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A Review of Bacterial Degradation of Azo Dyes

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ABSTRACT

Biodegradation of synthetic dyes using different bacteria is becoming an accepted approach for treating Azo dye wastewater to mitigate many environmental problems. Azo dye has a negative impact on the environment by increasing biochemical oxygen demand (B.O.D.) and chemical oxygen demand (C.O.D.). The Biodegradation of azo dyes by different bacteria was evaluated. Biodegradation of synthetic dyes not only results in the decolorization of the dyes but also in the disintegration of the dye molecules into smaller and simpler parts. Decolorization of the dye arises when the chromophoric center of the dye is slashed. Various microorganisms, including fungi, bacteria, yeasts, and algae, have been used to decolorize and Degradation of synthetic dyes. Bacterial decolorization is usually faster than fungal decolourization. It is well known that bacteria degrade azo dyes reductively under anaerobic circumstances to colorless aromatic amines, which are carcinogenic compounds. These colorless aromatic amines must also be degraded because they may be toxic, mutagenic, and carcinogenic to humans and animals Bacteria are capable of removing dyes by adsorption and absorption. Biosorption rates are directly matched with the composition of heteropolysaccharides and the cell wall lipids. Dead cells are more useful in Biosorption than living cells because they do not need nutrients and can be stockpiled for a long time. The azoreductases are considered to be the most potent group of enzymes active in the Biodegradation of synthetic azo dyes. They accomplish the reductive cleavage of synthetic azo dyes bonds. Laccases enzymes become more interesting and have more focus recently due to their ability in generating much lower toxic aromatic amines. Laccases do not need other cofactors. Many factors affecting the process are temperature, pH, salinity, dye concentration, and Bioremediation in aerobic and anaerobic environments.

Keywords: Biodegradation, azo dyes, biochemical oxygen demand (B.O.D.), chemical oxygen demand (C.O.D.), bacterial enzymes and Bacterial Laccases enzymes.

BACKGROUND

3,500 years ago natural dyes and pigments were already in use. The significance of colour was due to it has been linked to hierarchy, authority, and leadership, is also demonstrated by cave paintings. Color is a visual language, a social and symbolic art form, and an expressive tool. [2–4].

Large amounts of aqueous waste and colours are released from the dyeing process in the textile industry. The admission of sunlight into water bodies is impeded by solid and stable hues, which has an impact on photosynthesis and biological oxygen consumption (B.O.D.). This is not acceptable for the environment. [1].

Initially, the only coloring agents available were dyes and pigments of natural origin obtained from mineral sources and vegetables [5, 6]. Natural dyes are a safer and more environmentally friendly, but they were and still are very costly and difficult to obtain and apply. Natural dyes are not practical and feasible for most commercial applications.

The first synthetic dye was created by William Henry Perkins when he accidentally and fortuitously discovered and synthesized mauveine [7]. Synthetic dyes have taken place of dyes with natural origin dyes over the years due to their wide range of colors, cost-effectiveness, perspiration resistance, and resistance to fading by sunlight, water, and different chemicals [8].

An example of a plant-based dye is the madder root, which has a long tradition as a dyestuff because of its bright red color. Napoleon's army's red pants and the English soldiers' red coats in the 18th and 19th centuries were dyed with madder. 10,000 new synthetic dyes had been developed and manufactured at the end of 19 century. Nowadays, India, the former USSR, Eastern Europe, China, South Korea, and Taiwan consume 600 thousand tons of dyes annually [9]. About 15% of these synthetic dyes are discarded into the environment.

It is estimated that thousands of dyes are being manufactured as a mass production annually (about 800 tons). About 10% to 15% of which are discarded into nature. This scenario generates severe consequences for the polluted environment. These compounds can be toxic, so if they are discarded without being treated, they can cause harm if they are ingested by people or animals either orally, through being breathed in, or even through skin contact [6, 10, and 11].

The azo dyes themselves and metabolites such aromatic amines that are generated as they break down or degrade are linked to the harmful effects of azo dyes, notably their capacity to promote mutations. The likelihood of the dyes degrading and releasing these carcinogenic amines when they come into contact with a natural opening in the human body, such as when they are consumed orally, the ability of intestinal bacteria to reduce the dye, and most likely the activity of the enzyme azoreductase, which is found in the liver or intestinal wall [7].

Consequently, it is important to be aware of the dangers associated with dumping these azo dyes in the environment without first treating them and to understand that utilizing microorganisms to bioremediate these pollutants is a realistic alternative. This review attempts to properly assess the biological processes involved in the degradation of azo dyes by bacteria, to pinpoint the variables influencing these processes, and to outline the microbial mechanisms. In order to avoid the discharge of untreated dyes, it is necessary to develop bacterial bioremediation strategies and increase awareness of situations in which contamination by these pollutants has been discovered in sewage.

Biodegradation of Azo Dyes

The term "biodegradation" refers to the biologically assisted breakdown of chemical substances. This energy-dependent process uses enzymes to break down the dye into several derivatives. Synthetic dyes are discoloured as a result of biodegradation, but the dye molecules are also broken up into smaller, simpler pieces. When the dye's chromophoric core is broken, the dye becomes discoloured. [12]. Synthetic dyes have been discoloured and degraded using a variety of microorganisms, including fungus, bacteria, yeasts, and algae. It has been demonstrated that different microorganism groups degrade certain colours to varying degrees [13]. The use of a correctly chosen strain and its utilization under favourable conditions are required to exploit the degrading potential in dye degradation biotechnology [14].

Treatment of Colored Wastewater

There are three management types (biological, chemical, and physical) for wastewater-containing dyes. Elimination of azo dyes through physical handling is most commonly applied. Physical approaches, such as adsorption, flotation, sedimentation, irradiation, membrane filtration (nanofiltration-ultrafiltration), and reverse osmosis, are simple and effective [20]. However, these are non-destructive

procedures that depend on the handling method. They are appropriate for synthetic dyes that, when processed, produce more toxic substances than the original dyes [21].
The Potential of Bacteria for the Degradation of Azo Dyes the restrictions associated with the use of physicochemical techniques for the management of sewage contaminated with azo dyes have necessitated the development of new management alternatives that are attractive, efficient, profitable, environmentally friendly, and that produce less sludge [22]. Table 1 compares efficiency, environmental impact, impact on creatures and costs between biological and physicochemical methods.

