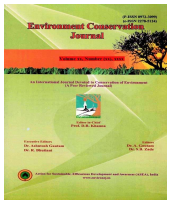
Environment Conservation Journal 24 (3): 274-282, 2023

Journal homepage: https://www.environcj.in/

Environment Conservation Journal

ISSN 0972-3099 (Print) 2278-5124 (Online)



Azo dye degrading bacteria and their mechanism: A review

Manasi Upadhyay
Department of Microbiology, Bijoy Krishna Girls' College, Howrah, India
Ahana Mondal
Department of Microbiology, Bijoy Krishna Girls' College, Howrah, India
Biswajit Saha ✉
Department of Microbiology, Bijoy Krishna Girls' College, Howrah, India

ARTICLE INFO

Received : 28 October 2022
Revised : 27 February 2023
Accepted : 20 March 2023

Available online: 28 June 2023

Key Words:
Synthetic dyes
Idols
Azo dye
Toxicity
Laccase, GMO

ABSTRACT

Major part of the aesthetics and beauty of idols, textiles, paper, paintings industries, etc. finds its roots in the use of colours (azo compound). These synthetic dyes can not degrade easily by physical and chemical means and are toxic for the environment and animals including humans. Even if they get degraded, it becomes difficult to get rid of the secondary toxic products. Microbes especially bacteria can be used which results cheap, eco friendly and complete degradation of azo dye products without production of any secondary toxic products (or secondary products with way lesser toxicity). Also, it requires no new chemical to be added (in an attempt to degrade azo dye) in an already polluted environment, as the bacterial enzymes would do the job without requiring any other added chemicals. This review article discusses the use of bacteria for azo dye degradation, the bacterial enzymes such as laccase etc. that degrade azo dye and how they work to decolourise the dyes, the common genetic elements found in the different bacteria that can degrade azo dye. This article also includes information on future prospects and some *genetically modified organism* (GMO) that are being/ (can be) brought to use for dye degradation and pollution reduction.

Introduction

The use of dye for the aesthetic enhancement of objects is an ancient practice. With the discovery of new pigments and dyes the dyeing technology has been evolved. Nowadays uses of azo dye are ever present & they are widely used in the textile, paper and food, painting industries. Releasing this untreated waste into the environment without prior treatment can pose significant environmental risks as azo dyes and their metabolites are *toxic*, *carcinogenic*, *mutagenic* and *highly recalcitrant*.

The synthetic design of these dyes is described by the presence of azo bonds (-N = N-), associated with naphthalene or benzene rings . These aromatic rings can have different substituents, for example, sulfonic corrosive (SO_3H), chloro (- Cl), hydroxyl (- Gracious), methyl (- CH_3), carboxyl (- COOH) , nitro (- NO_2) and amino (NH_2) groups. These substituents make azo dyes *water-soluble* and degradation resistant under environmental conditions and they cause serious harm to the marine environment. This is not only a threat to the marine animals but consumption of such water may cause *cancer* in humans.So, this is important to treat the dye containing effluent before discharging them in to water bodies (Ruhela *et al.,* 2021; Bhutiani *et al.,* 2021; Bhutiani *et al.,* 2022). The chemical, physical methods to treat this effluent but they are expensive and produces amine residue containing sludge. So, utilization of microorganisms for remediation design is consequently a potential answer for ecological contamination since it incorporates reasonable remediation innovations. Under certain conditions several microorganisms helps to decolorize and mineralize the azo dye. Bacteria have become the main tool for the degradation of the azo-dye containing waste water as they produce various intracellular redox enzymes, can easily be cultured and reproduce at a rapid rate (Garg, 2012). Also they can survive in high salt concentration and high

Corresponding author E-mail: *biswajit.saha1402@gmail.com*
Doi:https://doi.org/10.36953/ECJ.15672490

temperature environment and they have high degradation and decolorization ratios.Studies have shown that most azo-dye degrading bacteria are commonly *mesophilic* (20-40 ^{0} C) (Sudha *et al.,* 2018) in nature. Although most azo-dye waste water produces in the dyeing bath and rinsing process with discharge temperatures reaching as high as 50 – 70^{0}C (Boonyakamol, 2009). So, this type of waste water can be treat effectively using thermophilic bacteria. Thermophilic bacteria are also have some advantages including fast metabolic ratios, high processing efficiencies and the capacity to effectively kill pathogens when azo dye were degraded at high temperatures and enzymes secreted by the thermophilic bacteria are stable and resistance to chemical degradation. In recent days, from the azo-dye containing waste water researchers isolate and screen some azodye degrading bacteria *Bacillus sp., Micrococcus sp., Lysinibacillus sphaericus sp., Aeromonas hydrophila sp. Anoxybacillus sp.*

What are azo dyes?

