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Review

Azo dyes degradation by microorganisms – An efficient and sustainable approach

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ABSTRACT

Synthetic aromatic compounds consisting of various functional groups are known as dyes. These colored compounds are often discharged in effluents, and they are very dangerous to aquatic life. Basically, the dye industry started by using natural plant and insect sources, and then suddenly turned into artificial manufacturing. Natural equilibrium of our environment gets changed by the reduction in photosynthetic activity due to the dyes. In China 900,000 tons of all kinds of dyes are usually produced, which are used in many industries like food, textile, food, paper and leather. Untreated wastewater contaminates aquatic bodies by causing eutrophication, change in water color, oxygen depletion which affect aquatic organisms to a great extent. Dye wastewater is now the key environmental pollution form. In recent eras an extensive study line has been developed to explore the dye decolorization and biodegradation under both aerobic as well as anaerobic conditions. In this review, the chemistry, toxicity and microbial biodegradation/decolorization are presented. Some recent studies along with the new techniques and methodologies of remediating the dye pollution are also discussed to provide the bases of their handling. Overall, efficient and high biodegradation potential make microbes an impending foundation for green chemistry to eradicate toxic dyes from industrial wastewater.

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1. Introduction

The aromatic compounds, azo dyes, consisted of one or many -N=N- groups. These aromatic compounds also have a major class of synthetic dyes utilized in various commercial areas. These man-made colored compounds are being utilized in many industries like fabric industry, food industry, leather industry and paper

Abbreviations: DNA, Deoxyribonucleic acid; p-DAB, p-dimethylaminobenzene; FADH₂, Flavine-adenine dinucleotide; NADH₂, Nicotinamide adenine dinucleotide; HRT, Hydraulic retention time; MFCs, Microbial fuel cells; PdNPs, Palladium nanoparticles; CAP oxidation, Centella asiatica phenolics oxidation.

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industry and are also used in other industries as cumulative in different products (Brown and De Vito, 1993). These dyes contribute up to 40 % of dyeing pollution in developing countries like India and Bangladesh (Rawat et al., 2018). Azo dyes have made their large use in the food industry as they impart significant appearance to processed foods and make them appealing to their users. Natural dyes are less stable as they degrade but azo dyes are much stable on the other hand to be used freely in food processing (Yamjala et al., 2016). In laboratories azo dyes are used as pH indicators or biological stains. It is being noted that only 85 % dye gets attached to clothes and remaining 15 % is wasted in water as an effluent (Tripathi and Srivastava, 2011).

Large amounts of industrial effluents are generated by these industries every year causing mainly the aquatic pollution quite dangerous for aquatic biota as well as for plants and other animals like human beings (Gao et al., 2018). Many different approaches (physical, chemical and biological techniques) are now being used by scientists for dyes removal from wastewater (Sarkar et al., 2020). Some examples of azo dyes are Trypan Blue, Lithol Red, Orange I, Orange II, 4-Phenylazo-Naphthylamine, Methyl Red, Allura Red, Sunset Yellow, Yellow AB, Carmoisine, Mercury Orange,

and Phenylazophenol with their reduced aromatic amines. The degradation and reduction of azo dyes produce such compounds that are harmful and mutagenic in nature (Rawat et al., 2018).

The holistic and large scale notion is required to handle these chronic dyes to know whether the toxicity is caused by degraded dye products or by the parent molecule itself. Carcinogenicity of some azo dyes is observed without being cleaved into aromatic amines (de Campos Ventura-Camargo and Marin-Morales, 2013) which are resistant to biodegradation due to their xenobiotic nature. Various physicochemical methods including photocatalysis, activated carbon adsorption, ion exchange, advanced oxidation, and ozonation have been used to decolorize such dyes (Malik et al., 2017; Rawat et al., 2018). These processes are very expensive, and require huge amounts of chemicals and energy. Moreover, use of such processes generate large amounts of sludge containing aromatic amines which become toxic after use to living organisms including humans (Vikrant et al., 2018; Ajaz et al., 2019; Shindhal et al., 2021).

The peculiarity of the present review is that it highlights the various aspects of azo dyes including dye nature, dye reduction, dye toxicity and dye degradation through various microbes including bacteria and fungi. Moreover, it also addresses the current and future prospects for the mitigation of azo dyes microbial-based remediation approaches.

1.1. Chemistry of azo dyes

Due to the high affinity of dyes with water, these colored substances are used in aqueous form. Chromophore group, present in the chemical structure of azo dye, mainly contributes in coloring and is commonly used in leather, textile, or food industry (Franco et al., 2018). By-products of petroleum and earth minerals are used to form the synthetic dyes. A variety of dyes like synthetic dye, basic dye, acidic dye, oxidation dye, anthraquinone dye, acridine dye are used in the textile industry but mostly the reactive dyes such as azo dyes are used. These dyes cover most of the area in synthetic aromatic dyes class which are excessively used in dyeing. Azo dyes are water soluble with huge commercial interest and have single or many azo ($-N=N-$) and sulfonic (SO_3^{3-}) groups. Azo dyes may have more than one azo linkage. The dyes consisting of one nitrogen-nitrogen bond ($N=N$) are called monoazo dyes; likewise are the, diazo dyes, triazo dyes, and polyazo dyes (Fig. 1), having the two $N=N$ bonds, three $N=N$ bonds or more than three $N=N$ bonds respectively (Muniyasamy et al., 2020).

