own limitations. For example, coagulation/flocculation although economically feasible but produces large amount of toxic sludge which faces disposal problems. Another widely used method is oxidation of wastewater, this method helps to decolorize the wastewater but leads to rise in COD and it adds on the extra cost as well. Adsorption of toxicants can also be done by activated carbon in order to eliminate the suspended solids and organic substances but adsorbents also face issues during disposal. During the chemical processing, ozonation is the main treatment which helps to remove azo dyes but it doesn't go well with disperse dyes and release aromatic amines as by-product. Also, chemical methods further add huge load of toxicants into environment rather eradicating them and generate massive amount of toxic sludge. On the contrary, biological processes not only works in combination with physical/chemical approach as a retrofittable technology but it is also economical, reduce sludge generation, degrade toxic/hazardous substances from textile effluent and eventually leads to healthy environment (Shah 2018).

Random mutagenesis

Wastewater discharged from textile industries contains various groups of azo dyes which makes them impervious to microbial degradation. Azo dyes are synthesized chemically with varying number of –N=N– groups. The structural complexity of these compounds makes them recalcitrant in the environment for a long period of time. The native bacterial strain is not capable of degrading the wide spectrum of azo dyes (alone or in combination) found in textile effluents. Eventually paves path to potential new strains for enhanced efficacy through technological interventions. Nowadays, genetically modified microorganisms are being used to effectively manage various issues associated with pollution. Despite the fact that bacteria are small organisms, they have a distinct set of genes that are responsible for the creation of numerous metabolic or regulatory enzymes that can improvise their bioremediation potential. The potential mutant candidates can be generated via physical/chemical treatment and the significant strains can further be exploited for their dye degrading capabilities. These mutants can perform better with respect to their wild relatives and can further be used in industrial scale capacities, respectively.

Efficacy of mutant over wild bacterial strains

Joshi et al. (2013) employed ultraviolet radiations as mutagen for *Pseudomonas* sp. LBC1 in order to amplify the activity of oxidoreductase enzymes in this bacterial strain. These ionic radiations induce genetic alterations in mutant bacterial species which was later confirmed by RAPD (random amplification of polymorphic DNA) profiling. In current context, the mutant strain obtained after UV exposure

shortened the time required for biodegradation of Green HE4B by 25% in contrast to wild type and produce slightly different metabolic end products due to the upregulation in the activity of various enzymes such as laccase, manganese peroxidase, lignin peroxidase and various oxidoreductases. Another study employed physical and chemical agents to induce mutations in *Bacillus* sp. to enhance industrial dye degradation and thereby eradicate the pollution from environment in excellent manner. *Bacillus* strain isolated from dye contaminated soil although had dye degradation potential but after exposure to various mutagenic agents like UV rays, Ethidium bromide and X rays, its activity increased in manifold (Pushpa et al. 2020). Another study by Mani and Hameed (2019) presented that the mutant bacterial strains produce larger amount of oxidoreductive enzymes as compared to wild strains. This may occur due to the alteration in genetic makeup of the microorganism as a result of induction of random mutation. In another investigation, Gopinath et al. (2009) stated the *Bacillus* sp. underwent mutation by UV and EtBr induced treatment, exhibited the significant increment in the azo dye degradation efficiency (from 200–1000 to 3000 mg/L) and minimize the duration of effective decolourization by 12–30%. Out of the pool of mutated *Bacillus* sp. isolates, only two strains viz., *ACT 1* and *ACT 2* were showing better efficiency. Lone et al. (2015) conducted the study on dye degrading *Bacillus* species isolated from soil. They exposed the bacterial petriplates to UV light as well as various concentration of EtBr and found that fewer colonies appeared in petriplates with increasing concentration of mutagens. Some studies investigated the use of error prone PCR as an approach for random mutagenesis. Bu et al. (2020) used *Bacillus licheniformis* to induce mutation in the gene responsible for laccase enzyme synthesis. Gene of interest is modified by error prone PCR and later expressed in *E.coli*. Further, these cloned isolates were tested against a bunch of dyes like acid violet, methyl orange and alphazurine A and it was inferred that the Lac^{ep69} mutant laccases outperformed wild type because they were more stable and efficient in nature. Comparative data for wild and mutant strains is discussed in Table 2.