Before we discuss this specific category of dye, the azo dye, we should know what is a dye. Dyes are synthetic, aromatic compounds used as colouring agents. They're used to colour various substances, giving them a new look, a new colour. One other substance used for colouring are pigments. Dyes are different from pigments in the way that pigments are mostly insoluble in substrate and their application is mostly in dispersed powder or crystal form.Dyes, on the other hand, get dissolved in the substrate at the molecular level. They're better colouring agents and find use in all the various industries— the textile industry, leather industry, cosmetic industry, paper industry, idol making industry etc.Dyes, in turn, are of many types of which, the azo dyes form the most widely used category. Their omnipresence can be credited to their characteristic strong colouration. They are synthetically made – organic chemical compounds with high stability and chemical versatility. The azo dyes consist of one or more (–N=N–) bonds which are referred to as azo. They also feature amino, chloro, nitro, hydroxyl and methyl substituent groups (Bell *et al.,* 2000).Though these dyes are used in almost all industries that deal with colouring, they cause a major environmental concern. The azo dyes that get dispersed into the nature, mostly into water bodies, as a part of industrial effluents or via idol dispersing etc. don't get degraded easily. They do break down via mineralization and form aryl-amine which are highly suspected carcinogens. As we already discussed, azo dyes are mostly water soluble and thus they can be absorbed directly via skin and inhalation, causing risks of cancer and allergic reactions. Toxic compounds of azo dyes can get absorbed into the aquatic organisms in form of water-dye mixture(He *et al.,* 2004; Asad *et al.,* 2007). When humans consume this water or the aquatic organisms from a dye polluted water body, the toxic substances reach man and cause various diseases like, *renal damage, acute tubular necrosis, sporadic fever, splenic sarcomas, hepatocarcinoma, oedema of the larynx, urinary bladder cance*r etc. Nuclear anomalies and chromosomal aberration in laboratory animals and mammalian cells dye to azo dyes has also been observed (Bayoumi *et al.,* 2010; Puvaneswari *et al.,* 2006; Mani and Bhargava 2016 a,b; Houk *et al.,* 1991; Haley, 1975). Dye effluents may also contain suspended solids particulate or heavy metal B which again are environmental and life hazard. Dyes are also known to absorb sunlight due to the presence of chromophoric group. This absorption of sunlight by the dye present in water hampers and has a detrimental effect on the photosynthetic process of water flora, like the phytoplankton, algae and water plants (Kagalkar *et al.,* 2010).

Such a group of compounds, which are toxic and detrimental to both flora and fauna including humans, should be gotten rid of. But it is not possible as we do need them for the aesthetic and colouring in various fields. Such is the condition that we can neither fully get rid of these dyes nor can we leave it as is. So, the best we can do is make sure that these dyes are degraded and don't persist in the environment in their own toxic form or even as toxic secondary products. The best way to achieve this is by biodegradation of the azo dyes rather than by chemical degradation. Use of chemical or physical means for the breakdown of the dyes comes forward as an expensive method and on top of that, considerable amount of chemical sludge waste is produced whose disposal, once again, is no less problematic. Thus biodegradation

or microbial decolourisation and degradation forms a cost effective method of pollutant-dye removal from the environment. Of the microbes, many fungal, algal, and bacterial species have been found with the capability of degrading azo dyes. This study focuses on the use of bacteria and their enzymes for biodegradation and decolourisation of azo dyes.

Azo dye degradation by bacteria:

As we have already mentioned earlier, bacteria form a very good mean of biodegrading the azo dyes. Bacterial enzymes and bioadsorption by bacteria form the two major biodegrading pathway for dyes.These microorganisms can completely degrade the azo dyes. They have developed enzyme systems and produce enzymes like azo reductase, laccase, hydrogenase, peroxidase and exo-enzymes which reduce the azo dyes. The reduced forms are then converted to simpler compounds and/or used as energy source by the bacteria (Stolz, 2001).The complete degradation of azo dyes by bacteria mostly occur as a two step process and is carried out by bacterial enzymes acting on the dues. The reactions involved in these two steps are partially aerobic and partially anaerobic. Thus, a coupled aerobic-anaerobic process brings about the full degradation of azo dyes. The 1st step is the reductive cleavage of azo bonds (–N=N–) which leads to production of aromatic amines, the toxic carcinogens. In the 2nd step, these aromatic amines (aryl-amines) are degraded and thus, the environment is fully cleared of any toxic dye product.One other process by which bacteria clear the environment of azo dyes is by biosorption. Biosorption by bacteria depends on the composition of heteropolysaccharides and lipids in the bacterial cell wall. The attraction between the various charged groups of the cell wall and the azo dyes forms the basis of biosorption. Though biosorption is helpful to some extent, it is not a complete solution for the problem as the dye does not gets destroyed but is only embedded inside the biomass matrix. So, bacterial enzymatic processes for dye degradation are the most preferred method for removal of azo dye compounds from the environment. Some bacterial species that have been isolated and screened for their dye degrading ability are *Bacillus sp, Pseudomonas sp, Rhabdobacter sp, Enterococcus sp, Staphylococcus sp, Xenophilus sp, Cornebacterium sp, Clostridium sp., Micrococcus sp, Dermacoccus sp, Acinetobacter sp, Geobacillus sp, Lactobacillus sp, Rhizobium sp, Proteus sp, Morganella sp, Aeromonas sp, Alcaligenes sp, and Klebsiella sp.(*Stolz, 2001; Olukanni *et al.,* 2006; Vijaykumar *et al.,* 2007; Chen *et al.,* 2008; Lin and Leu, 2008*).*