Table-1: Comparison between biological and physicochemical degradation methods for Azo dyes.

Criteria	Biological Degradation	Physico-chemical Degradation
Efficacy	They are able to completely degrade many azo dyes under certain environmental factors. [25]	They have low color removal efficiency. They do not entirely degrade azo dyes. [26]
Impact on the Environment	They are eco-friendly because they use microorganisms or enzymes. [27]	They generate a significant amount of sludge that can cause secondary pollution problems. [29]
Energy	They require less water and energy consumption[28]	They are energy-intensive
Costs	They have low operating costs[28]	They are economically unviable. A large amount of sludge generated substantially increases the cost of these methods. [30]
Impact on living creatures	Not toxic [27]	Toxic

The effectiveness of microorganisms in the Degradation of compounds depends on various factors such as survival, adaptability, the activity of the microorganism, and the chemical Structure [23]. One of the natural alternatives for dye removal is phytoremediation, which uses plants - such as *Aster amellus* - to remove azo dyes primarily through their roots [24].
Bacterial decolorization is usually faster than fungal decolorization [15]. It is well known that under anaerobic circumstances, bacteria degrade azo dyes reductively to colorless aromatic amines. The carcinogenicity of an azo dye may be due to the dye itself, or the aryl amine derivatives produced during the reductive biotransformation of an azo linkage [16]. These colorless aromatic amines must also be degraded because they may be toxic, mutagenic, and/or carcinogenic to humans and animals [17]. Aromatic amines formed during anaerobic cleavage of the azo dyes could be additionally degraded in an aerobic handling system [18]. According to the combined anaerobic–aerobic treatment, azo dyes should be removed from the water phase by (anaerobic) reduction followed by (aerobic) oxidation of the dyes' constituent aromatic amines. This technique promises to altogether remove azo dyes from wastewater [19].
Bacterial bioremediation is frequently used in the degradation of synthetic azo dyes because it may work in groups or in conjunction with other biological inducers or promoters. Microorganisms, especially in large-scale operations, have stronger resilience to abiotic factors and lower reaction rates to enzyme inactivation, making them even more suitable options for increasing the discoloration rate of effluents polluted with azo dyes [24].
The most important competitive advantages and disadvantages that make bacteria the most effective microorganisms for the degradation of azo dyes are listed below:-

Advantages

-They have short life cycles and generate faster discoloration processes.
-They have a higher growth rate and adaptability.
-They are inexpensive, viable, and ecologically adaptable.
-Their degradation capacity is boosted when used in consortia.
-They detoxify the aromatic amines. [34]

Disadvantages:

-The discoloration process depends not exclusively on these microorganisms but also external variables such as agitation, oxygen, temperature, pH, dye structure, dye concentration, carbon and nitrogen sources, electron donors and redox mediators [30, 32].

-Under anaerobic conditions, the azo dye penetrates with difficulty through the cell membrane, affecting the rate of Degradation [30, 38].

-As a result of the degradation process, bacteria generate noxious and recalcitrant aromatic amines [39].

Pure crops do not fully degrade azo dyes, so bacterial pools are required to make the process more productive [30, 40].

-Also, the sole source of bacteria used for Bioremediation affects the Degradation rate.

METHODS

This review article analyses and summarizes the efforts of eminent specialists on azo dye categories and their detrimental effects on the environment. The systematic review article explains the biological technique, in particular the bacterial strategy, to breakdown the Azo dyes. And explains which procedure produces less toxic aromatic amines. It highlights several downsides, such as high cost, high energy demand, low efficiency, and restricted versatility. And concentrate on the benefits of bacterial biodegradation.

In recent years, innovative integrated processes called hybrid technologies have emerged. They provide a new treatment system that eliminates the individual limitations of physical, chemical, and biological methods [31]. Among the most widely used adsorbents is activated carbon, which enhances the degradation process when combined with bacteria. Sometimes, however, carbon particles become trapped in the matrix floc, and lose their adsorption properties, hindering bacterial growth and dye removal. This process allows C.O.D. and color removal from textile wastewater in a single step without additional physicochemical treatment [42].

RESULTS AND DISCUSSIONS

Biodegradation of Dyes by Bacteria

Biosorption is described by both the adsorption and absorption processes. Bacteria offers an ability to get rid of different azo dyes by adsorption property. Biosorption rates are directly compared with the composition of heteropolysaccharides and lipids of the cell wall; different groups with different charges result in attraction between them and the synthetic azo dyes. Both the living cells and the dead cells can undergo the biosorption process. In this situation, the process is called “bioaccumulation.” The number of charged groups existing in the Structure of the cell walls of microorganisms, and the distinctive structures of the azo dyes, must be considered [43]. Dead cells are more useful in Biosorption than living cells because they do not need nutrients, and could be reserved for a long time, and the application of surfactants and solvents can reuse them. The biosorption process is not the most appropriate mechanism for synthetic azo dye treatment because the large volumes of contaminated waste treatment would result in large volumes of biomass containing azo dyes, and potentially other toxic products that must be appropriately disposed of [44].

Enzymatic Degradation

The initial step for decolorizing solutions with azo dyes is the cleavage of the azo bond (-N = N-) in a chromophore group. This step, which can occur extracellularly or intracellularly, includes the transfer of four electrons in two stages. In each stage, two electrons are transferred from the azo dye to its final electron acceptor, leading to its decolorization. Azoreductases and laccases are enzymes that are capable of the reduction of azo dyes [45]. Figure 1 shows the general action mechanisms of those two enzymatic groups, plus the peroxidase

group, which also performs on the azo chromophore group [46].

