Plant-based Enzyme-mediated Biodegradation of Azo dyes: A Review

Sunita Singh¹, Pragya Singh², Aman Saifi³, Shalini G. Pratap, Pramod K. Singh¹*

Abstract

Azo dyes are a popular group of dyes in the printing, food, leather, cosmetic, textile, and pharmaceutical sectors and are the largest and most important group of colored chemicals due to their facile production procedure. It is characterized by the presence of an azo group (-N=N-). The stability of azo dyes makes them recalcitrant. Discharge of untreated waste water that contains colored compounds with azo dye reaches water streams and affects the organisms due to the toxicity of the dyes. Many physicochemical and chemical approaches have been used for the removal of dye from polluted water. Although these procedures effectively treat polluted water but become costly and may result in the formation of hazardous compounds. A microbial enzymatic decolorization is an environment-friendly approach that shows excellent removal efficiency at low operating costs however, it has certain downsides, such as a slow process rate and a longer assimilation phase. Alternatively, enzymes derived from various sources can be employed to biodegrade and decolorize azo dyes. For the treatment of dye-based chemicals, enzymes extracted from plants have benefits over other approaches. They are believed to have a significant potential to degrade the recalcitrant pollutants present in the effluent from industries i.e., laccases, polyphenol oxidases, azoreductases, and different peroxidases like manganese peroxidase, lignin peroxidases, and decolorizing peroxidases, are the common enzymes that are isolated from plants and have the potential in the biodegradation of colored compounds. The importance of these enzymes in the treatment of industrial wastewater is unquestionable.

Keywords: Azo-dyes, Enzymatic degradation, Laccase, Peroxidase, Polyphenol oxidase, Toxicity, Wastewater.

Introduction

More than 50,000 tons of colored chemicals are emitted into the environment each year around the world (Lewis, 1999). Various industries release untreated water that contains colored compounds with azo dye as a major component reaching the water streams. The textile industries release untreated wastewater into the commercial drain, eventually emptying into the rivers. This untreated water contains most of the recalcitrant compounds. The major portion of these effluents contains azo compounds. Due to their characteristic properties, such as stability in the environment and xenobiotic nature, these dyes are not completely discolored and degraded by conventional wastewater treatment processes like the activated sludge process and make them recalcitrant (Chung and Cerniglia, 1992). In all the dyes synthesized, azo dyes constitute (Pereira et al., 2014) the maximum portion and are used in the printing, food, leather, cosmetic, textile, and pharmaceutical industries (Chang and Lin, 2001). Due to its simple synthesis process, azo dyes used in industries form the most vital group of colored compounds. An estimated 15% (1–5% decrease in production and 1–10% loss during usage), or 280 kilotons of dyes, remain untreated and transferred via wastewater (Pereira et al., 2014). These dye molecules do not fade when exposed to light. According to the Ecological and Toxicological Association of the Dyestuffs Manufacturing Industry, over 90% of commercial dyes have LD50 (Lethal dosage 50) values more than 2000 mg/kg (Won et al., 2004). If untreated water containing such colored substances is dumped into the environment, it dramatically raises the risk of health problems (Won and Yun, 2008). This affects the organisms that are exposed to these dyes due to the toxicity of its breakdown product and reduction in photosynthetic activity because of decreased oxygen solubility and availability of light that enters water due to less absorbance (Durán and Esposito, 2000). The discharge of complex-colored compounds into nature is a concern due to their biodegradation products’ mutagenicity, carcinogenicity, and toxicity. This also affects several parameters such as pH, BOD (biochemical oxygen demand), and COD (chemical oxygen demand). Azo dyes are made up of one or more azo groups (-N=N-), which are reduced with the help of enzymes (Langhals, 2004). As a result, treatment of wastewater containing dyes and their intermediate products is required before disposal into the surrounding environment. Numerous studies have been conducted to reduce the environmental impact of industrial effluent. Wastewater treatment methods involving colors are classified as chemical, physical, or biological. For the removal of color from polluted water, many physicochemical and chemical approaches have been used. These methods, however, have some drawbacks.
and limits. They are unable to destroy the dyes, and their partial degradation generates a huge amount of sludge that must be disposed of safely and may cause additional pollution problems (Forgacs et al., 2004). Several biotechnological approaches have focused on the elimination of dyes from the effluent. Biological treatment methods of the dyes are most appropriate in the present scenario and are widely used due to their low operating cost, less amount of sludge production, and environmentally friendly nature (Song et al., 2003). Degradation using yeast, algae, bacteria, and fungi is advantageous for treatment because of their high rate of degradation, environmentally friendly nature, lower energy consumption, and lower chemical requirement. However, some limitation of microbial degradation includes a longer acclimatization phase, slow process rate, and reliance on external parameters such as pH and salt concentration for mechanistic performance (Husain and Husain, 2012; Wang et al., 2009; Kalyani et al., 2009). These drawbacks could be overcome by adopting the enzymatic technique, which has been shown to offer promising and satisfactory outcomes within minimum time, no environmental impact, and low energy demand (Michniewicz et al., 2008). Many studies have been published in peer-reviewed journals that demonstrate the use of enzymes extracted from sources such as fungi, algae, bacteria, and plants for the treatment of dye-contaminated wastewater (Sun et al., 2015; Patil, 2014; Levin et al., 2005).