On the basis of hydrophobicity, azo dyes are categorized into two classes;

(a) Hydrophobic azo dyes: these are reduced intracellular after bacterial cell uptake.

(b) Hydrophilic azo dyes: these are reduced extracellular.

Azo dyes are non-fluorescent in nature, so to track their pathways the fluorescent probes are used. The alkyl bond binds these probes to azo dyes. On the basis of different azo dye usage purposes various types of azo dyes are used for dyeing. All the dye used for dyeing does not get fixed to the fabric but releases outside as a waste and by mixing in water causes environmental pollution with various health maladies.

2. Mechanism of azo dye reduction

For azo compounds, reduction is the most important reaction which occurs in mammalian liver in the presence of azoreductases. It is estimated that bacterial azo dye reduction might be non-enzymatic. But bacterial azo dye degradation is observed under anaerobic or aerobic conditions both. Reduction involves the $-N$

N - bond breakage with aromatic amines metabolites formation (Cao et al., 2018).

Reduction processes may occur by different methods, such as enzymes, redox mediators of low molecular weight, and chemical reduction through biogenic reductants. This reaction could be intracellular or extracellular. In mammals, the intestinal microorganisms in the gastrointestinal tract degrade the water soluble azo dyes but liver enzymes can metabolize the water insoluble azo dyes. Anaerobic bacteria are mostly present in the gastrointestinal tract, which are quite active in metabolizing compounds including azo compounds. These microflora reduce the azo dyes into mutagenic and toxigenic substances. Thus, the reduction of azo dyes, a significant process of azo compounds, plays a key role in mutagenicity and carcinogenicity of such dyes (Chung, 1983).

3. Textile coloration with azo dye

The dispersive dyes are used through suspension as they are insoluble in water and are used on cellulose fiber and other hydrophobic fibers. The dye is hydrolysed and the precipitation of basically insoluble formation occurs in dispersed form on the cellulose acetate in the dyeing. Usually, all of this happens with long chains dispersing agents used for the dye suspension stabilization, which facilitates addressing with the hydrophobic fiber (Gottlieb et al., 2003). To dye the manufactured fibers i.e., cellulose acetate, polyester, nylon, and polyamide azo dyes are commonly used. It is observed that almost 3,000 types of dyes are present in the market and almost 50 % of them belong to azo dyes class. Azo dyes present a great variety of colors with permanence properties having excellent fixation characteristics when compared to the natural dyes.

4. Toxicity of azo dyes

Many research laboratories used animals as a model organism to check the exact toxic effects of these azo compounds and their

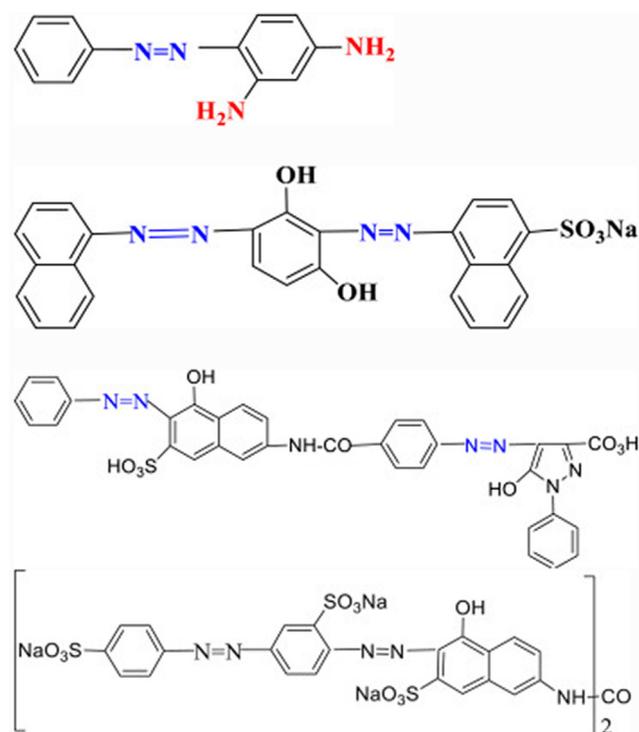


Fig. 1. Classification of azo dyes bonds as monoazo, diazo, triazo and polyazo (Benkhaya et al. 2020).

derivatives on living organisms (Parrott et al., 2016). Several studies related to degradation and metabolites formation have been conducted to establish the strategies to lessen the dangerous impacts of these compounds on living organisms. The biological and toxicological activities of each dye differ greatly, despite the fact that they may belong to similar groups with the same chemical structure (Chen et al., 2018). Extreme water pollution, affecting the aquatic biota is the most deleterious impact of uncontrolled azo dyes released in water bodies. Under these stressed environmental conditions, the living organisms face many problems, like for light absorption reduction in organisms and production of different amines under anaerobic conditions (Rawat et al., 2018).