Enzymatic degradation of azo dyes:

Enzymes form a very effective bioremediation tool. Decolouration of dyes by bacteria depends fully on their capability to produce the various dye degrading enzymes. Azo reductases are known for their anaerobic dye degrading ability yet, some specific azo reductase enzymes have the potential to degrade azo dyes under aerobic conditions (Stolz, 2001; Naik and Singh , 2012). Laccases are known for dye degradation by free radical mechanism. Peroxidase enzymes in addition to laccases show an extra 25% decolourisation rate (Hadibarata *et al.,* 2012). These three enzymes, azo reductase, laccase and peroxidase form the most studied groups of dye degrading enzymes.

Azo reductase:

Azo reductases form the largest group of dye degrading enzymes. They specifically cleave the azo bonds producing aryl-amines. They use reducing agents like FADH, NADH and NADPH as an electron donor for the degradation reactions (Tian *et al.,* 2014; Jadhav *et al.,* 2008). These enzymes are mostly oxygen sensitive. As aerobic respiration also uses the same reducing agents as that of the azo reductase catalysed reaction, presence of oxygen impedes the azo bond degradation steps by competing for the reducing agents. So, Azo reductases are more related to anaerobic degradation of dye. Despite of this, there have been certain examples of aerobic azo reductases found in *Pseudomonas sp.*(Zimmermann *et al.,* 1982,1984).

Laccase:

Laccases are copper containing oxidases. One of their main advantage over other dye degrading enzymes is that they function through a free radical mechanism and form phenolic compounds rather than the toxic aromatic amines (Chivukula and Renganathan, 1995). The reduction mechanism of laccase entails the removal of a hydrogen ion (H^+)

from the amino and hydroxyl group of the substituted ortho and para position of phenolic compounds and aromatic amines.Laccases are non-specific enzymes and work on a wide variety of azo dyes. On top of that, laccases don't require cofactors for their activation. Redox mediators help expand the range of azo dyes that a laccase enzyme can degrade. Bacterial laccases also have properties like wide pH range of function and high thermal stability. All these qualities make them an enzyme of great industrial interest and they play important roles in textile industry, cosmetic industry, bioremediation and biodegradation etc. (Tian *et al.,*2014).

Peroxidases:

Peroxidases are exo enzymes mostly present in fungi but they do occur in some bacteria as well (Kandelbauer and Guebitz, 2005). They are hemoprotein and need hydrogen peroxide (H_2O_2) to catalyse reactions. Reduction mechanism of peroxidases is very similar to that of azo reductases. Only difference is in the electron donor used.Of the peroxidases, lignin peroxidase and manganese peroxidase are directly involved in biodegradation and decolouration of azo dyes (Paszczynski *et al.,* 1991; Pasti *et al.,* 1992).Some examples of dye degrading bacteria and enzymes is given in Table 1.

Table 1: Some examples of dye degrading bacteria and enzymes

Bacterial strains	Dye	Enzyme	References
Lysinibacillus fusiformis	Methyl red	Enzymatic-Laccase, Azoreductase and Lignin Peroxidase	Sariand Simarani, 2019
Pseudomonas extremorientalis	Congo red	Laccase	Neifar *et al.,* 2016
Bacillus subtilis	Acid blue 113		GuruLakshmi *et al.,* 2008
Comamonas sp.	Direct Red 5B	Enzymatic-Laccase and Lignin Peroxidase	Jadhav *et al.,* 2008
EnterococcusFaecalis YZ 66	Reactive Orange II		Sahasrabudhe and patthade, 2011
Proteus mirabilis	Reactive Blue 13	Enzymatic- Laccase, azoreductase and veratryl alcohol oxidase	Olukanni *et al.,*2010
Aeromonashydrophila, Lysinibacillussphaericus	Reactive Red 195	Enzymatic-Laccase and Azoreductase	Srinivasan and Sadasivam, 2018
Aeromonas sp.	Methyl orange	laccase, NADH-DCIP reductase, and azoreductase	Du *et al.,* 2015

Isolation and screening of azo-dye degrading bacteria:

A large number of azo dye degrading bacteria have already been isolated from soil and water from industrial area, and effluents from water bodies by researchers. Literatures present many examples of such various strains that can degrade and decolourize the azo dyes. For example, one process used for isolating and screening dye degrading bacteria was carried out by Khan and Joshi (2020) from Rajasthan, India. The authors isolated azo dye degrading bacterial strain from water and soil from textile industries in Jodhpur province. *Bacillus pumilis* and *Paenibacillus thiaminolyticus* were the two bacterial strains which were screened and identified by theirbiochemical characteristics. Serial dilution and streaking method was used to obtain several colonies which were, separately inoculated into nutrient broth media. A 10% (v/v) inoculum was transferred into 100 mL of mineral salt medium (MSM) and incubated at 37^0C at 125 rpm for 24-48 hrs. 10% (v/v) of the sample was sub-cultured into 10 mg/L MSM of the respective dyes and further incubated after 24 hrs. Strains which were able to utilize the fresh dyes as their source of nutrient were plated on Nutrient agar plate and incubated at 37^0C. After that pure bacterial cultures were transferred into the Nutrient broth subsequently (Khadijah *et al.,*2009).

Alternatively we can take some carbon free media like Bushnell Haas agar media and enrich it with respective dyes at different concentrations. After the inoculation, we need to check whether the growth has appeared or not. If growth appears, we can surely say that organism is capable of degrading the dyes. This is because Bushnell Haas media lacks carbon and here we are using the dye in the place of carbon, so if the bacteria are able to

utilize the dyes only then they will show growth. Many other processes have also been developed by other researchers that have been used to isolate and screen various other dye degrading bacterial species.

Laccase activity assay :

Laccase plays an important role in the bioremediation of several aromatic compounds. Especially they help in dye degradation and waste water detoxification. The basic reaction mechanism of laccase involves the formation of two water molecules upon the accompanying electron loss of a single oxygen molecule. This abstracting electron leads to the oxidation of several benzene ring-containing compounds (Solomon *et al.,* 1996; Chandra and Chowdhary 2015). With the cation generation catalytic activity of the laccase plays an important role in the degradation of aromatic compounds. As these cations are less stable, in the presence of laccase they converted to the stable product (quinine → phenol). Due to the presence of four copper atoms that forms the central part of this reaction, the redox mechanism takes place. The mechanism is given in Figure number 1. They are classified into three types – type 1 copper (T1Cu), type 2 copper (T2Cu) and type 3 copper (T3Cu). Near the T1- copper center (shallower than the oxygen binding center) substrate molecules are bound. One-electron abstraction occurs from the substrate to T1 copper by an outer-sphere process. That's why, substrate molecules are converted to free radicals which can undergo further oxidation or radical coupling reactions. This leads to the formation of polymers or oligomers. Via a cysteine–histidine pathway that is highly conserved among multi-copper oxidases the abstracted electron moves from the T1 center to the trinuclear cluster. This so-called super-exchange pathway is created by overlapping redox active molecular orbitals of the T1 coordinating cysteine, the backbone atom, and the T3 copper coordinating histidine residue (Solomon *et al.,* 2008). Type 2 and type 3 copper make up the trinuclear center. After oxygen molecules attach to the trinuclear cluster and prevent further entry of any other molecules the catalytic process begins. The T2Cu site interacts with two histidine molecules and one water molecule, whereas the T3Cu interacts with three histidines and a hydroxide molecule. In the last step, laccase converts the oxygen molecule to water in two steps. In the first step, the first electron is reduced by T2Cu and T3Cu, while the reduction of the second electron is assisted by a peroxide mediator that protects the T2Cu site and T1Cu is linked to T3Cu through a Cys-His covalent bonds. As we have already mentioned, enzymes like azoreductase, Peroxidase degrades the dyes with the production of aromatic amines which are toxic in nature (carcinogenic). But laccase reduce the azo dyes to the phenolic compounds. This property is advantageous as well as of industrial interest in dye degradation process. Therefore various scientists did laccase activity assay. For example, Fatemeh Sheikhi *et al.* (2012) Centrifuged (6000 * g) bacterial cultures at 4^0c for 20 min to obtain cellular debris precipitate and clear supernatants. After that they washed bacterial pellets with phosphate buffer(0.1 M, pH 6.5) containing 10mM of phenylmethylsulfonyl fluoride (PMSF). They used PMSF because it helps to inhibit the activity of protease before sonication. Then they obtained the cell extract by centrifugation (14000 * g) for 20 min at 4^0c , which was used as a crude intracellular enzyme. To remove the effect of H_2O_2 produced by bacteria assay solution was incubated with catalase (1,000 U/mL; Sigma-Aldrich) for 1 h at 37^0c. At 436 nm spectrophotometrically laccase activity was determined as described by Niku-Paavola et al. with 2, 2- azino - di- [3- ethylbenzo- thiazolin-sulphonate] (ABTS) as a substrate. The amount of enzyme that oxidized 1 μmol of ABTS per min at 25 °C was defined as one activity unit (U) and in terms of U/L activities were expressed. Also laccase activity was determined at 465 nm spectrophotometrically using a substrate (2 mM guaiacol) in a reaction mixture containing 50mM phosphate buffer. The amount of enzyme that increased the absorbance by 0.001 units per min at 55 °C was defined as one unit of enzyme activity (Bains*et al.,*2003). Here also activities were expressed in U/L. Sondhi *et al.* (2014) also measured the laccase activity by governing the oxidation of 2 , 6-Dimethoxyphenol, (2, 6-DMP) buffered with 100 mM phosphate buffer for 1 min at 450 nm. An absorption coefficient of 14,800 M/cm was used to calculate the enzyme activity. According to Ang *et al.* (2012)the amount of enzyme required to oxidise 1μM of 2, 6-DMP per minute is defined as the enzyme activity.