Various research studies have discovered that treatment of dye-based compounds by enzymes isolated from plants is advantageous over other methods due to its highly selective nature. They are found to substantially affect the elimination of recalcitrant pollutants present in the industrial effluent (Husain, 2006; Novotny et al., 2001). Enzymatic treatment methods can be practiced at extreme conditions such as over varied ranges of temperatures, pH, and salinity and in a high concentration of pollutants (Torres et al., 2003). The selection and extraction of the proper enzyme is a critical step in the biological component of dye degradation (Sudha et al., 2014). The present paper reviews the role of various plant-based enzymes and their enzymatic mechanism involved in biodegradation and decolorization of azo colored compounds also describes the importance of biological methods for the same.

**Azo Dyes**

The most common dyes used in industrial operations are azo dyes. According to statistics, around 1 million tons of azo dye are manufactured each year (Fatima et al., 2017). Currently, over 1500 distinct azo dyes are employed in the industry. There are various types and forms of azo dyes. The chemical structure of the dye may have more than one azo bond. If there is only one azo link, the dye is termed mono-azo. If there are two, diazo, and if there are three, the dye is called tri-azo. Because of the varied aromatic side groups, the same dye has distinct colors with varying color intensities (McMullan et al., 2001). Because of their azo bond, azo dyes are electron deficient xenobiotics in nature. The sulfonate group, or any other electron-withdrawing group, causes electron shortage. As a result, the azo dye is less susceptible to microorganism biodegradation (Kuberan et al., 2011; Hsueh et al., 2009; Kurade et al., 2011; Enayatizamir et al., 2011; Barragán et al., 2007). The sulfonated azo dyes have a sulfonate group as a substituent. Both sides of these azo groups are either connected with aromatic ring structures like naphthalene and benzene or ionizable moieties which creates a complex structure of the dye (Fig. 1). This complex structure results in a huge variation in the expression of colors in dyes (Langhals, 2004; O’Neill et al., 2000).

**Toxicity of Azo Dyes**

Azo dyes are an important and substantial material for several industries to work. Though industries’ increasing demand and dependence on complex-colored compounds, the negative impact of industrial effluents containing these dye molecules on nature has raised major environmental concerns (Copaciu et al., 2013; de Aragao Umbuzeiro et al., 2005). A dye's toxicity is determined by its structure and chemical characteristics. The toxicological character of the dye is determined by the parent molecule included in the chemical structure and its lipophilicity feature, which permits dye to pass through the cell membrane. Toxic dyes have an immediate effect on living organisms, as they do not require the enzymatic degradation of the azo link to release breakdown products (Brown and De Vito, 1993). Due to increased water solubility, these dyes remain present in the ecosystem and are not completely removed by the currently used physical or chemical methods (Pinheiro et al., 2004). Hydrophilic moieties are obtained on biodegradation of dyes. These moieties can easily cross the cell membrane barrier and affect the cell (Dias et al., 2010; Gottlieb et al., 2003). These hydrophilic metabolites catalyze oxidative Genotoxicity (Sweeney et al., 1994). These moieties cause cytotoxicity, inhibit microbial community formation (Chung et al., 1997), and trigger the unwanted formation of DNA in organisms (Joachim and Decad, 1984). The biodegradation and destiny of azo dyes are influenced by external stimuli such as reduction, oxygenation, photolysis, enzyme interactions, and hydrolysis. Toxic intermediates such as 1-amino-2-naphthol, 2,4-dimethylaniline, 3,3'-dimethoxy benzidine, and aniline can be produced by azo dyes in both oxidizing and reducing circumstances (Michniewicz et al., 2008).

Various intermediates and end breakdown products are formed by the symmetrical and asymmetrical breakage of the azo bond (Thakur et al., 2014). Aromatic amines are not formed as a product in the asymmetrical breakage of azo bonds (Telke et al., 2009). When dyes are not processed and released into water bodies, as is the case in many developing-country enterprises, the consequences can be quite severe and drastic. The biodegradation products of dyes are determined by factors such as enzyme types, dye degradation pathways, the presence or absence of redox mediators as degradation catalysts, and inorganic complex substances (Husain, 2006; McMullan et al., 2001; Chacko and Subramaniam, 2011). Organic or inorganic artificial redox mediators can catalyze the reduction of the azo bond by the enzyme either directly or indirectly. Oxidative enzyme inducers (indole, veratrole, etc.) can be used to increase the degradation rate of azo dyes (Fig. 1).
o-Tolidine, CaCO₃, and vanillin) and electron donors for reductases, e.g., improved the rate of biodegradation reaction (sodium citrate, sodium pyruvate, sodium acetate, sodium formate, and sodium succinate) (Dawkar et al., 2009). As a result, several dye biodegradation end products might be found in the environment. When calculating the toxicological effect of azo-colored compounds, the consequences of such chemical moieties were not considered. Once metabolically activated, azo substances can cause the generation of free radicals (Mansour et al., 2009), formation of micronuclei (An et al., 2007), attaching with strands of DNA (Wang et al., 2012), DNA adduction formation (Kojima et al., 1994), cause genotoxicity by damaging DNA (Carmen and Daniela, 2012), and chromosomal disorders (Prasad et al., 2013). Toxicological impact on living organisms has been widely accessed by performing various assays on model organisms. Toxicological studies have been carried out on plants such as Vigna mungo, Allium cepa, and Sorghum (Jadhav et al., 2011; Prasad and Rao, 2013). Prior knowledge about the chemical structure of dye and toxicological impact by performing assays may assist in finding biological processes which determine the specific product of an azo dye in the ecosystem. Such research studies are important, and their results may help in designing ecologically relevant studies.