Different studies have presented that azo dyes are accumulated to an alarming level into the environment with toxic, mutagenic and carcinogenic effects, and different damages to organisms occur due to their biotransformation products (Ismail et al., 2019). Recently the genetic toxicology of some azo dyes has been reviewed by researchers (Hassan and Carr, 2018; Shindhal et al., 2021).

After azo dyes reduction mutagenic activity is observed. With relation to the original compound, the aromatic amines formed as metabolites during reduction can show their toxic effects according to their chemical structure. Azo dye reduction can produce the DNA adducts, which could be toxic even to discolourizing microorganisms. Probably toxicity involves more than one reduction process (Alderete et al., 2021). The produced sludge also contains dyes which can leach into landfills and by mixing with water harm the aquatic creatures (Ganesh et al., 1994).

4.1. Toxicity of azo dyes in humans

Azo dyes are compounds having diazotized amine combined to phenol or amine with more than one azo linkage with the precursor of aromatic amines. In humans' azo dye benzidine is associated with bladder cancer with high risk in dye workers. Azo dyes pose harmful effects like genotoxicity, mutagenicity, and carcinogenicity to humans along with other animals (Chung and Cerniglia, 1992). Human skin microflora, intestinal microflora and liver azoreductase are able to reduce azo dye. Toxicity is also observed in some azo dyes that do not reduce into aromatic amines. But various human tumors are observed due to carcinogenic effects of cleaved aromatic amines like benzidine. Human oral exposure to azo dyes results in aromatic amines formation, by intestinal microflora and liver azo reductases with carcinogenic properties. In highly industrial countries, intestinal cancer is prevailing, so there is a large connection between high cancer cases and huge azo dye uses in these countries. Beside this many other human maladies have also been observed (Chung, 2016).

4.2. Toxicity in plants

Azo dyes presence and toxicity have been seen in plants due to release of azo dyes into the environment. Azo dyes show their effects in the following three ways.

- a-By reducing the photosynthetic activity.
- b-By reducing plant growth.
- c-By decreasing soil quality.

In water stressed countries it is common practice to irrigate soil with grey water. The soil irrigated with grey water has the following properties i.e. reduction of soil pH, increase in soil salt and organic matter content with change in macro or micro nutrients concentrations. Dye contaminated textile wastewater can accelerate ethylene synthesis in plants. Red HE3B (Reactive Red 120) toxicological study showed the oxidative stress and a high abundance of chromosome aberrations with micronuclei in the root cells of some plants. The rate of damage induced was much higher in

DNA by the dye itself, instead of its metabolites. The genotoxicity and mutagenicity of azo dyes are treated much effectively by chlorination (Xu and Li, 2010).

4.3. Toxicity in other animals

Azo dyes toxicological effects are also observed in other animals. For example, cytotoxic and genotoxic effects of p-dimethylaminobenzene (p-DAB) in bone marrow cells and spermatozooids of rats are shown by the mitotic index and chromosome aberrations. In rats fed with p-DAB, many chromosomal aberrations and nuclear abnormalities in germ cells are observed. Researchers have concluded that clastogenicity, a mechanism of micronuclei formation within mammalian cells, is induced by the textile dyes. The azo dye C.I. Disperse Blue 291 dye toxicity, by fragmentation of DNA, micronuclei formation and increase in rate of apoptosis in mammalian cells (HepG2) is also being observed. Sudan azo dye genotoxic and mutagenic effects in humans and bladder of other mammals are also observed. The aneugenic and clastogenic actions of azo dyes are also observed for the test organisms (Han et al., 2020).

The mutagenic and carcinogenic effects of 3-methoxy-4-aminobenzene against bacteria and rats have been reported (Abe et al., 2018). Other cancers like splenic sarcomas, bladder cancer, hepatocellular carcinomas, cell anomalies and chromosome aberrations are also observed due to some azo dyes exposure. These hazardous effects of azo dyes and their metabolic products occur due to their direct effect on the cells (Abe et al., 2018) which interacts with DNA molecules, and damages them. In rodents the carcinogenic and teratogenic effects with malfunctioning of reproductive organs is also observed due to azo dyes (Parrott et al., 2016). In benthic organisms including mammals and fishes these hydrophobic compounds produce lethal effects for their survival and growth (Milani et al., 2018).

5. Azo dyes degradation by microbes

Microorganisms have devised some mechanisms to degrade azo dyes (Fig. S1). The microbial effectiveness to degrade azo dyes totally depends on the selected strain ability. Microbial biodegradation is cheap and economical with less sludge production (Meerbergen et al., 2018). Mineralization of azo dyes is reported in Basidiomycetes. By different mixed and pure cultures of bacteria, decolorization of azo dyes is being reported under anoxic conditions (Guo et al., 2020).