GMO for dye degradation:

Nowadays, dye degradation is becoming more and more challenging as they are being produced in such a way that they resist degradation. Dyes degradation is either achieved by a single treatment method or by an integration of physical, chemical and biological methods. Interestingly, microbiological treatment methods have found acceleration due to environmental friendliness, improved performance and low cost nature (Moharikar *et al.,* 2005). Under environmental conditions improvement of dye degradation can be achieved using GMOs. A GMO is obtain by modifying their genetic material using genetic engineering. Functional genes from several bacterial strains such as *Sphingomonas desiccabilis*, *Escherichia coli*, *Bacillus idriensis*, *Pseudomonas putida*, *Mycobacterium marinum*, *Ralstoni aeutropha* are transferred into other species to produce GMO for dye degradation (Saxena *et al.,* 2019). For example, Jin *et al.*(2008) and (2009), produced *Escherichia coli* JM109(pGEX-AZR), a GMO that was able to degree the dyes– Acid red GR and Direct blue 71. Azo reductase gene from a subspecies of *Rhodobacter sphaeroides* was isolated and inserted in the vector pGEX4T-1 and then expressed in *Escherichia coli* JM109 under the control of lac operon.In another attempt, Dixit and Garg (2019) reported Methyl orange degradation by a GMO *Escherichia coli* BL21. Azok gene from a *Klebsiella pneumoniae* strain was expressed in *Escherichia coli* DH5 to produce this GMO.Many more such GMOs have been developed which degrade a variety of dyes. These GMOs demonstrate high dye decolorization and degradation rate and are instrumental in effective bioremediation.

Future prospects:

Bioremediation using microbes has emerged as a promising approach and an eco friendly technology of degrading dyes. Enhancing this technology via further research is the need of time. Genetic engineering and molecular biology tools are already being exploited for potential benefits in the field of dye degradation and environmental purification. Still, more in-depth study of the genetic aspect of dye degradation by bacteria along with the study of mechanisms of degradation is very much required to have a more clear understanding on how to take help from the bacterial world for more efficient and complete degradation of dyes, which still stands as a challenge. Future research should focus on reducing limiting factors upon dye degrading microbial activities and improving and enhancing the efficiency of already existing bioremediation processes. In future, studies can focus on introducing non-indigenous bacteria in sites where critical dye degrading microbes are absent. Or, novel genetic engineering techniques, i.e. CRISPR/Cas9, can be used for modifying indigenous bacteria into bacteria with dye degrading ability.Use of microbial consortia instead of a single bacterial species should also be explored in the coming years. It would present higher and more complete degradation of dyes in shorter time as synergistic effects would come into play. Searching out the various bacterial strains whose enzymatic activity would positively influence and supplement each other in degrading dyes can be an open wide field for future research. These bacteria will be useful in reclaiming the polluted soils around dye-related industries and cleaning the water bodies where the effluents are dumped. Although, use of microbial consortia or a non-indigenous bacteria and GMO to assist dye degradation is a scope for future research, care should be taken as adaptation of microbial community to a new environment is not always an efficient process. Factors like stress due to different environmental condition, competition with the indigenous species etc. affect the adaptation/ acclimatization process. Apart from that, the introduced bacteria may also cause changes in the environment that might influence the survival of both the introduced bacteria as well as the indigenous microbial species either positively or negatively. This acclimatization process and it's after effects can be monitored by using diversity indexes such as Shannon diversity index (1949), which is a way to mathematically measure the species diversity in a community. Shannon index depends on both, species richness as well as species evenness and it's value for ecological studies typically varies between 1.5 to 3.5. It increases with the increase in both, the richness as well as the evenness.

It is calculated as:

$$H = -\Sigma[pi * ln(pi)$$

Where, H– Shannon diversity index; Σ– Summation; ln– Natural log; pi– proportion of entire community made up of species 'i'.