**Physiochemical and Chemical Methods for Biodegradation**

As shown in Fig. 2, a variety of physicochemical and chemical approaches have been used to treat dye-containing wastewater. Physical procedures such as sedimentation, coagulation, and flocculation of dyestuff are essential treatment methods for removing Sulphur-containing colors (Riera-Torres et al., 2010; Shakir et al., 2010; Zodi et al., 2010). Reverse osmosis and filtration methods like nanofiltration and ultrafiltration are used for the recovery of the chemicals present in water (Al-Bastaki, 2004; Capar et al., 2006; Amini et al., 2011). The utilization of Advanced Oxidation Processes is a significant approach to dye decolorization (AOPs). Ozonation (Turhan et al., 2012), photolysis (Feng et al., 1999; Elmorsi et al., 2010), photocatalysis (Husain and Husain, 2012), and the Fenton process (You et al., 2012) are examples of oxidation processes. These treatment processes have various limitations and are not much efficient in dye biodegradation. They are not very economically feasible, produce secondary pollutants and sludge, are inefficient to break down a wide range of colors and require the use of energy (Abdessalem et al., 2010; Hai et al., 2007).

**Enzymatic Biodegradation of Dyes**

Microbial decolorization has exhibited great treatment efficiency at very cheap operating costs and less hazardous products. However, microbial decolorization has some drawbacks, including a slow process pace, a prolonged acclimation phase, and a reliance on external conditions like pH and salt content for process effectiveness (Ramachandran et al., 2013). The reduction of the azo bond is the initial step in breaking azo-colored compounds. In phytoremediation of the azo-colored compound, oxidoreductive enzymes appear to be the most effective. Laccases are extensively studied to degrade and decolorize a different industrial dye (Rodriguez et al., 1999). The plant-based enzymes like laccases, DCIP reductase, polyphenol oxidase (PPO), Lignin peroxidase, horseradish peroxidase, soybean peroxidase, tyrosinases, and azoreductase, etc. have the potential to degrade and decolorize the dyes (Karagoz et al., 2011; Yu et al., 2006; Schückel et al., 2011). These enzymes produce a variety of compounds, each with a different toxicological capacity and susceptibility to subsequent degradation. Enzymatic biodegradation is a newer alternative approach against conventional methods, where organic pollutants are degraded into lower toxic products. Enzymes isolated from plants for degradation can oxidize the chemical structure of dyes and transform them into low molecular weight compounds and eventually decolorize the dyes. These can work in milder conditions and have a higher rate of reactions. Microbial decolorization using bacteria or fungi has attracted significant interest. Although a considerable amount of research has been done on the biodegradation of azo dyes by enzymatic method, information regarding plant-based enzymes and their pathway for biodegradation of dyes is still non-existent. To effectively utilize this approach, it is necessary to understand the chemical structure of the intermediate compounds created in the breakdown route.

**Laccases**

These enzymes belong to the MCO protein family, which is a group of copper-containing polyphenol oxidases (PPO). These enzymes were first isolated from the sap of the Japanese lacquer tree (Rhus vernicifera), and Bertrand discovered their unique property as a metal-containing oxidase in 1985 (Giardina et al., 2010). Laccases were later discovered in a range of plants, including lacquer, mango, mung bean, peach, pine, prune, and sycamore (Flickinger and Drew, 1999). Plant and fungal laccases are extracellularly located, whereas bacterial laccases are intracellularly located (Diamantidis et al., 2000). Laccases isolated from plants have a molecular weight of 60 to 130 kDa and an average amino acid composition of 500 to 600. Plant laccase’s optimal pH ranges from 5 to 7, and its isoelectric point (pl) ranges from 5 to 9.6; the enzymes are heavily glycosylated (22%–45%) (Wang et al., 2015; Harvey 1997; Berthet et al., 2012). The carbohydrate region of plant laccase contains monosaccharides such as hexosamines, glucose, mannose, galactose, fucose, and arabinose (Rogalski and Leonowicz, 2004), and glycosylation seems necessary for secretion, copper retention, proteolytic susceptibility, thermal stability, and enzymatic activity (Flickinger and Drew, 1999). Plant laccases and fungal laccases share the same molecular architecture and reaction processes as bacterial enzymes.
laccases but have a lower reduction potential E° (0.41 V) for Rhus vernicifera and a pl ranging from 7.0 to 9.6 (Mate and Alcalde, 2015; Frasconi et al., 2010; Morozova et al., 2007).

Laccases have recently received a lot of interest for their role in the biodegradation of colored compounds found in wastewater (Husain, 2006). Laccase is an oxidoreductase (EC1) that plays a critical role in a variety of biological processes since it can be used directly in biochemical reactions. They don’t need any cofactors, oxygen, or mediators as substrates or mediators (Telke et al., 2011). Majorly fungi or plant origin-based laccases are known (Gianfreda and Bollag, 1999).