5.1. Azo dyes degradation by bacteria

Extensive studies are done to get to know about the role of bacteria in dye decolorization. The xenobiotic compounds like azo dyes are recalcitrant to biodegradation and are mineralized by certain microorganisms including the bacteria (Naraginti et al., 2016). They have some enzyme systems which decolorize and mineralize the azo dyes under certain environmental condition (Zhang et al., 2010). Several aerobic and anaerobic bacteria i.e. *Bacillus subtilis*, *Pseudomonas* sp., *Lactobacillus* sp., *Staphylococcus* sp., *Enterococcus* sp., *Corneybaterium* sp., *Xenophilus* sp., *Clostridium* sp., *Acinetobacter* sp., *Rhizobium* sp., *Micrococcus* sp., *Rhodobacter* sp., *Dermacoccus* sp., *Klebsiella* sp., *Proteus* sp., *Alishewanella* sp., *Morganella* sp., *Aeromonas* sp., *Alcaligenes* sp., *Shewanella* sp., and *E. coli* have been reported to degrade or decolorize azo dyes (Ajaz et al., 2019; Chang and Kuo, 2000). For decolorization *Pseudomonas* sp. is widely used and several genera of Basidiomycetes for azo dyes mineralization are used (Hsueh and Chen, 2007).

Very few aerobic bacteria are reported to reduce azo dyes, with diminutive knowledge of their process (Rakkan and Sangkharak, 2020). On the other side a notable number of anaerobic bacteria, which can reduce azo dyes are reported. In bacterial metabolism the reductive breakage of dyes leads to aromatic amines formation (Srinivasan et al., 2020). Many mixed and pure cultures of bacteria are used to decolorize azo dyes under aerobic and anoxic environments (Ambrosio and Campos-Takaki, 2004). Under these conditions with respect to organisms and dyes the reaction is somewhat non-specific. In this non-specific reductive cleavage, enzymatic and low molecular weight redox mediators are used (Fig. 2). There are very few bacterial strains known which can use azo dyes as growth substrate (Pandey et al., 2007; Sarkar et al., 2017).

5.1.1. Azo dye degradation under anaerobic environments

Dye degradation in aerobic environments is not much studied and known but anaerobically dye degradation is studied extensively (Seshadri et al., 1994). Acidogenic and methanogenic bacteria are studied for their dye degradation and decolorization activity. Many different bacterial groups like acidogenic, acetogenic and methanogenic bacteria are involved in methanogenic dye for complex organic compounds degradation. Carbon in the form of starch, glucose, acetate, ethanol and complex ones including whey and tapioca is utilized as energy source in dye decolorization process (Manu and Chaudhari, 2002). In methanogenic conditions, glucose is considered as the most preferable substrate in anaerobic decolorization of dye. But for anoxic dye decolorization by facultative anaerobes and fermenting bacteria the glucose suitability may vary. Adverse effect of glucose by reduction in pH (due to acid formation) under anoxic conditions is observed in some bacterial cultures. In anaerobic conditions, azo reductases use redox mediator as electron transporter by bounding with cell membrane. Membrane azoreductase mediators binding mechanism is different from cytoplasmic azoreductase. As the azoreductase is oxygen sensitive in nature so degradation occurs more efficiently in anaerobic conditions.

Dye structure and used carbon source affect the rate of decolorization. Under anaerobic decolorization, the dye might work as electron acceptor by electron transport chain carriers. The dye color change depends on the carbon source added and dye structure. Otherwise reduced compounds (formed through anaerobic biomass) in non-specific extracellular reactions may be attributed for dye decolorization (Türgay et al., 2011). The anaerobic azo reduction is also achieved by immobilized redox mediators, it increases the rate of reduction due to its high catalytic potential (Olivo-Alanis et al., 2018).

5.1.2. Azo dye degradation under the aerobic conditions

Many strains of bacteria which can degrade azo dyes under aerobic environments are known to scientists. Bacteria cannot degrade azo dyes freely in aerobic environments (Ajaz et al., 2020). Organic carbon source is required to get energy as azo dyes cannot be utilized as substrate (Chittal et al., 2019). Azo dyes are electron deficient, decolorization under reducing conditions form aromatic amines (Fig. S2) and they are further converted into non-toxic end products under the oxidizing-conditions. Thus aerobic-anaerobic treatment techniques seem to be more efficient and economical for decolorization of azo dyes as these are used as a source of carbon and nitrogen by some aerobic bacteria during metabolic activities (Sponza and İşik, 2002; Ajaz et al., 2019). A study has been reported that *A. aquatilis* 3c metabolized Synazol red 6HBN enzymatically into various end products. Firstly, pyrrolo [1,2-a] pyrazine-1,4-dione derivative like structure formed through desulfonation and oxidative deamination which can be involved in amino acid metabolism and eventually produce 3C compound i.e.