Conclusion

Dye, the important component involved in colouring and decorating the items used in our daily lives, becomes intractable and hazardous colouring pollutant, toxic for environment and human, animals and plant lives, when it is dumped untreated into water bodies as dispersed dyed items or industrial effluents. In this review article, the problem as well as the solution has been discussed and we can conclude that, degradation of dyes is a huge problem in today's world and bacterial bioremediation is a good way to get rid of it. It is a sustainable and green process but a lot still needs to be studied and researched. There are factors to consider including the maintenance and implementation cost of bioreactor, available space for using bioreactor, availability of nutrients under optimal conditions, presence of redox mediator for proper functioning of azo-dye reducing enzymes etc. Despite the limiting factors, the knowledge of the bacterial enzymes and the mechanism of dye degradation that has been acquired till date has been put to use and it has proved to the most economic and eco friendly way to overcome dye pollution. In recent times, creation of new GMOs and use of extensive genetic engineering tools, along with the efforts to produce useful microbial consortium has been another successful step and has additional benefits to improve degradation process efficiency. With the fast, new developments happening in this field, we can be hopeful of more improved processes and maybe even complete dye degradation can be accomplished in the near future.

Conflict of interest

The authors declare that they have no conflict of interest.

References

Ang, T. N., Ngoh, G. C., Chua, A. S. M., & Lee, M. G. (2012). Elucidation of the effect of ionic liquid pretreatment on rice husk via structural analyses. *Biotechnology for biofuels*, *5*(1), 1-10.

Asad, S., Amoozegar, M. A., Pourbabaee, A., Sarbolouki, M. N., &Dastgheib, S. M. M. (2007). Decolorization of textile azo dyes by newly isolated halophilic and halotolerant bacteria. *Bioresource technology*, *98*(11), 2082-2088.

Bains, J., Capalash, N., & Sharma, P. (2003). Laccase from a non-melanogenic, alkalotolerant γ-proteobacterium JB isolated from industrial wastewater drained soil. *Biotechnology Letters*, *25*, 1155-1159.

Bayoumi, R. A., Musa, S. M., Bahobil, A. S., Louboudy, S. S., & El-Sakawey, T. A. (2010). Biodecolorization and biodegradation of azo dyes by some bacterial isolates. *Journal Of Applied Environmental and Biological Science, 1*(1), 1-25.

Bhutiani, R., Ahamad, F., & Ruhela, M. (2021). Effect of composition and depth of filter-bed on the efficiency of Sand-intermittent-filter treating the Industrial wastewater at Haridwar, India. *Journal of Applied and Natural Science*, *13*(1), 88-94.

Bhutiani, R., Tiwari, R. C., Chauhan, P., Ahamad, F., Sharma, V. B., Tyagi, I., & Singh, P. (2022). Potential of *Cassia fistula* pod-based absorbent in remediating water pollutants: An analytical study. In *Sustainable Materials for Sensing and Remediation of Noxious Pollutants* (pp. 261-272). Elsevier.

Bell, J., Plumb, J. J., Buckley, C. A., & Stuckey, D. C. (2000). Treatment and decolorization of dyes in an anaerobic baffled reactor. *Journal of Environmental Engineering*, *126*(11), 1026-1032.

Boonyakamol, A., Imai, T., Chairattanamanokorn, P., Higuchi, T., Sekine, M., &Ukita, M. (2009). Reactive Blue 4 decolorization under mesophilic and thermophilic anaerobic treatments. *Applied biochemistry and biotechnology*, *152*, 405-417.

Chandra, R., &Chowdhary, P. (2015). Properties of bacterial laccases and their application in bioremediation of industrial wastes. *Environmental Science: Processes & Impacts*, *17*(2), 326-342.

Chen, C. H., Chang, C. F., Ho, C. H., Tsai, T. L., & Liu, S. M. (2008). Biodegradation of crystal violet by a Shewanella sp. NTOU1. *Chemosphere*, *72*(11), 1712-1720.

Chivukula, M., &Renganathan, V. (1995). Phenolic azo dye oxidation by laccase from Pyriculariaoryzae. *Applied and Environmental Microbiology*, *61*(12), 4374-4377.

Dixit, S., & Garg, S. (2019). Development of an efficient recombinant bacterium and its application in the degradation of environmentally hazardous azo dyes. *International Journal of Environmental Science and Technology*, *16*, 7137-7146.

Du, L. N., Li, G., Zhao, Y. H., Xu, H. K., Wang, Y., Zhou, Y., & Wang, L. (2015). Efficient metabolism of the azo dye methyl orange by Aeromonas sp. strain DH-6: characteristics and partial mechanism. *International Biodeterioration& Biodegradation*, *105*, 66-72.

Garg, S. K., Tripathi, M., Singh, S. K., & Tiwari, J. K. (2012). Biodecolorization of textile dye effluent by Pseudomonas putida SKG-1 (MTCC 10510) under the conditions optimized for monoazo dye orange II color removal in simulated minimal salt medium. *International biodeterioration& biodegradation*, *74*, 24-35.

Gurulakshmi, M., Sudarmani, D. N. P., &Venba, R. (2008). Biodegradation of leather acid dye by Bacillus subtilis. *Advanced Biotech*, *7*, 12-19.

Hadibarata, T., Yusoff, A. R. M., Aris, A., Hidayat, T., &Kristanti, R. A. (2012). Decolorization of azo, triphenylmethane and anthraquinone dyes by laccase of a newly isolated Armillaria sp. F022. *Water, Air, & Soil Pollution*, *223*, 1045-1054.