An electron acceptor is necessary for the catalysis of the oxidation reaction of phenolic and non-phenolic substances, and in the case of laccases, molecular oxygen acts as an electron acceptor (Sharma et al., 2007). Laccases can degrade the xenobiotic compounds in industrial effluent. These enzymes degrade azo dyes and form phenolic compounds by a free radical mechanism. Toxic aromatic amines are not formed by these enzymes (Chivukula and Renganathan, 1995). A phenoxy radical is generated by the oxidation of phenolic groups of azo dye with one electron. Sequentially a carbonyl ion is produced by oxidation of this radical by the enzyme. A nucleophilic assault of water on the phenolic ring connected to azo linkage results in the formation of two compounds: 1, 2-naphthoquinone, and 3-di-azonyl-benzensulfonic acid (III). The enzyme’s radicals generated during dye oxidation react with 1, 2-naphthoquinone. Cross-coupling among the reactive species results in the formation of -N-N- and -C-N- links between aromatic amines and C-C and C-O bonds between phenolic compounds. An electron is removed by cross-coupling the phenolic group from the hydroxyl group, resulting in an alkoxy radical. These radical forms react with the hydroxyl groups at the ortho and parasites to form dimers. Phenolic radicals can be further oxidized to produce oligomeric compounds. Under specific conditions, C-C dimers can participate in coupling processes to generate extended quinine (Camarero et al., 2005; Zille et al., 2005). Only a few cases have been reported so far, although plant laccases are not widely used in dye degradation.

Purified laccase from Blumea malcolmii was discovered to break down five structurally diverse textile dyes in the presence of 2,20 Azino-bis 3-ethyl-benzothiazoline 6-sulfonic acid (ABTS): Brilliant Blue R, Malachite Green, Reactive Red 2, Direct Red 5B, and Methyl Orange (Kagalkar et al., 2015). Arca et al., (2009) investigated the potential of immobilized laccase isolated from R. vernicifera to degrade the textile dye Reactive Red 120 in a batch mode enzyme reactor and discovered that the immobilized enzyme could maintain its activity over a wide temperature range when compared to the free form.

**Polyphenol Oxidases (PPO)**

PPO is an oxidoreductive enzyme that aids in the removal of aromatic pollutants from a variety of contaminated areas. PPO enzymes function on a wide range of substrates, including organic contaminants found in the environment in moderate concentrations (Husain and Jan 2000; Ali and Qayyum, 2007). This enzyme has four copper atoms per molecule, binding sites for oxygen, and two aromatic chemicals. As a result, it is a tetramer enzyme. Plant polyphenol oxidases and peroxidases have been considered for the breakdown of dyes on a modest scale, but both have been practiced on a small scale due to the high cost of purification and low enzymatic activity (Zilly et al., 2002). Plant peroxidases require expensive H₂O₂ as a co-substrate for decolorization of the wide spectrum of dyes (Bhunia et al., 2001; Shaffique et al., 2002). Polyphenol oxidases are less expensive than microbial oxidases for the decolorization of aromatic contaminants in plants because they utilize free molecular oxygen as an oxidant (Wada et al., 1995; Arabaci and Usluoglu, 2014). Enzyme immobilization technology is an important and effective method for improving polyphenol oxidase activity and stability. Different types of immobilizing materials are used to immobilize the enzyme (Table 1). The PPO enzyme was partially isolated from quince leaves and immobilized on alginate beads. Immobilization improved stability throughout a wide pH and temperature range. In comparison to the immobilized PPO, the free enzyme was unable to completely decolorize the textile dye (Jadhav et al., 2011). Khan and Husain (2007) investigated the potential of PPO derived from potato (Solanum tuberosum) immobilized on elite to decolorize colored compounds. PPO extracted from potato, and brinjal precipitated by ammonium sulfate showed very high activity. These PPOs have a lot of potential for biodegrading textile and non-textile colors (Ali and Qayyum, 2007). For decolorization of a mixture of Brown R and Yellow 5G dyes, PPO extracted from the plants, Alternanthera sessilis, Jatropha curcas, and Parthenium hysterophorus, was effective (Shinde et al., 2012). These plants are fast-growing plants. Purified PPO extracted from the banana pulp degraded 85% textile dyes direct Blue GLl within 90h and 90% direct Red 5B within 48 h as shown in Fig. 3 (Jadhav et al., 2011).

**Peroxidases**

Peroxidases are EC1 enzymes that contain haem groups in their structure. In the presence of hydrogen peroxide, they accelerate a process (Durán, 2002). A similar reaction mechanism is present during the catalytic cycle of lignin and manganese peroxidases (MnP). Plant peroxidases are involved in a variety of pathways and provide a variety of functions. They are used in wound healing (Kumar et al., 2007), lignin biosynthesis (Gross, 2008), fruit ripening (Huang et al., 2007), suberization (Bernards, 1999), auxin metabolism, and disease resistance (Veitch, 2004). Peroxidases are categorized into three superfamilies, according to the research. Intracellular peroxidases such as ascorbate peroxidase and bacterial catalase peroxidases are found in Class I, secretory fungal peroxidases such as lignin peroxidase (LiP) and manganese peroxidase (MnP) are found in Class II, and plant-based secretory peroxidases such as bitter gourd peroxidase (BGP), turnip peroxidase (HRP) (Ghaem et al., 2010; Husain, 2010). Class III enzymes require calcium ions for their activities. Enzymes of this class are monomeric glycoproteins having four disulfide bonds (Schuller et al., 1996).

These enzymes catalyze the oxidation process of a wide range of substrates in the presence of hydrogen peroxide. Degradation of azo dyes by peroxidase has been described in the literature to occur via symmetric or asymmetric azo bond cleavage (López et al., 2004). Peroxidase has a variety of applications, including the treatment of wastewater containing phenolic compounds and stubborn contaminants. Plant-derived peroxidases oxidize a wide range of phenolic compounds, including pyrogallol, catechin, o-dianisidine,
Biodegradation of Azo Dyes

**Fig. 3:** Depicts the method of Direct Red 5B degradation by Polyphenol oxidases derived from the banana pulp (Jadhav et al. 2011).