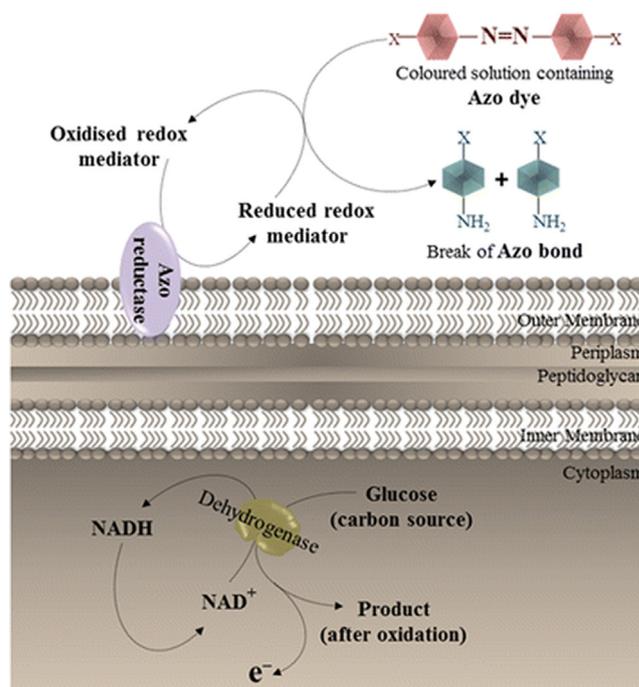


Fig. 2. Bacterial azo dye reduction by azoreductase and redox mediators (Sarkar et al. 2017).

pyruvate which ultimately can be changed into acetyl-CoA to continue energy yielding process, Krebs cycle (Fig. 3). Secondly, phthalate derivatives are produced as a result of desulfonation, carboxylation, and oxidative deamination which can be converted into aldehydes and fatty acids. Such molecules can enter directly/indirectly into fatty acid catabolism to yield acetyl-CoA, FADH₂ and NADH₂ (Ajaz et al., 2019).

An improved mixed culture of bacteria and fungi is in focus for azo dye degradation purposes (Mani and Hameed, 2019). Under anaerobic conditions the redox mediators like flavins or quinones are produced which then are reduced either by cellular enzymes or by the environmentally abundant reductants. Few bacterial strains that use azo dyes as growth substrates (narrow range) are reported. Generally, two stages are followed by bacteria to degrade azo dyes. First stage is reductive breakdown of azo linkages which results in-generally colorless but potentially harmful-aromatic amines (You and Teng, 2009). This reduction requires only the anaerobic conditions whereas the aromatic amines biodegradation is an entirely aerobic process. So for the efficient treatment of wastewater a combination of aerobic and anaerobic conditions is therefore the best scheme to remove azo dyes from wastewater.

5.2. Azo dye degradation by fungi

The selective fungi have the potential for azo dye degradation by different mechanisms and play an important role in cleaning up our environment (Table 1). Bioremediation is considered as the most effective technique for dye degradation rather than physicochemical processes. A salt tolerant yeast strain *Galactomyces geotrichum* is known to degrade and detoxify azo dye aerobically (Guo et al., 2019). Likewise, a potentially harmful azo dye Methyl orange which gets absorbed into the skin and could be inhaled is degraded by brown rot fungi (Purnomo et al., 2019). Some other strains of fungi like *Penicillium* sp., *Aspergillus niger*

and *Cladosporium* sp. are identified as azo dye Red 3BN degraders (Praveen and Bhat, 2012).

Minimal media was used as a selective method to isolate the azo dye degradation. Because of huge production of biomass and rapid growth characteristics, many species of fungi are investigated for their ability to azo dye degradation. Mixed fungal cultures may benefit the azo dye degradation by attacking at diverse positions on dye molecules and by production of in-between metabolites for next mineralization and conversion into neutral forms (Krishnamoorthy et al., 2018). For azo dye degradation a variety of different enzymes like laccase, azoreductase, manganese dependent or independent peroxidase and lignin peroxidases are secreted by microorganisms. Fungal biosorption methods without metabolites formation by forming complexes with dye are used for removing dye from the solutions (Almeida and Corso, 2019).

Wood-rotting fungi, a dominant agent of wood degradation, are distinguished by their degrading ability of lignin and cellulose. Some wood-rotting fungi, mainly white-rot fungi, have capacity for bioremediation applications with vague systems. To depolymerize and mineralize the complex and headstrong lignin polymers this system is developed by fungi. This system of lignin degradation in fungi is not substrate specific. These fungal strains are capable of mineralizing a variety of environmental pollutants as well (Sen et al., 2016).