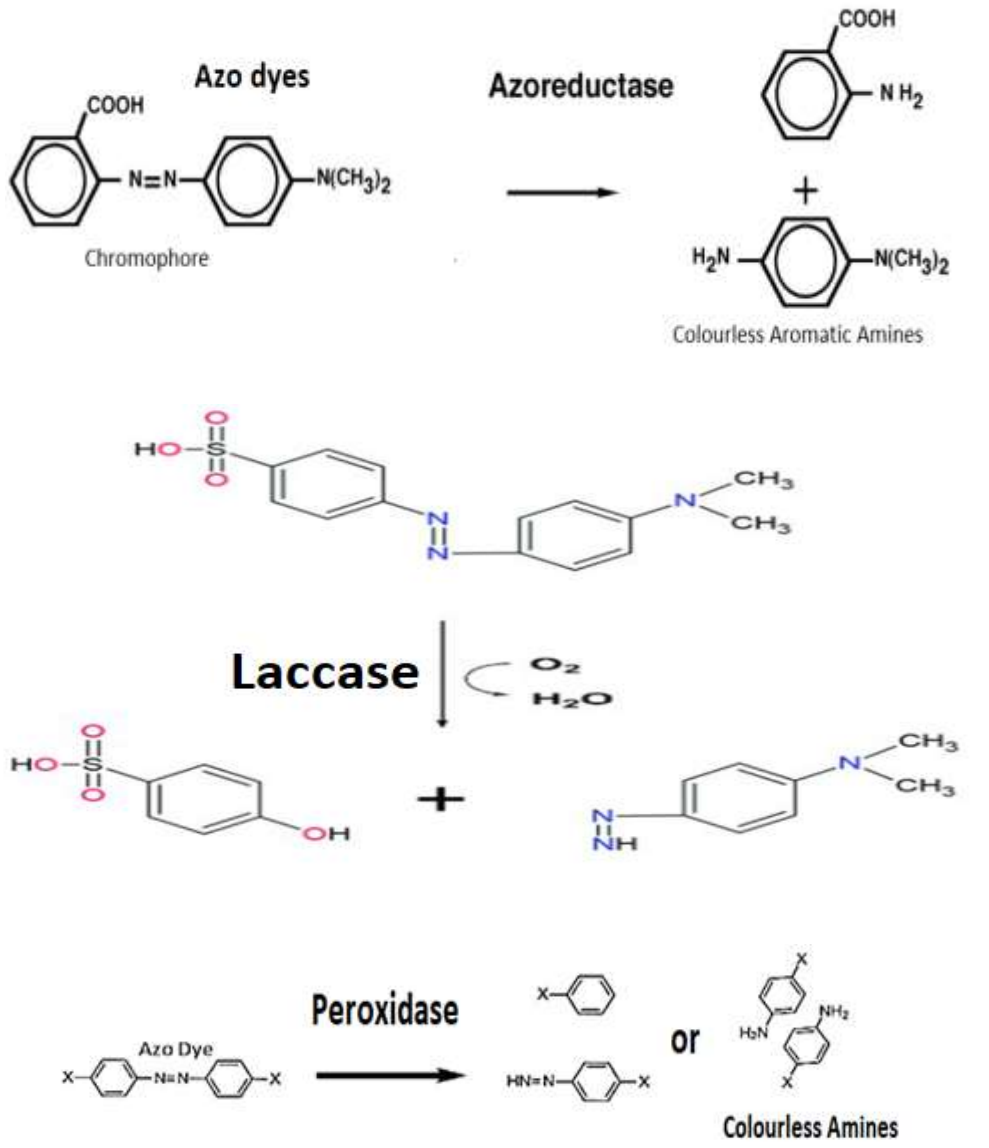

Figure 1: Schematic representation of three bacterial enzymatic degradation mechanisms of azo chromophore group

Figure 1 depicts the enzymatic disintegration caused by azoreductases. In this illustration, azoreductase serves as a crucial reducing agent to break azo bonds, produce aromatic amines, and subsequently discolour the medium. The catalytic reaction cycle mediated by laccase generates an oxidized substrate instead of potentially toxic aromatic amines, which do not acquire cofactors. Finally, lignin peroxidase or manganese peroxidase illustrate some possible products according to the breakdown of their bonds, which can be symmetric or asymmetric.

Degradation by Azoreductases enzyme

The azoreductases are the most promising group of enzymes for the Biodegradation of synthetic azo dyes. They are the reductive cleavage of synthetic azo dye bonds, producing aromatic amines. However, azoreductases need reducing agents such as NADH, FADH2, and NADPH to induce this reaction. They are strongly associated with the anaerobic degradation process of

azo dyes because oxygen impairs the azo characteristic bond reduction stage. Reducing agents are required as electron acceptors for the azo bonds and are also used in the aerobic respiration process. These enzymes are divided into flavin-dependent and flavin independent, based on function. The first class is divided into those enzymes that need coenzymes: (1) NADH only; (2) NADPH only; or (3) both NADH and NADPH [47]. This group varies depending on its source, i.e., the organism or species from which it was extracted. There are differences in, for example, catalytic activity, cofactor requirement, and biophysical characteristics. Therefore, there is specificity between the substrate and the types of azoreductase enzymes. These vary in their requirement for cofactors and reducing agents and in their ability to resist oxygen [48].

Most azo dyes have high molecular mass, and they are unable to cross the plasma membrane of cells. Nevertheless, because microorganisms have a reduction mechanism that is related to the electron transfer of azo dyes in the extracellular media, an interaction between the intracellular electron transfer methods and the dye molecules is required to achieve the degradation process. The action of azoreductase enzymes in the intracellular medium has also been recognized, and enzymes of this group have been shown to exist in both halophilic and halotolerant bacteria. [47].

Degradation by Laccase Enzymes

Laccases are oxidases with a condensed copper ion structure and great industrial value due to their ability to accommodate different substrates. They can non-specifically induce the degradation process of azo dyes by reacting to the phenolic group of azo dye by using free radicals to produce phenolic compounds, thereby generating much lower levels of toxic aromatic amines. These enzymes do not require other cofactors for their activation [49]. Although laccases do not need other cofactors, they are more reactive if cofactors are in the medium. Redox mediators dramatically enhance the degradation process and potentially expand the variety of azo dyes that this enzyme can degrade. A lot of research has been done on bacterial laccases as bio-catalysts due to their characteristics as pH working range, Thermal stability and denaturation resistance ability of surfactants that are used to remove synthetic textile dyes and treat industrial effluents [49].