**Table 1:** Showing the sources of plant polyphenol oxidase applied in dye degradation.

<table>
<thead>
<tr>
<th>Source</th>
<th>Free/immobilized</th>
<th>Dyes</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Potato (Solanum tuberosum) and Brinjal (Solanum melongena)</td>
<td>Free</td>
<td>PAGE Blue 83, Coomassie Brilliant Blue G 250, Coomassie Brilliant Blue R 250, Methylene Blue, Naphthaquinone-4-sulphonic acid, Carmine, Naphthalene Black 1.2 B, Tropaeolin and Evans Blue</td>
<td>(Ali and Qayyum, 2007)</td>
</tr>
<tr>
<td>Banana pulp (Musa paradisiac)</td>
<td>Free</td>
<td>Direct Red 5B, Direct Blue GLL, Direct Yellow 5GL, Reactive Blue HERD, Reactive Navy Blue, Blue 2RNL, and DK Red 2B</td>
<td>(Jadhav et al., 2011)</td>
</tr>
<tr>
<td>Potato (S. tuberosum)</td>
<td>Free and immobilized on Celite 545</td>
<td>Textile dyes (Reactive Blue 4, Reactive Blue 160, Reactive Blue 171, Reactive Orange 4, Reactive Orange 86, Reactive Red 11, Reactive Red 120, and Reactive yellow 84) Non-textile dyes (Bromophenol Blue, Carmine, Chelpark Blue, Chelpark Black, Chelpark Red, Coomassie Brilliant, Blue R 250, 1,2-Naphthaquinone-4-sulphonic acid, Naphthalene Black 12 B, PAGE Blue 83, and Tropaeolin)</td>
<td>(Khan and Husain, 2007)</td>
</tr>
<tr>
<td>P. hysterophorus, A. sessilis, and J. curcas</td>
<td>Free</td>
<td>Reactive blue 52, Reactive green 15, Reactive yellow 125 and Congo red (CR)</td>
<td>(Lončar et al., 2012)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Yellow 5G and Brown R</td>
<td>(Shinde et al., 2012)</td>
</tr>
</tbody>
</table>
Biodegradation of Azo Dyes

catechol, chlorogenic acid, and guaiacol (Passardi et al., 2005). These enzymes can be used for the phytoremediation of various aromatic contaminants as well as detoxification of azo and non-azo dyes found in effluent from industries such as paper, textile, and printing (Jadhav et al., 2009).

According to Bhunia et al., 2001, the HRP may effectively degrade and precipitate industrially important azo dyes. They demonstrated HRP selectivity toward many dyes, including Remazol Blue and Cibacron Red. For the elimination of Remazol Blue, enzyme activity at pH 2.5 was shown to be considerably superior to that at neutral pH. Remazol Blue was a powerful competitive inhibitor of HRP at neutral pH. The enzyme showed broad substrate specificity towards a variety of azo dyes. The enzyme showed broad substrate specificity towards a variety of azo dyes. The use of horseradish peroxidase (HRP), soybean peroxidase (SBP), and radish peroxidase to partially decolorize industrial colors in textile effluent have been documented in the literature (Forni et al. and de Souza, 2007; Knutson et al., 2005; Habib, 2012).

HRP can effectively biodegrade and precipitate industrial azo-colored compounds. The activity of the enzyme HRP in the biodegradation of Remazol Blue was higher at pH 2.5 than at neutral pH. Decolorization efficiency of HRP was reported in dyes Lanaset blue 2R (94%), Remazol turquoise blue G (59%), and textile effluents (52%) (Schückel et al., 2011).

Methylene Blue (MB) and Azure B (AB) oxidation by horseradish peroxidase (HRP) and lignin peroxidase (LiP) were examined. The results demonstrated that HRP can N-demethylate both dyes, although it has considerably slower reaction kinetics than LiP and requires higher H₂O₂ concentrations. Furthermore, unlike LiP, HRP was unable to produce aromatic ring cleavage (Ferreira-Leitão et al., 2003).

Horseradish peroxidase enzyme (HRP) has also been found to be capable of removing Eriochrome Blue Black R and Fluorescein via a chemical transformation and polymerization process (Pirillo et al., 2010). HRP decolorization of Lancet Blue 2R dye was achieved at pH 4.0. However, the same enzymatic decolorization of Remazol Blue and Red Cibacron was strongly impeded above pH 6.0 (Knutson et al., 2005). The decolorization of bromophenol and methyl orange dyes by an HRP/H₂O₂ system revealed a decrease in HRP efficiency as the temperature was increased from 30 to 80°C (Liu et al., 2006).

The oxidation of commonly found dye yellow-12 in textile effluent was done by the Horseradish peroxidase and H₂O₂ coupled reaction. The enzyme's activity was examined concerning parameters such as HRP and H₂O₂ concentrations, contact time, and pH. At pH 4, with the increase in concentrations of H₂O₂ and HRP, the transforming potential of the enzyme increased. An immobilized enzyme in gel/alginate reduced the azo dye. Immobilized peroxidase beads decolorized the same dye with lower potential and were used two-three times (Maddhini et al., 2006).