Uptill now, the white rot fungi, particularly *Phanerochaete chrysosporium* is known as the common microorganism for dye degradation. *Penicillium* genus is also reported as the azo dye degrader. At the same time, in large number of bacteria such as *Sphingomonas xenophaga* BN6, *Pigmentiphaga kullae* K24 and *Caulobacter subvibrioides* C7-D, the azoreductase activity has been recorded (Solís et al., 2012). As there are a huge variety of azo dyes under use in many different fields, there is a need to identify more microorganisms which can degrade and decolorize them under optimal conditions with suitable enzyme production (Abd El-Rahim et al., 2017). The treated water by all these means should be reused in irrigation and other areas to pace water and protect our environment (Singh et al., 2019). It will also save the transfer of dye pollutants into other media (Zhu et al., 2000).

A large number of experiments, with use of crude extracellular enzymes preparations of ligninolytic fungi systems or whole cultures are done on dye effluents degradation. By these enzymatic and non-enzymatic systems, the decolorization of azo, anthraquinone, heterocyclic, triphenylmethane and polymeric dyes is accounted for (Jarosz-Wilkolazka et al., 2002; Mishra and Maiti, 2018). Laccase, a multi-copper oxidase, is present in many species of bacteria, fungi, insects, and higher plants. Together with all of them, laccase is significantly produced by fungi. Because of the huge number of organic compounds degradation it can also reduce azo dyes significantly. Fungal laccase synthesis is affected by both carbon and nitrogen nutrients supply (Liu et al., 2020).

Fungal process of azo dyes biodegradation is most effective and economical as it is low cost and eco-friendly with less sludge production. Mechanism of fungi decolorization occurs by adsorption, enzymatic degradation or by both (Yang et al., 2009). Extracellular ligninolytic enzymes including the laccase, manganese and lignin peroxidase are formed by fungi for degrading the complex organic compounds. Industrial wastewater produces such chemicals which harm plant growth. The microbes produced in these effluents can degrade azo dyes effectively. These microbes should be isolated, because in developing countries like Pakistan the untreated water is used for irrigation which is more hazardous for human health (Shafiq et al., 2017).

5.3. Azo dye degradation by plants

In recent studies the plant's ability to degrade azo dyes has gained much attention. As the untreated industrial water is released in water bodies it poses many lethal effects on the living organisms. So with other bioremediation processes plants should also be utilized for this purpose. Plants can degrade azo dyes as they can produce enzymes like lignin, peroxidases and manganases. The aerobic and anaerobic environment needed for dye degradation is provided by roots in the rhizosphere and plants can absorb the dye from wastewater which can help in wastewater decoloration (Zhou and Xiang, 2013; Goud et al., 2020).

The anaerobically produced aromatic amines are carcinogenic and mutagenic in nature but they could be reduced under aerobic conditions. A combination of plants and microbes can facilitate this process effectively. Azo dye, Methyl red, is treated by plant-microbe association resulting in up to 92 % effective decolorization (Jayapal et al., 2018).

6. Recent and future work

The old or conventional ways of azo dye effluents degradation are not enough to get rid of this massive pollution created by these toxic dyes. The conventional methods include the physical, chemical and biological process which includes adsorption, filtration and coagulation (Abramian and El-Rassy, 2009). Many factors of dye i.e. pH, temperature and dye concentration itself limit the application of these conventional methods (Ince and Tezcanli-Güyer, 2004). So, various recent techniques are now in use to degrade these recalcitrant synthetic dyes (Selvaraj et al., 2020). In recent searches some studies presented the potential of brown-rot fungi for different azo dyes removal (Ali et al., 2010).

For azo dye degradation some physical and chemical methods have also been used including the reductive degradation, ozonation, adsorption, electrochemical, photocatalysis and the Fenton reaction. Some of these are explained as following.

a. Use of microbial fuel cells (MFCs).

Microbial fuel cells (MFCs) are used by many scientists in present time for dye treatment with electricity generation. Various factors like dye concentration, dye structure, microbial fuel cells characteristics e.g. architecture, types of electrode, external resistance, pH, hydraulic retention time (HRT), auxiliary carbon source, composition of wastewater, and used microorganisms influence the microbial fuel cells performance (Pietruk et al., 2019). In MFCs, an enzyme i.e. laccase which is significantly produced by fungi can be used with an air cathode as a catalyst in place of noble metal (Oon et al., 2020).

b. Use of laccase.

In recent years, MFCs cathode has used laccase as a redox catalyst. Traditional catalysts made of precious metals like gold and platinum are replaced due to laccase strong redox properties. So, laccase is significantly used with an air cathode in MFCs (Liu et al., 2020).

c. Use of Fenton reaction.

Fenton is basically a low iron oxidation process for azo dye degradation. It is the best technology combined with catalytic oxidation process with mixture of ferrous ions (Fe^{2+}) and hydrogen peroxide (H_2O_2) which produces the strongest oxidant hydroxyl radicals ($\cdot\text{OH}$). These radicals are commonly used for a large number of pollutant degradation in biological systems. High levels of H_2O_2 are required for azo dye reduction (Hsueh et al., 2005; Purnomo et al., 2019; Zhuang et al., 2020).