Laccases Oxidoreductase

Laccases are multicopper oxidoreductase enzymes [50] the responsible of the reduction of molecular oxygen (O2) to water (H2O) [51]. Laccase was first identified by Yoshida in 1883 [52] in the sap of the Japanese tree *Rhus vernicifera*. Laccases are ubiquitous oxidases that exist in plants, lignin-degrading fungi (Basidiomycetes, Ascomycetes, and Deuteromycetes), [53-55], bacteria [56], algae [57], and insects [58].

Laccases are well-studied enzymes, due to their excellent redox concern and biotechnological applications focus on fungal laccases. Fungal laccases are involved in lignin breakdown, detoxification processes, mineralization, pigment formation, fruiting body construction, fungal morphogenesis, detoxification, sporulation, and some pathogenesis processes [59]. In plants, laccases are responsible for the polymerization of lignin [60], cytokinin homeostasis, polymerization of flavonoids in seed coatings, resistance to phenolic contaminants [57], and the formation of insoluble protective barriers, thereby contributing to the plant's defense. In bacteria, laccases contribute to melanin production, spore resistance, morphogenesis, copper detoxification, and manganese oxidation [61, 62]. In insects, laccases are responsible for sclerosing and pigmenting the cuticle [63]. Finally, in algae, laccases are associated with detoxifying phenolic compounds,

synthesizing biopolymers associated with the cell wall, and acquiring nutrients through transforming lignocellulosic substrates [39].

Structure of the Active Centre of Laccase

The active site of laccase has four Cu^{2+}, distributed in three sites [63], assigned in three types CuT1, CuT2 and CuT3 where CuT2 copper and a CuT3 copper pair form the trinuclear copper center (T.N.C.), [64, 65]. Laccases have the same structure architecture of three cuppredoxin-type domains, CuT1 is the Domain [66].

The CuT1 site is mononuclear and responsible for the enzyme's blue color due to its spectroscopic properties. CuT1 is in Domain 3, almost 6.5 Å beneath the surface of the enzyme. [67]. The coordination of CuT1 is varied due to the microorganism source. Several studies have shown that Cu(II) ions prefer square-to-flat and square-to-pyramid coordination [68]. In most fungal laccases, the axial quarter ligand in CuT1 is absent [69]. CuT1 with another three ligands forming a coordination structures from two histidine and one cysteine ligand [70, 71]. The triple coordination is much favored due to the tetra-coordination limits cationic exposure to the solvent and consequently reducing its polarization [72]. A strong Cu-S link

Compensates for the missing fourth ligand.

Fungal laccases give a non-coordinated residue as leucine or phenylalanine instead of axial methionine. These potential changes in laccases structures will affect the electronic configuration and the transfer of electrons to CuT1, as shown in Figure 2 [73].

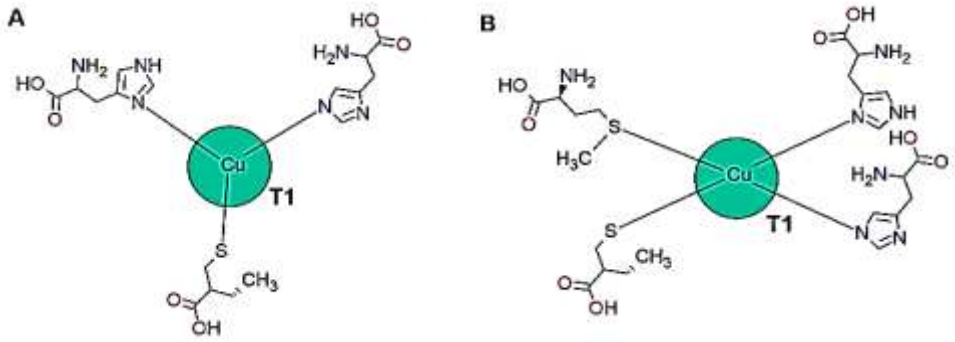

Figure 2: coordinated ligands of copper (T1). (A) Tri-coordination of laccases from fungal sources (B)

Tetra-coordination laccases from non-fungal source.

CuT2 is a mononuclear copper site with no characteristic absorbance but a parallel hyperfine coupling constant, similar to a typical tetragonal copper core [70]. This copper is coordinated by two histidine residues and an oxygen atom as a hydroxide (O.H.-) ligand and is strategically located near the T3 copper, forming a coplanar trigonal configuration [63].

The CuT3 copper site is dual nuclear of a pair of copper atoms identified as CuT3_ and CuT3_ [89]. CuT3 site has a weak U.V. absorbance at 330 nm and no electronic paramagnetic resonance (E.P.R.) signal due to the antiferromagnetic (A.F.) coupling that results from a ligand forming a hydroxide bridge between the coppers [70]. Each one of the coppers on the site is coordinated with three histidines and participates in the hydroxide group.

Laccases commonly contain all four copper atoms, but some laccases that do not have the identified blue color may have iron or zinc atoms instead [55]. Yellow laccase does not generate the absorption spectrum produced by the CuT1 site [74]. The U.V. visible spectrum did not show the typical CuT1 peak (near 600 nm) for the pure laccase of *Phellinus ribis* [75]. Atomic absorption measurements of pure laccase (POXA1) *Pleorotus ostreatus* show 0.2 _ 0.2 zinc/mol, 0.7 _ 0.2 copper/mol, and 0.7

_ 0.2 iron/mol protein, suggesting an isoenzyme with a copper/iron/zinc stoichiometry of 1:1:2 [76].

Catalytic Mechanism of Laccases

It has been assumed that laccases must be resting with all copper in the oxidized state (Cu++) [66], with the four copper atoms representing the catalytic machinery of these enzymes [51]. The oxidation of the substrate step is the starting point in the catalytic cycle in laccases with CuT1 (Cu++), which is responsible for transferring electrons, and transferring them to the trinuclear copper center via His-Cys-His [65, 66]. After oxidation of the four electrons, the fully reduced form (Cu+) is produced. Oxidation and intramolecular electron transfer are simultaneous to proton transfer, allowing the O_2 reduction to H_2O through ionizable groups within the T.N.C. channels, as shown in Figure 3 [66].