The review highlights the comparative efficacy of the wild versus mutant strains on azodyes and utilization of these positive mutated strains to develop novel bioremediation technology to deal with environmentally noxious pollutants from textile industries through translational research.

Conclusion

Textile industry is among the third largest sector across globe, and due to the discharge of the hazardous effluent, the industry is placed in the red category. With the growing demand of the textile based product, these industries are expanding voluminously generating huge amount

Table 2 Comparative efficacy of wild and mutant strains of dye degrading bacterial isolates

Specification		Mutagen	Time of exposure	Decolourization efficacy		Enzymes involved	References
Strain	Dye			Wild	Mutant		
Pseudomonas sp. LBC1	Green HE4B	UV light	75 s	100% in 48 h	100% in 36 h	Lignin peroxidase, laccase, tyrosinase	Joshi et al. (2013), Kalme et al. (2007)
Bacillus sp.	Red dye	UV light	15 min	–	38%	laccase, manganese peroxidase, lignin peroxidase and NADH-DCIP reductase	Pushpa et al. (2020)
		X- rays (R4 exposure)	–		39%		
		EtBr	20 min		40%		
	Blue dye	UV light	5 min		34%		
		X- rays (25 RT exposure)	–		35%		
		EtBr	40 min		36%		
Bacillus licheniformis	Methyl orange	Site directed mutagenesis (Error prone PCR)	–	15%	18%	Laccase	Bu et al. (2020)
	Acid Violet			40%	78%		
	Alphazurine A			30%	70%		
Bacillus sp. Strain *ACT 1* and *ACT 2*	Congo red	UV light	3 min	Dye degradation from 200–1000 mg/L	Dye degradation upto 3000 mg/L and duration minimized by 12–30%	Azo-reductase	Gopinath et al. (2009)
		EtBr	18 min				

of contaminants and toxicants. Present treatment technologies treat the same but are not potent as per the regulatory compliance limits governed by various agencies as Pollution Boards, Ministries and Environmental Protection agencies to name a few. The present review proposes an insight to explore the random mutagenesis to handle the menace created by the toxicants discharged. Limited study on the mutagenic context suggests a need to explore the methodology to benefit the textile ecosystem. The use of dyes since antiquity to nowadays has grown up and popularize in response to increase in the population, their need, industrial growth, and leads to an increased use of these recalcitrant dyes. These synthetic azo compounds have a significant impact on environment, and require special consideration due to its extensive exploitation and arduous degradation process. Among various bioremediation technologies, bacteria stand out for being most resilient organisms that can be used in a variety of ways *i.e.* axenic cultures or in their consortium. These characteristics go synonymously with the potential of various bacterial strains to act on diverse range of physico-chemical parameters along with optimal performance under compliance of concerned authorities in case of translational research from lab to industry. This review provided a comprehensive account on the utilization of bacteria for their enhanced biodegradation and detoxification of textile effluent. It also focusses on inducing random mutations by various physical and chemical methods for bacterial strain improvement by targeting genes for enhancing the production of azo dye degrading (oxidoreductase) enzymes that control the downregulated degradative pathway. In order to get profound insight into the induced changes in base pairs of DNA, further molecular studies can be conducted so as to achieve improved mitigation. As it is a random mutagenesis approach, the mutations can be positive, negative or neutral but only positive mutation were recommended to be carried forwarded that will leads to increase in gene expression of oxidoreductive enzymes for quicker biodegradation. Hence, positively mutated bacterial strains may be used in near future to combat conventional technologies for bioremediation of azo dye contaminated environment. The significant strains wild vs mutants were suggestive to be used in the industrial scale capacities for being commercially employed as novel remediation strategy, after approvals from the competent regulatory bodies in the near future.

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Declarations

Conflict of interest The authors wish to declare no conflict of interest in the study conduct and its publication. The study was carried out as per the standards of ethics.

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