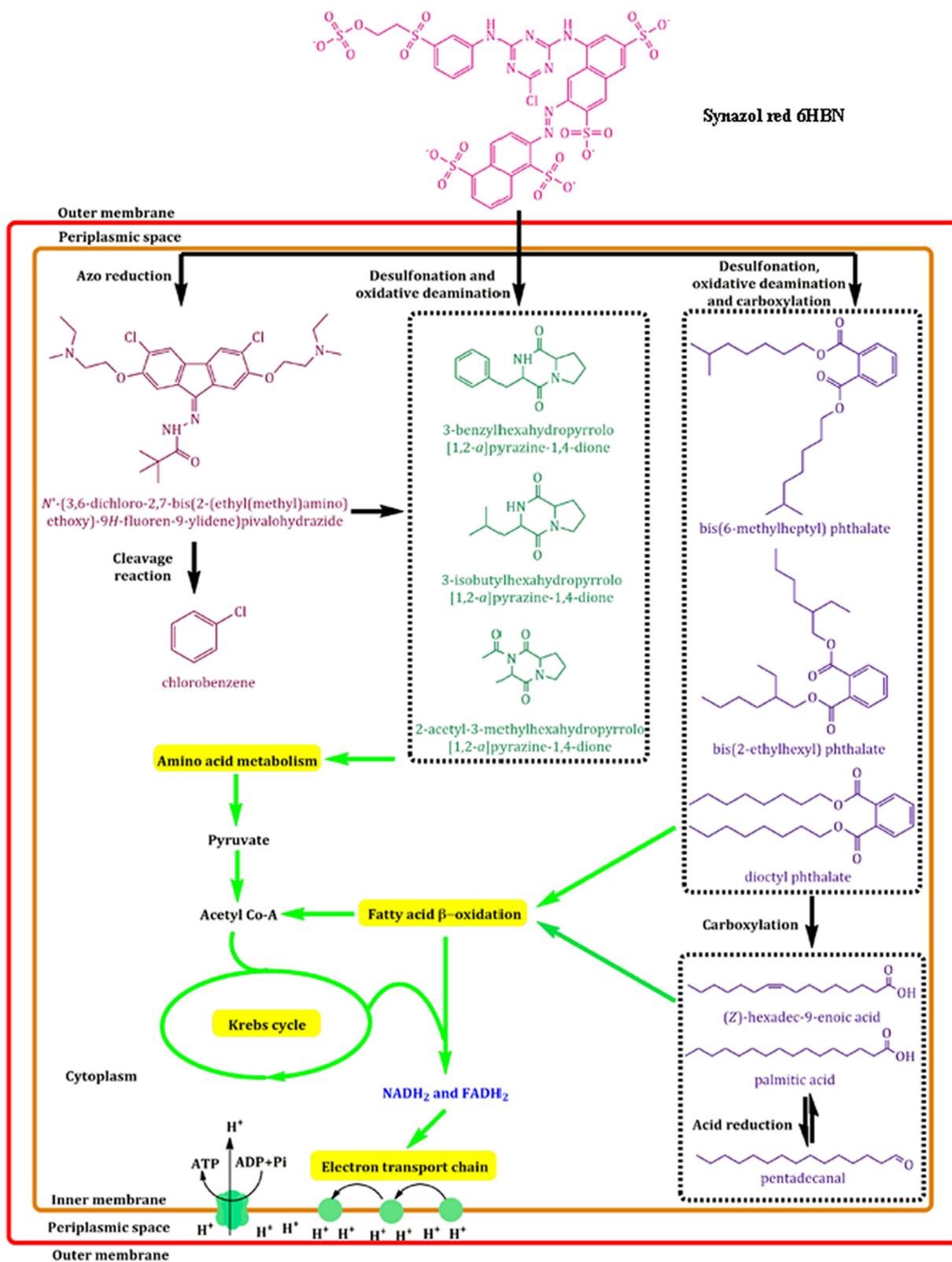


Fig. 3. Degradation of Synazol red 6HBN through desulfonation, carboxylation, and oxidative deamination by *A. aquatilis* 3c (Ajaz et al. 2019).

d. Use of Zero-Valent iron (ZVI).

For water decontamination, zero-valent iron (ZVI) is also evolved. Sulfidation enhances the ZVI efficacy by increasing its reactivity towards its pollutants (Zhang et al., 2019).

e. Use of nano synthetics.

The feasible and quite effective way of azo dye removal was created by synthesizing the Nickel (II) oxide nanoparticles. The more advancing analytical techniques i.e. transmission electron micro-

Table 1
Azo dyes degradation potential of various fungal strains reported in literature.