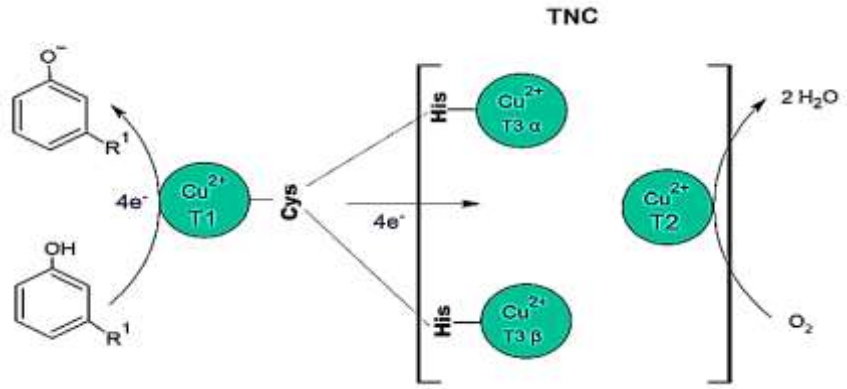

Figure 3; Oxidation reaction process in laccases.

Bacteria could be used to treat the contamination resulting from azo dyes. They can use both prior to release into the environment and for the Bioremediation of an already contaminated environment. Table 5 shows how efficiently bacteria can be applied in remediating actual samples in confirmed cases of contamination.

Factors Affecting Biodegradation of Dyes

Ecosystems are very dynamic environments with variable abiotic conditions, like pH, temperature, and the presence of oxygen, metals, salts, etc. Microorganisms, which have a crucial role in the global C, N, and S cycles, are influenced by any changes in the above parameters. As a result, their decomposition rates are also affected. The effects of these parameters should be considered when evaluating different microorganisms for degrading particular organic xenobiotics. Optimization of such abiotic conditions will significantly help in the development of industrial-scale bioreactors for Bioremediation. In this section, some of the factors affecting the Biodegradation of synthetic dyes are discussed.

Pure Bacterial Colonies

Degradation Process in the Presence of Different Salts.

As previously mentioned, bacteria can break down dyes in different salt concentrations. Inorganic salts used as mordants in dyeing processes to fix color include sodium sulfate (Na_2SO_4) and sodium chloride (NaCl). The degradation process of bacteria has proven effective for dyes at salt concentrations ranging from 0.25% to 10%. Most countries have no explicit regulations stating the proper amount of salts that can be disposed of in the environment. Even if they do, they may allow the release of these salts in high concentrations. An essential criterion for choosing microorganisms with a true biotechnological potential for use in the bioremediation of dyes is the capacity of bacteria to breakdown colours in salty conditions. [78].

In their research, Xu and co-workers [79] used a strain of *Shewanella marisflavi*, which is an exoelectrogenic bacterium with extracellular electron transfer pathways, and that has been isolated from marine aquatic sediments in China. Testing with the dye Xylidine Ponceau 2R revealed that this strain is capable of decolorizing the medium at NaCl concentrations ranging from 0% to 20%. Higher salt concentrations also affect the solubility of the azo dye by increasing the ionic strength of the mixed solution, leading to a floc formation. Flocculation was also noticed at lower NaCl concentrations in the presence of *Shewanella marisflavi*. The researchers reported that the tested bacteria had two decolorization mechanisms: Degradation and flocculation. There was only Degradation in dyes with up to 6% NaCl, degrading and flocculating in dyes with between 8% and 10% NaCl in the medium, and only flocculation in mediums with more than 10% NaCl.

The ability of laccases in fungi and plants to break down phenolic compounds is well known. Some bacteria that already contain these types of enzymes can perform in environments with high salt concentrations and an alkaline pH, presenting an extra advantage over fungi and plant laccases [80]. Neifar and co-workers [80] used a laccase produced from a Pseudomonas *extremorientalis* strain isolated from oil-contaminated sediments in Tunisia to assess the Degradation of Congo red dye. This strain's enzyme has good resistance to salt, keeping 90% of its activity in the presence of NaCl at 17.5%, as well as alkalinity, maintaining its activity in pH 7–10.

Wang and co-workers [81] shown that a strain of the marine bacteria Aliiglaciecola lipolytica is capable of discolouring a medium that contains the dye Congo red. at a salt concentration of 4%. The strain did not adapt to pH increase and had glucose as the best carbon source for its dye degradation process activity. The study also represents the mode of action of bacteria in the degradation mechanism of azo dyes that involves the first stage of the adsorption of azo dye in their cells through the extracellular polymeric substances (EPS), When a portion of it is broken down by a process involving the co-metabolism of glucose, the azoreductase enzymes, and intracellular laccase, the remaining colour is encapsulated in the bacterial cell.

In a study by Zhuang and co-workers [82], four bacteria, including Enterococcus faecalis, Shewanella indica, Oceanimonas smirnovii, and Clostridium bufermentans, were utilized in the Degradation of azo dyes. These bacteria were obtained from the shore of the Republic of China. The isolated samples of these strains were capable of degrading eight different synthetic dyes, with most of them achieving high removal rate percentages of above 70%.

Even at higher salt concentrations of up to 7%, E. faecalis and C. bufermentans were still able to decolorize dye at rates greater than 80%. The four bacteria were also demonstrated to retain their decolorization activity rate in the presence of various ions, with the majority of those examined having no effect on them. Cadmium (Cd2+) and Copper (Cu2+) were the ions that most adversely affected the decolorization results. This could be a serious problem because metals are frequently found in industrial dye effluents.