Soybean peroxidase (SBP) has several advantages, including a low cost and abundance, resistance to irreversible inactivation by hydrogen peroxide, and increased conformational stability (Kamal and Behere, 2002; Kamal and Behere, 2003; Al-Ansari et al., 2009; Al-Ansari et al., 2010; Stevensz et al., 2009). SBP has been demonstrated in studies to oxidize a variety of substrates and to be highly stable in terms of chemical and thermal denaturation, making it appropriate for industrial applications (Geng et al., 2001; Boscolo et al., 2006). SBP was employed to catalyze the breakdown of Remazol Turquoise Blue G 133 (RTB) by breaking the phthalocyanine ring, releasing ammonium and Cu(II) ions, and generating sulfo-phthalimide (Marchis et al., 2011). The soybean peroxidase (SBP)/H₂O₂ system degraded Turquoise Blue G 133 with the maximum SBP activity at pH 3. The percentage degradation of the dye reduced with increasing pH values, reaching nearly zero at pH 8 (Ferreira-Leitão et al., 2003). In the degradation of dye, SBP was more effective than HRP. SBP, which has a wider substrate access channel and thus more exposure of the catalytically essential delta heme edge, can accept more substrates than HRP (Knutson et al., 2005; Alpeeva and Sakharov, 2005).

Plant peroxidase has a high manufacturing cost and a low output when compared to fungal or bacterial enzymes. The cost of enzymes can be reduced by extracting them from inexpensive plant sources or by lowering the cost of manufacture. Enzymes derived from the plants Ipomea palmata and Saccharum spontaneum can be employed in the biodegradation of textile colors in place of currently available commercial sources of enzymes such as horseradish and soybean peroxidase. These plant peroxidases were used to decolorize eight textile dyes and seven non-textile dyes. Peroxidase isolated from Ipomea leaf destroyed Methyl Orange (26%), Crystal Violet (36%), and Supranol Green (68%) within 2–4 h, and Direct Blue (15%), Chrysoidine (44%), and Brilliant Green (54%) after 1–2 h of treatment. Procion Brilliant, Direct Blue, Chrysoidine, and Blue H-7G were all decolorized by an enzyme derived from S. spontaneum leaf in less than an hour, although Procion Green HE-4BD and Supranol Green were eliminated (Shaffi et al., 2002).

Bitter gourd peroxidase (BGP) isolated from Momordica charantia was employed to effectively remove dispersion colors used in industries (Satar and Husain, 2011). In the presence of the free radical mediator 1-hydroxy benzotriazole, the rate of dye decolorization rose dramatically when the enzyme content in the reaction mixture was raised (HOBT) (Akhtar et al., 2005). Peroxidases isolated from three macrophytes, Phragmites australis, Arundo donax, and Typha angustifolia, were used to decolorize Direct azo dyes amido black and amaranth (Haddaji et al., 2015). Crude peroxidases isolated from biowaste of plants: luffa fruit peels (Luffa acutangula) and soybean seed hulls (Glycine max) served as the low-cost source of peroxidase. The enzyme effectively decolorized Methyl Orange. The degradation potential was dependent on factors like reaction pH, substrate, temperature, and enzyme concentration in the reaction mixture. Using low-cost methods and a cheap source for isolation of peroxidase and less use of harsh chemicals creates an environmentally friendly and sustainable operation with low process economics (Chiong et al., 2016).

Acidic azo dye Acid Black 10 BX was studied for the catalyzation by peroxidase extracted from the plant. Horseradish roots were used to isolate the enzyme. The dye enzyme's decolorization effectiveness was found to be dependent on the dye concentration, reaction time, H₂O₂ dose, enzyme concentration, and aqueous phase pH. Alginate immobilized enzyme yielded low activity over the free crude enzyme, whereas acrylamide matrix immobilized enzyme yielded...
ineffective activity as compared to the free one (Mohan et al., 2005).

The dyes Reactive 5, Reactive 4, Reactive 2, Disperse Black 9, and Disperse Orange 25 have been demonstrated to be degraded by peroxidase isolated from Brassica oleracea (cauliflower bud) (Jamal et al., 2015).

For the biodegradation of azo dyes, both free and immobilized enzymes have been utilized. However, due to some limitations of free peroxidases, such as stability against denaturants, suppression of activity, protease degradation, and reusability, these free peroxidases are not widely used (Feng et al., 1999; Husain et al., 2007). The use of an immobilized enzyme is the best way to overcome these limitations. This has the potential to be exploited on a large scale (Durán and Esposito, 2000; Husain and Kulshrestha, 2009). Because immobilized enzymes can be reused in continuous reactors, treatment costs are reduced (Akhtar et al., 2005; Kulshrestha and Husain, 2006; Kulshrestha and Husain, 2006; Matto and Husain, 2006).

Peroxidases isolated from several plant sources (bitter gourd, horseradish, turnip, etc.) were immobilized utilizing various methods of immobilization such as entrapment, covalent attachment, adsorption, and so on. The immobilization of enzymes has been accomplished using a variety of organic and inorganic supporters. The biodegradation and decolorization of dyes were accomplished using these immobilized enzymes (Husain and Ulber, 2011). It was claimed that HRP may be immobilized utilizing a covalent attachment approach to several polysulfone substrates. Different polysulfones were methacrylate using methacryloyl chloride for immobilization, and nonwoven fabric samples were subsequently coated with methacrylate polysulfone solutions. For 63 days, the enzyme was employed seven times with freshly produced dye solutions. The dye biodegradation efficiency of the immobilized enzyme remained high after three reuses (70%) (Celebi and Kaya, 2013).