Sr. #	Organism	Dye type	Degradation time (h/d)	Decolorization efficiency (%)	Reference
1	<i>Aspergillus niger</i>	Synazol red HF-6BN	24 d	88.0	Ilyas and Rehman (2013)
2	<i>Nigrospora</i> sp.	Synazol red HF-6BN	24 d	96.0	Ilyas and Rehman (2013)
3	<i>Oudemansiella canarii</i>	Congo red	60	80.0	Lark et al. (2019)
4	<i>Peurotus florida</i>	Remazol blue	72 h	94.6	Montiague et al. (2020)
5	<i>Aspergillus terreus</i>	Direct Blue-1	168 h	98.4	Singh and Dwivedi (2020)
6	<i>Aspergillus fumigatus</i>	Disperse red1	-	85.0	Ameen et al. (2021)Ameen et al. (2021)
7	<i>Aspergillus flavus</i>	Acid Blue 29	-	86.0	Alam et al. (2021)Alam et al. (2021) Khan et al. (2020)
8	<i>Trametes hirsute</i> D7	Reactive blue 4 Acid blue 129	-	90.0	
9	<i>Trametes hirsute</i> D7	Synozol Red HF-6BN	-	96.0	
10	<i>Aspergillus nidulans</i>		-	90.0	

scopy, scanning electron microscopy, X-ray power diffraction, selected area electron diffraction, energy-dispersive X-ray spectroscopy, elemental mapping analysis and X-ray photoelectron spectroscopy are used to characterize it. By batch absorption method the methyl orange dye is removed (Baig et al., 2020; Xiong et al., 2020). Likewise, palladium nanoparticles PdNPs with high surface area and better catalytic activity are used in azo dye reduction (Xiong et al., 2020).

f. Use of aerobic-microaerophilic bacterial system.

It was proposed that *A. hydrophila* MTCC 1739 and *L. sphaericus* MTCC 9523 are good in their ability to decolorize and biodegrade the textile wastewater azo dyes (Srinivasan and Sadasivam, 2018). So, the modern method consisting of an *in silico* screening and bacterial dye degradation is considered most effective for both perspective i.e. economical and time effective (Guo et al., 2020).

g. Use of oxidation.

Advanced oxidation process shows an effective result in recent studies of removing wastewater effluents. This oxidation removes the organic pollutants from alkaline wastewater. For example, better AO7 decolorization by CAP oxidation is reported in laboratory experiments (Li et al., 2018; Muniyasamy et al., 2020).

h. Use of photocatalysts.

A photocatalyst consisting of two dimensional conductors as organic semiconductor carbon nitride and inorganic semiconductor CdS, work as the light harvesting units and heterogeneous catalyst. Both materials are the light active semiconductor. A heterojunction catalyst forms having ability to remove dye upto 97 % (Ayodele et al., 2021; Leeladevi et al., 2021). Methyl orange, azo dye, is degraded by this method. A ZnO photocatalyst impregnated on fungi is also used for azo dyes photocatalytic oxidation (Bouras et al., 2019).

i. Use of corncob via biosorption.

During corn processing a huge amount of corn waste is generated. The biosorption process carried out with this corn waste helps to reduce the dye wastes (Berber-Villamar et al. 2018).

j. Use of modern biofilms electrode reactors (BERs).

BERs are basically a combination of microorganisms with electrochemistry. It can degrade azo dyes, nitro compounds and antibiotics (Cui et al., 2017). It works by electrical supply at anode which reduces the refractory substances (azo dyes having more than one – N=N – bonds) at cathode (Cao et al., 2018).

k. Use of catalytic.

Catalytic degradation of azo dyes in minimal media with least energy consumption is a more efficient way to deal with this massive pollutant. A complete set of heterogeneous catalysts oxidize azo dyes under less harsh conditions. Activated carbons, zeo-

lites, clay minerals, ashes, alumina and silica are used as a support in heterogeneous catalysts preparation (Torres-Luna et al., 2019).

7. Conclusions

From the available water sources the textile industry uses a large portion and releases tons of wastewater with dangerous effluents every day. Azo dyes, the most famous and excessively used chemicals in the textile industry, are now reaching alarming concentration levels in our environment due to their massive release outside directly. Many different approaches i.e. oxidation, photocatalyst, bioadsorption and nanotechnology are used to eliminate these dyes from wastewater effluents to prevent living organisms. These methods are costly and are not suitable to use at low azo dye concentration. Microbial use for dye polluted wastewater treatment is often considered as an eco-friendly method instead of conventional methods. Bacterial, yeast, and fungal capabilities to reduce these dyes are being investigated. Mixtures of aerobic and anaerobic bacteria are considered more efficient to degrade azo dyes. Microbial enzymes like lignin and azoreductase have capacity to reduce azo dyes under aerobic and anaerobic conditions. Now it is well understood that the microbial strains are very helpful to reduce/degrade these toxic compounds into nontoxic forms. So the microbial consortia, their processes and enzymes are in use with other advancing technologies as an effective sustainable strategy for azo dye decolorization and biodegradation, indicating that bioremediation is an efficient and sustainable option for green chemistry to exterminate azo dyes from the ecosystem.

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Appendix A. Supplementary data

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