Biodegradation in Alkaline Medium

Sodium hydroxide and other essential substances are frequently used in the textile dying industry, which is one of the three sectors with the most extensive release rate of azo dye-contaminated effluent into the environment. So we must use an alkali-resistant bacterium that for the treatment of these azo dyes effluents can reach a pH of 11.5. Guadie and co-workers [83] tested the decolorization capacity of a Bacillus sp. strain obtained from alkali lakes. This strain could decolorize mediums with pH from 9-11 with above 90% efficiency. Also, it worked well against the seven different dyes that were put to the test, completely decolorizing the sample in anoxic and

anaerobic conditions. There was essentially little environmental medium decolorization when oxygen was present.

Several scientists have discovered and isolated bacterial strains that may discolour colours in alkaline solutions. Tests on an Aeromonas hydrophila strain that was capable of destroying the azo dyes Reactive Red 198 and Reactive Black 5 throughout a pH range of 5.5 to 10 should be presented. Jadhav and his colleagues [84] isolated a strain of *Comamonas* sp. from contaminated soil in an industrial environment. It could biodegrade the dye Direct Red 5B in a pH range of 6–12, but it performed best in a neutral pH. This differed from the results of the Asad group [85], who worked with three different *Halomonas* sp. strains isolated from textile effluents. They discovered that the best decolorization was obtained by all of them in an alkaline media, with pH 11 having the maximum activity. In an aerobic medium, these three investigations revealed little to no decolorization action.

Bioremediation in Anaerobic and Aerobic Environments

The generation of very poisonous aromatic amines during the biodegradation of azo dyes in anaerobic conditions is one of the possible issues. (e.g., Benzidine and 4-bi-phenylamine) as byproducts that are only degraded in aerobic environments [86]. Shah's research involved evaluating the Reactive Black dye's biodegradation using a strain of Aeromonas sp. in a microaerophilic environment until the colour vanished from the medium. Next, he induced an aeration process that encouraged the oxidation of the aromatic amines formed by the dye's degradation into non-toxic products, obtaining a discoloured medium with low toxicity. These results demonstrated that a dye bioremediation procedure may also be successful in environments other than anaerobic ones. Franca and his coworkers [87] adopted a distinctive strategy. Following the full removal of the colour, they employed an aerobic setting to assess an Oerskovia paurometabola strain's capacity to metabolize the hazardous compounds produced by the preceding stage. They conducted the decolorization experiments in an anaerobic medium. Positive results were found, with decolorization rates above 90% and harmful product elimination rates above 63%.

Aeromonas hydrophila and Lysinibacillus sphaericus strains were used in Srinivasan and Sadasivam's [88] research in order to degrade an azo dye, in this case Reactive Red 195, utilizing the aerobic-microaerophilic method. This research also used a molecular docking tool and decolorization trails to clarify the interaction between the amino acid residues of the enzymes laccase, azoreductase, and the dye. The research indicated that these strains were highly efficient in biodegrading the azo dye showing an apparent positive correlation between the score of the docking studies and the results of dye biodegradation. In silico approaches for azo dye, biodegradation studies have been increasingly employed as a strategy to use resources and research efforts [89-91].

Despite the fact that the mechanism of azo dye decolorization frequently takes place in anaerobic environments, Sari and Simarani's research [92] indicates that there are bacteria that can perform azo dye biodegradation in an aerobic environment. The study highlighted the capacity of the strain of Lysinibacillus fusiformis to obtain a 96% decolorization rate of Methyl Red dye in two hours in a medium with a neutral pH and temperature of 30° and 2° C under aerobic conditions. The research also came to the conclusion that lignin, azoreductase peroxidase, and laccase enzymes were all engaged in the oxidoreduction process of the bacteria. As can be seen, laccase and azoreductase are frequently discussed in research on the biodegradation of azo dyes by bacteria, such as the mechanism of action of Pseudomonas stutzeri in the biodegradation of the dye Acid Blue 113. [93]. This strain demonstrated effective

decolorization and strong resistance to azo dye at high doses. The presence of both enzymes in the microorganism's decolorization pathway was confirmed by genomic research. These two enzymes were shown in several studies to be involved in the bioremediation of azo dyes. [94-97].

The Effect of pH on Bioremediation

Generally, fungi and yeasts show better decolorization and biodegradation activities at acidic or neutral pH, while bacteria show better decolorization and biodegradation activities at neutral or basic pH. Nozaki et al. [98] studied the decolorization of 27 dyes, including monoazo, diazo, phthalocyanine, and tri-phenylmethane dyes, using 21 different basidiomycetes. They pointed out that the optimum pH for decolorizing the dyes was 3–5. While studying the Biodegradation of Methyl Red by *Galactomyces geotrichum* (yeast), Jadhav et al. [84] found that this strain could completely decolorize Methyl Red at a pH range of 3–5. The optimum pH for decolorization was 3. Sarnthima et al. [99] studied the decolorization of synthetic dyes by the white-rot fungus *Lentinus polychrous*. They discovered that Indigo Carmine, Remazol Brilliant Blue R (RBBR), Bromophenol Blue, and Methyl Red decolorized best at pH values of 9.0, 3.0, 4.0, and 4.0-5.0, respectively. In line with Yang et al. [100], the optimum pH for decolorization of RBBR (200 mg L−1) by a white-rot fungus Trametes sp. SQ01 was 4.5. Similarly, according to Shedbalkar et al. [101], the optimum pH for decolorizing Cotton Blue (50 mg L−1) by *Penicillium ochrochloron* MTCC 517 was 6.5.

Raghukumar et al. [102] reported the Effect of pH on color removal by three marine fungi, and the effective pH was 4.5. While studying the Effect of pH on decolorization and Biodegradation of Navy Blue HER by *Trichosporon beigelii* (yeast), they found that the decolorization rate was optimum at pH 7. These studies show that fungi and yeasts degrade synthetic dyes mostly at low or acidic pH. They point out that the fungal ligninolytic enzymes show maximal activity at low pH; efficient dye decolorization is also observed at low pH. Furthermore, fungi can grow at low pH, ranging from 4–5 [103].