One of the cheapest alternatives of horseradish peroxidase is Turnip peroxidase (TP). TP enzyme was used for biodegradation of Reactive Blue 21 dye (RB21). Under the optimized conditions, a decolorization efficiency of 95.83% was observed. High-performance liquid chromatography-mass spectrometry coupling (LC–ESI/MS) was used to investigate the end products formed during the decolorization of RB21. The breaking up of the chromagenous system caused the color removal of the dye RB21 by turnip peroxidase, according to the results (Silva et al., 2012).

Decolorization potential of free and immobilized peroxidase extracted from Cucurbita pepo (courgette) was determined for

---

**Fig. 4:** McMullan et al. (2001) suggested a mechanism for the breakdown of sulfonated azo dyes by peroxidase. The compounds in parenthesis have not been discovered in this pathway, but their existence is rationalized as required intermediates for the observed final products. The compounds represented by the numbers in brackets have been found in the reaction mixture in reaction mixtures. R1=R2=O and B=O substitution pattern (as in I); R1=H, R2=OCH, and B=NH substitution pattern (as in II). [2a] 2, 6 dimethyl-1,4-benzoquinone, [4a] 4 nitrosobenzenesulfonicacid, [6b] 2 methoxyhydroquinone, [7b] 2-methoxy-4 aminophenol, [8a] sulfanilic acid, [8b] sulfanilamide, [9a] 4-hydroxybenzenesulfonic acid, [9b] 4-hydroxybenzenesulfonamide, [10a] benzenesulfonic acid, [10b] benzenesulfonamide, [11a] azobenzene-4,40 disulfonic acid, [12] ammonia.
the azo dye, Direct Yellow (DY106). Calcium alginate beads were used to immobilize the enzyme. The Free peroxidase was able to destroy more than 87 percent of the dye in less than 2 minutes under optimum conditions. The degradation of immobilized peroxidase was up to 75% within 15 minutes (Boucherit et al., 2013). Fig 4 depicts the mechanism by which sulfonated azo dye is degraded by peroxidase. Numerous studies have been published on the degradation of textile colors utilizing a free and immobilized version of plant peroxidases without the use of any kind of mediator. Some significant results are discussed in Table 2.

**Use of Plant Tissues Culture and Phytoremediation for Dye Degradation**

Phytoremediation is the technique of using plants to reduce the impact of toxins in the soil, water, and air. This method's long-term usability and cost-effectiveness make it a potential solution for decolorizing textile dyes (Rauf and Ashraf, 2012).

Plant tissue cultures are an effective approach for dye phytoremediation. In phytoremediation studies, plant parts such as hairy roots, cell suspensions, and calli, as well as complete plants, have been routinely employed. Tissue cultures provide a clear benefit in comprehending the selected plant’s metabolic capabilities, toxin tolerance, and phytoremediation systems. Tissue culture techniques have the advantage of growing plants under controlled conditions and establishing them with unlimited propagation (Khandare et al., 2015). Tissue culture techniques allow work to be done in a sterile environment without the presence of unwanted microbes, which can help to distinguish the actual potential and responses of plants (Doran, 2009) phytoremediation, on the other hand, has certain limitations because the actual pollutant load and hydraulics

<table>
<thead>
<tr>
<th>Enzyme/ plant sources</th>
<th>Free/ Immobilized</th>
<th>Dyes</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Horse Radish Peroxidase (HRP)</td>
<td>Free</td>
<td>Remazol Turquoise Blue G and Lanaset Blue 2R</td>
<td>(Forgiarini and de Souza, 2007)</td>
</tr>
<tr>
<td></td>
<td>immobilized in calcium alginate beads</td>
<td>Orange II (OII)</td>
<td>(Urrea, Gimenez et al. 2021)</td>
</tr>
<tr>
<td></td>
<td>immobilization by copolymerization into a cross-linked polyacrylamide gel</td>
<td>methyl orange</td>
<td>(Bilal, Rasheed, et al. 2018)</td>
</tr>
<tr>
<td></td>
<td>horseradish peroxidase-laccase co-enzymes nanoflower</td>
<td>malachite green (MG) and acid orange 7 (AO7)</td>
<td>(Gül and Ocsoy 2021)</td>
</tr>
<tr>
<td>Hampshire peroxidase-laccase co-enzymes nanoflower</td>
<td>cross-linked enzyme aggregates (HRP–CLEAs)</td>
<td>Reactive Blue 160</td>
<td>(AYHAN and AKPOLAT 2021)</td>
</tr>
<tr>
<td>Ipomea palmate and Saccharum spontaneum</td>
<td>Free</td>
<td>Supranol Green, Procion Green HE-4BD Direct Blue, Procion Brilliant Blue H-7G and Chrysoidine, Methyl Orange, Crystal Violet</td>
<td>(Shaffiquet al., 2002)</td>
</tr>
<tr>
<td>Saccharum spontaneum</td>
<td>Immobilized on the modified polyethylene matrix</td>
<td>Procion Navy Blue HER, Procion Brilliant Blue H-7G, Procion Green HE-4BD, and Supranol Green</td>
<td>(Shaffiquet al., 2002)</td>
</tr>
<tr>
<td>Bitter gourd peroxidase (BGP) from Momordica charantia</td>
<td>Free</td>
<td>Naphthalene Black 128, Coomassie Brilliant Blue R 250, Evans Blue, Eriochrome Black T, Carmine, Methyl Orange, Coomassie Brilliant Blue G 250, Rhodamine 6G, Methylene Blue and Methyl Violet 6B and Celestine</td>
<td>(Akhtar et al., 2005)</td>
</tr>
<tr>
<td>P. australis, A. donax, and T. angustifolia.</td>
<td>Free</td>
<td>Direct azo dyes amido black and amaranth</td>
<td>(Haddajiet al., 2015)</td>
</tr>
<tr>
<td>luffa fruit peels (Luffa acutangula) and soybean seed hulls (Glycine max)</td>
<td>Free</td>
<td>Methyl orange</td>
<td>(Chionget al., 2016)</td>
</tr>
<tr>
<td>HRP extracted from horseradish roots</td>
<td>Free and immobilized</td>
<td>direct yellow-12</td>
<td>(Maddhinntet al., 2006)</td>
</tr>
<tr>
<td></td>
<td>Free and immobilized on alginate and acrylamide matrix</td>
<td>Acid Black 10 BX</td>
<td>(Mohan et al., 2005)</td>
</tr>
<tr>
<td></td>
<td>Immobilized onto various polysulfone supports.</td>
<td>Acid Black 1</td>
<td>(Celebi, Kaya, et al. 2013) Celebi et al., 2013</td>
</tr>
<tr>
<td>Turnip peroxidase</td>
<td>Free</td>
<td>Reactive Blue 21 dye</td>
<td>(Silva et al., 2012)</td>
</tr>
<tr>
<td>Turnip peroxidase from (Brassica rapa)</td>
<td>Free</td>
<td>Crystal Ponceau 6R (CP6R)</td>
<td>(Almaguer, Carpio et al. 2018)</td>
</tr>
<tr>
<td>Cucurbita pepo (courgette)</td>
<td>Free and immobilized on calcium alginate bead</td>
<td>direct yellow-106</td>
<td>(Boucherit et al., 2013)</td>
</tr>
</tbody>
</table>
### Table 3: Showing the sources of plant-based enzymes used in phytoremediation of dye.