Haley, T. J. (1975). Benzidine revisited: a review of the literature and problems associated with the use of benzidine and its congeners. *Clinical toxicology*, *8*(1), 13-42.

He, F., Hu, W., & Li, Y. (2004). Biodegradation mechanisms and kinetics of azo dye 4BS by a microbial consortium. *Chemosphere*, *57*(4), 293-301.

Houk, J.M., Doa, A., Dezube, M., and Rovinski, J.M. (1991). Evaluation of dyes submitted under the toxic substances control act new chemical programme. In: Color Chemistry. *Elsevier Science Publishers Ltd., London*, pp. 135 150.

Jadhav, U. U., Dawkar, V. V., Ghodake, G. S., &Govindwar, S. P. (2008). Biodegradation of Direct Red 5B, a textile dye by newly isolated Comamonas sp. UVS. *Journal of Hazardous Materials*, *158*(2-3), 507-516.

Jadhav, S. U., Kalme, S. D., &Govindwar, S. P. (2008). Biodegradation of methyl red by Galactomycesgeotrichum MTCC 1360. *International Biodeterioration& Biodegradation*, *62*(2), 135-142.

Jin, R. F., Zhou, J. T., Zhang, A. L., & Wang, J. (2008). Bioaugmentation of the decolorization rate of acid red GR by genetically engineered microorganism Escherichia coli JM109 (pGEX-AZR). *World Journal of Microbiology and Biotechnology*, *24*, 23-29.

Jin, R., Yang, H., Zhang, A., Wang, J., & Liu, G. (2009). Bioaugmentation on decolorization of CI Direct Blue 71 by using genetically engineered strain Escherichia coli JM109 (pGEX-AZR). *Journal of hazardous materials*, *163*(2-3), 1123-1128.

Kagalkar, A. N., Jagtap, U. B., Jadhav, J. P., Govindwar, S. P., &Bapat, V. A. (2010). Studies on phytoremediation potentiality of Typhoniumflagelliforme for the degradation of Brilliant Blue R. *Planta*, *232*, 271-285.

Kandelbauer, A.; Guebitz, G.M. (2005). Bioremediation for the Decolorization of Textile Dyes—A Review. In: Lichtfouse, E., Schwarzbauer, J., Robert, D. (eds), *Environmental Chemistry* (269–288). Springer: Berlin/Heidelberg, Germany. (ISBN 978-3-540-22860-8)

Khadijah, O., Lee, K. K., &MohdFaiz, F. A. (2009). Isolation, screening and development of local bacterial consortia with azo dyes decolourising capability. *Malaysian Journal of Microbiology,* 5, 25-32.

Khan, S., & Joshi, N. (2020). Molecular identification of dye degrading bacterial isolates and FT-IR analysis of degraded products. *Environmental Engineering Research*, *25*(4), 561-570.

Lin, Y. H., &Leu, J. Y. (2008). Kinetics of reactive azo-dye decolorization by Pseudomonas luteola in a biological activated carbon process. *Biochemical Engineering Journal*, *39*(3), 457-467.

Mani, S., &Bharagava, R. N. (2016,a). Exposure to crystal violet, its toxic, genotoxic and carcinogenic effects on environment and its degradation and detoxification for environmental safety. *Reviews of Environmental Contamination and Toxicology Volume 237*, 71-104.

Mani, S. and Bharagava, R.N. (2016,b). Microbial degradation and decolorization of dyes from Textile industry wastewater. In: Bharagava, R.N. and Saxena, G. (1stEd) *Bioremediation Of Industrial Pollutants.* Write & Print Publications New Delhi, 110 015. (ISBN: 978-93-84649-60-9).

Moharikar, A., Purohit, H. J., & Kumar, R. (2005). Microbial population dynamics at effluent treatment plants. *Journal of Environmental Monitoring*, *7*(6), 552-558.

Naik, C., & Singh, C. R. (2012). Isolation screening and development of Bacillus sps with decolorization and degradation capabilities towards reactive dyes and textile effluents. *Recent Research in Science and Technology,* 4, 1–5.

Neifar, M., Chouchane, H., Mahjoubi, M., Jaouani, A., &Cherif, A. (2016). Pseudomonas extremorientalis BU118: a new salt-tolerant laccase-secreting bacterium with biotechnological potential in textile azo dye decolourization. *3 Biotech*, *6*, 1-9.

Olukanni, O. D., Osuntoki, A. A., &Gbenle, G. O. (2006). Textile effluent biodegradation potentials of textile

effluent-adapted and non-adapted bacteria. *African Journal of Biotechnology*, *5*(20), 1980-1984.

Olukanni, O. D., Osuntoki, A. A., Kalyani, D. C., Gbenle, G. O., &Govindwar, S. P. (2010). Decolorization and biodegradation of Reactive Blue 13 by Proteus mirabilis LAG. *Journal of Hazardous Materials*, *184*(1-3), 290-298.