Wang et al. [104] studied the decolorization of Reactive Black 5 by a bacterial strain Enterobacter sp. EC3. According to their results, Enterobacter sp. EC3 showed a high decolorization rate at pH seven after 108 hours of incubation. Similar decolorization efficiency was observed from pH 8–12 in 120 hours, whereas the rate of color removal was much lower under acidic conditions (pH 4–6). The authors concluded that this could be because of the optimum pH for Enterobacter sp growth. EC3 was neutral. Decolorization and Biodegradation of Scarlet R by a microbial consortium-GR consisting of two bacterial strains - Proteus vulgaris NCIM-2027 and Micrococcus *glutamicus* NCIM-2168 - were studied. They found that the percentage of decolorization of Scarlet R at pH 5, 6, 7, and 8 was 62, 82, 100, and 100 after 24, 24, 14, and 36 hours, respectively, by P. vulgaris. For M. *glutamicus*, it was 55, 65, 100, and 100 after 24, 24, 20, and 48 hours respectively. From the previous results, it is clear that the most favorable pH range for dye decolorization by these bacterial strains was 7–8, with the optimum pH being 7 [105].

Temperature

Temperature is a potential environmental factor because the biodegradation activities of microorganisms are affected by changes in temperature. It is a common observation that dead animal bodies decompose faster in summer than in winter. This is because, in summer, the warmer environment favors the growth and multiplication of the decomposers, mostly soil bacteria and fungi. The relationship is not linear above a specific temperature - the optimum temperature for the growth and reproduction of the concerned microorganisms. Above the optimum temperature, the degradation activities of

the microorganisms decrease because of the slower growth and reproduction rate and deactivation of the enzymes responsible for Degradation. Hence, microorganisms will operate most effectively during biodegradation at the temperature that is ideal for their development, reproduction, and activity.

Different fungi have different optimum growth temperatures, most growing at 25–35 °C [106]. According to Shedbalkar et al. [101], the optimum temperature for the decolorization of Cotton Blue by P. *ochrochloron* MTCC 517 was 25°C. Jadhav et al. [84] studied the decolorization of Methyl Red by G. *geotrichum* (yeast) at different temperatures, that is, 5 °C, 30 °C, and 50 °C. The optimum temperature for decolorization was found to be 30 °C. A quicker decolorization was noted at 37 °C within 24 hours of incubation when the decolorization of Navy Blue HER by T. beigelii (yeast) was tested at various temperatures (30-50 °C) [106]. Reactive Black 5 is discoloured by an Enterobacter sp. bacterial strain. EC3 was studied [104]. They found that with an increase in temperature from 22–37 °C, the decolorization rate increased, and a further increase in temperature to 42 °C drastically affected the decolorization activity of Enterobacter sp. EC3. The optimum temperature for decolorization was found to be 37 °C. The authors concluded that the significant suppression of decolorization activity at 42 °C might be due to the loss of cell viability of the enzymes responsible for decolorization. Saratale et al. [105] studied the Effect of temperature on the decolorization of Scarlet R by a microbial consortium consisting of two bacterial strains—P. vulgaris and M. *glutamicus*. They found that the percentage of decolorization of Scarlet R by P. vulgaris at 30, 37, 40, 45, and 50 °C was 100, 100, 90, 82, and 45 after 24, 14, 24, 36, and 48 hours, respectively. For M. *glutamicus*, it was 100, 100, 94, 70, and 50 after 36, 20, 30, 36, and 48 hours, respectively. These results show that these strains' favorable temperature range for decolorization was 30–37 °C, with the optimum temperature being 37 °C. All the studies mentioned above indicate that microorganisms degrade synthetic dyes best in the 25–37 °C.

Initial Dye Concentration

Synthetic dyes are subject to microbial decolorization, which is widely researched in relation to the effect of initial dye concentration. With a rise in dye concentration, the rate of decolorization reduces. The findings of certain research examining the impact of initial dye concentration on microbial decolorization of synthetic colours are.

From Table 6, it is clear that increasing the initial dye concentration decreases the decolorization considerably. This decrease in decolorization with an increase in initial dye concentration is attributed to the toxicity of the azo dyes to the growing microbial cells at higher dye concentrations. According to Gopinath et al. [107], By using a strain of Bacillus sp. that was isolated from the effluent of the tannery industry to biodegrade Congo Red, it was discovered that lowering the decolorization rate as initial dye concentration increased, and inhibition was observed at high concentrations (1,500 and 2,000 mg L−1). Dye decolorization decreased sharply at 1,000 mg L−1; these strains achieved only about 20% decolorization.

Nitrogen Content in Medium

The supplementary sources of N in the growth and decolorization media may affect the microbial decolorization of synthetic dyes. The amount of nitrogen present in the media affects dye decolorization by altering the enzyme production by fungi; for several fungal species, the ligninolytic enzyme activity is suppressed rather than inspired by great nutrient N concentrations (25–60 mM). Decolorization and mineralization of azo dyes have been reported to be more enhanced in nitrogen-limited than in nitrogen-sufficient cultures. The addition of supplemental nitrogen only inhibited the

decolorization of Congo-Red in plates containing high amounts of nutrient nitrogen [108].

Salts

Textile effluents contain various acids, alkalis, salts, or metal ions as impurities [109]. Wastewaters from textile processing and dyestuff manufacturing industries contain substantial amounts of salts in addition to azo dye residues. Salt concentrations of up to 15–20% have been measured in wastewater from dyestuff industries (E.P.A., 1997). Thus, microbial species capable of tolerating salt stress will be beneficial for treating such wastewater. Finding salt-tolerant bacteria that can break down azo dyes may make it easier to create biological treatment processes for saline azo dye solutions in bioreactors. Khalid and associates [110]. studied the potential of *Shewanella putrefaciens* strain AS96 for decolorizing four structurally different azo dyes (100 mg L−1) at different concentrations of NaCl. The results showed that Reactive Black 5, Direct Red 81, Acid Red 88, and Disperse Orange 3 were 100% decolorized in 8 hours when NaCl concentration was 0–40 gL−1. When NaCl concentration was increased to 60 g L−1, more time was needed for decolorization, and in the case of Acid Red 88 and Disperse Orange 3, the percentage decolorization also decreased significantly. Thus, at NaCl concentration of 60 g L−1, Reactive Black 5, Acid Red 88, and Disperse Orange 3 were decolorized 100%, 53%, and 58%, respectively, in 24 hours. Decolorization was not observed at NaCl concentrations above 60 gL−1.