Pasti-Grigsby, M. B., Paszczynski, A., Goszczynski, S., Crawford, D. L., & Crawford, R. L. (1992). Influence of aromatic substitution patterns on azo dye degradability by Streptomyces spp. and *Phanerochaete chrysosporium*. *Applied and Environmental Microbiology*, *58*(11), 3605-3613.

Paszczynski, A., Pasti, M. B., Goszczynski, S., Crawford, D. L., & Crawford, R. L. (1991). New approach to improve degradation of recalcitrant azo dyes by Streptomyces spp. and Phanerochaetechrysosporium. *Enzyme and Microbial Technology*, *13*(5), 378-384.

Puvaneswari, N., Muthukrishnan, J., &Gunasekaran, P. (2006). Toxicity assessment and microbial degradation of azo dyes. *Indian journal of experimental biology*, *44*(8), 618–626.

Ruhela, M., Jena, B. K., Bhardawaj, S., Bhutiani, R., & Ahamad, F. (2021). Efficiency of Pistia stratiotes in the treatment of municipal solid waste leachate in an upwards flow constructed wetland system. *Ecology Environment & Conservation 27 (February Suppl. Issue): 2021;* pp. (S235-S244).

Sahasrabudhe, M. M., &Pathade, G. R. (2011). Biodegradation of sulphonated azo dye CI reactive orange 16 by Enterococcus faecalis strain YZ 66. *European Journal of Experimental Biology*, *1*(1), 163-173.

Sari, I. P., &Simarani, K. (2019). Decolorization of selected azo dye by Lysinibacillus fusiformis W1B6: Biodegradation optimization, isotherm, and kinetic study biosorption mechanism. *Adsorption Science & Technology*, *37*(5-6), 492-508.

Saxena, G., Kishor, R., Saratale, G.D., Bharagava, R.N., (2019). Genetically Modified Organisms (GMOs) and their Potential in Environmental Management: Constraints prospects, and Challenges. In: Bharagava, R., Saxena, G. (Eds.). *Bioremediation of Industrial Waste for Environmental Safety* (1-19). Springer, Singapore.

Shannon, C.E. and Weaver, W. (1949). The Mathematical Theory of Communication*. Urbana, IL: The University of Illinois Press*, 1-117.

Sheikhi, F., RoayaeiArdakani, M., Enayatizamir, N., & Rodriguez-Couto, S. (2012). The determination of assay for laccase of Bacillus subtilis WPI with two classes of chemical compounds as substrates. *Indian journal of microbiology*, *52*, 701-707.

Solomon, E. I., Augustine, A. J., & Yoon, J. (2008). O 2 Reduction to H2O by the multicopper oxidases. *Dalton Transactions*, (30), 3921-3932.

Solomon, E. I., Sundaram, U. M., &Machonkin, T. E. (1996). Multicopper oxidases and oxygenases. *Chemical reviews*, *96*(7), 2563-2606.

Sondhi, S., Sharma, P., Saini, S., Puri, N., & Gupta, N. (2014). Purification and characterization of an extracellular, thermo-alkali-stable, metal tolerant laccase from Bacillus tequilensis SN4. *PloS one*, *9*(5), e96951.

Srinivasan, S., &Sadasivam, S. K. (2018). Exploring docking and aerobic-microaerophilic biodegradation of textile azo dye by bacterial systems. *Journal of water process engineering*, *22*, 180-191.

Sudha, M., Bakiyaraj, G., Saranya, A., Sivakumar, N., &Selvakumar, G. (2018). Prospective assessment of the Enterobacteraerogenes PP002 in decolorization and degradation of azo dyes DB 71 and DG 28. *Journal of environmental chemical engineering*, *6*(1), 95-109.

Stolz, A. (2001). Basic and applied aspects in the microbial degradation of azo dyes. *Applied microbiology and biotechnology*, *56*, 69-80.

Tian, Y. S., Xu, H., Peng, R. H., Yao, Q. H., & Wang, R. T. (2014). Heterologous expression and characterization of laccase 2 from Coprinopsiscinerea capable of decolourizing different recalcitrant dyes. *Biotechnology & Biotechnological Equipment*, *28*(2), 248-258.

Vijaykumar, M. H., Vaishampayan, P. A., Shouche, Y. S., &Karegoudar, T. B. (2007). Decolourization of naphthalene-containing sulfonated azo dyes by Kerstersia sp. strain VKY1. *Enzyme and Microbial Technology*, *40*(2), 204-211.

Zimmermann, T., Gasser, F., Kulla, H. G., &Leisinger, T. (1984). Comparison of two bacterial azoreductases acquired during adaptation to growth on azo dyes. *Archives of Microbiology*, *138*, 37-43.

Zimmermann, T., Kulla, H. G., &Leisinger, T. (1982). Properties of purified Orange II azoreductase, the enzyme initiating azo dye degradation by Pseudomonas KF46. *European Journal of Biochemistry*, *129*(1), 197-203