Shaking

There are contradictory reports about the Effect of shaking or agitation on microbial decolorization of synthetic dyes. Some writers contend that shaking enhances decolorization, whereas others contend that static circumstances do. According to Kaushik and Malik [12], higher color removal is observed in shaken cultures because of better oxygen transfer and nutrient distribution compared to stationary cultures. However, the agitated culture of Pseudomonas sp. SUK1 showed almost no decolorization in 24 hours, while the static culture decolorized more than 96% of the initial dye concentration (300 mg L−1) of Reactive Red 2 in 6 hours.

Similarly, Husseiny [111], while studying the bio-degradation of Reactive Red 120 and Direct Red 81 by *Aspergillus Niger*, found that the static conditions were more efficient than the shaken. Higher enzymatic activities were observed in static conditions [12]. The Effect of liquid-medium culture type depends on the synthesis of ligninolytic enzymes and the rate of decolorization of synthetic dyes by *Irpex lacteus*. They used Reactive Orange 16 and Remazol Brilliant Blue R at an initial concentration of 150 mg L−1. After seven days, they measured the maximal enzyme activity (U L-1) of MnP, laccase, and LiP and percent decolorization. They found that, in stationary culture, the maximal enzyme activity of MnP, laccase, and LiP was 76±4.4, 2.0±0.0, and 1.1±0.2U L-1, respectively, while in submerged culture, these were 0.2±0.1, 1.0± 0.1, and 0 UL-1, respectively. Reactive Orange 16 and Remazol Brilliant Blue R decolorized similarly, with stationary culture showing a decolorization rate of 85.8% and 99.7% respectively, and immersed culture showing a decolorization rate of 16% and 97.8%. According to these findings, stationary culture performed better than submerged culture in terms of maximum enzyme activity and decolorization. Navy Blue HER by T. beigelii NCIM-3326 was subjected to a study by Saratale et al. [106] to examine decolorization and biodegradation. They discovered that shaking circumstances resulted in just a 30% decolorization of Navy Blue HER, but static conditions resulted in a 100% decolorization. The growth of T. *beigelii* was also observed to be more under static conditions (9.2 gL−1) than shaken conditions (4.2 gL−1).

Future Perspectives

A possible method of treating dye wastewater is the biodegradation of synthetic colours utilising various fungus, bacteria, yeasts, and algae. Green plants are nature's factories; they fix inorganic chemicals (CO_2 and H_2O) into organic forms (glucose and other complex molecules) through photosynthesis and other reactions. Microbes are nature's tools; they convert the organic materials (dead bodies of plants and animals) back to inorganic forms (CO_2, H_2O, and salts) through decomposition and mineralization. Consequently, maintaining a balance between the organic and inorganic worlds is the responsibility of green plants and bacteria. With the increasing production of synthetic chemicals and their ultimate release into the environment, natural microbial populations cannot decompose them. As a result, such chemicals are accumulated in the ecosystem and affect the quality of life of plants, animals, and humans. Keeping in mind the increasing production of these chemicals and their persistence in the natural environment, their removal is of utmost necessity. By exploiting the biodegradation potential of different microbes, it is possible to manage the problem in the most effective and socially acceptable way. A better understanding of the Biodegradation of synthetic dyes requires knowledge of chemistry and microbiology, whereas its application on an industrial scale requires knowledge of biochemical engineering as well. As a result, research in this area is quite multidisciplinary. Since interdisciplinary research is highly encouraged and valued worldwide, especially in broad-minded communities, it is fully hoped that the science and technology of Biodegradation of organic xenobiotics will emerge as a leading one for controlling environmental pollution. An understanding and knowledge of Biodegradation are helpful in pollution abatement and in the production of biofriendly and environment-friendly products like biodiesel, bioethanol, biopesticides, biopolymers, etc.

The biodegradation abilities of microorganisms can be enhanced by gradually exposing them to higher concentrations of synthetic organic chemicals. Adapting a microbial community toward toxic or recalcitrant compounds is found to be very useful in improving the rate of the decolorization process [112]. Adapting microorganisms to higher concentrations of pollutants is called acclimatization, which leads to forced or directed evolution. Microorganisms exposed to higher levels of pollutants evolve mechanisms and pathways for handling (degrading) them. This happens through an expression of genes encoding for the enzymes responsible for Degradation. Alternatively, identifying, isolating, and transferring genes encoding for derivative enzymes can greatly help design microbes with enhanced degradation capabilities. Thus, both acclimatization and genetic engineering can help design super-degraders. Among the two methods, acclimation is more natural since just a few components are activated, preserving the microorganism's inherent genetic configuration. In genetic engineering, on the other hand, the natural genetic setup of the microorganism is changed by incorporating new gene(s). Therefore, many scientists (primarily environmental scientists) are sceptical about the usefulness of genetically modified organisms. They worry that these altered species may bring up fresh environmental issues. Time will determine if these worries are justified or not.

CONCLUSION

Biodegradation of synthetic dyes using different methods is a promising approach for treating dye wastewater to help the climate change phenomenon. Bacterial Biodegradation is the most environmentally friendly method. Bacterial Bioremediation is commonly applied to the Degradation of synthetic azo dyes because they can

perform with consortiums - or in associations with other biological promoters or inducers - through the three bacterial enzymatic degradation mechanisms of the azo chromophore group. Azoreductase yellow enzyme, NADH, is used as an essential reducing agent. The catalytic reaction cycle is mediated by the laccase blue enzyme with a generation of the oxidized substrate instead of potentially toxic aromatic amines and lignin peroxidase and manganese peroxidase as green enzymes. Dead cells are more useful in Biosorption than living cells because they do not need nutrients, they can be reserved for a long time, and the application of surfactants and solvents can reuse them. Ecosystems are very dynamic environments with variable abiotic conditions like pH, temperature, and presence of oxygen, metals, salts, etc., that affect the Biodegradation of dyes. The effects of these parameters need to be taken into account.

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