<table>
<thead>
<tr>
<th>Source</th>
<th>Enzymes present in plant extract</th>
<th>Free/ immobilized</th>
<th>Dyes</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Soybean Peroxidase</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Trypsinase, lignin peroxidase, NADH-DCIP reductase, Riboflavin reductase, and azoreductase</td>
<td>Free</td>
<td>Remazol turquoise blue G 133 (Marchis et al., 2011)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Peroxidase, laccase, tyrosinase, azoreductase, riboflavin reductase and NADH-DCIP reductase</td>
<td>Free</td>
<td>Remazol brilliant blue R and its synthetic effluent (Silva et al., 2013)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Soybean peroxidase–catalyzed reactions using response surface methodology on a Box–Behnken design (BBD)</td>
<td>Free and immobilized on polyacrylamide gel</td>
<td>Trypan Blue (Umme et al., 2013)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Soybean peroxidase–catalyzed reactions using response surface methodology on a Box–Behnken design (BBD)</td>
<td>Acid blue 113 (AB113) and Direct black 38 (DB38) (Cordova-Villegas, Cordova-Villegas et al., 2019)</td>
<td></td>
</tr>
<tr>
<td><strong>Garlic Peroxidase</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Free</td>
<td>Methyl Orange (MO) and CI Direct Yellow 12 (DY12) (Kaur, Taylor, et al. 2021)</td>
<td></td>
</tr>
<tr>
<td><strong>Orange (Citrus reticulata)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Free</td>
<td>Vat Yellow 2, Vat Orange 11, Vat Black 27, Vat Green 9 (Osuji et al., 2014)</td>
<td></td>
</tr>
<tr>
<td><strong>Ginger peroxidase</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Immobilized on polypryrole-cellulose–graphene oxide nanocomposite</td>
<td>Immobilized on polypryrole-cellulose–graphene oxide nanocomposite</td>
<td>Drimarene Orange KGL (Nosheen et al., 2014)</td>
<td></td>
</tr>
<tr>
<td><strong>Citrus Limon</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Free</td>
<td>Trypridium Yellow 4</td>
<td>Direct yellow 4 (Nouren et al., 2017)</td>
<td></td>
</tr>
<tr>
<td><strong>Miscanthus giganteus</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Free</td>
<td>Ponceau S, Amidoblock 10B and Coomassie brilliant blue R250 (CBB R250). (Nouren et al., 2017)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Azadirachta indica</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Free and immobilized</td>
<td>Free and immobilized</td>
<td>methyl orange (azo group), congo red, trypan blue (Pandey et al., 2011)</td>
<td></td>
</tr>
<tr>
<td><strong>Cabbage Leaves</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Immobilized on magnetic cross-linked peroxidase aggregate</td>
<td>Immobilized on magnetic cross-linked peroxidase aggregate</td>
<td>methylene blue, Congo red, indigo carmine, and malachite green (Mehde, 2019)</td>
<td></td>
</tr>
</tbody>
</table>

Biodegradation of Azo Dyes
Biodegradation of Azo Dyes

### Table

<table>
<thead>
<tr>
<th>Source</th>
<th>Enzymes present in plant extract</th>
<th>Free/immobilized</th>
<th>Dyes</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Typha angustifolia</em> and <em>Paspalum scrobiculatum</em></td>
<td>Lignin peroxidase (LiP), veratryl alcohol oxidase (VAO), laccase, DCIP reductase, tyrosinase, azo reductase, and riboflavin reductase</td>
<td>Free</td>
<td>Rubine GFL, Green HE4B, Scarlet RR, and Congo Red</td>
<td>Chandanshiv <em>et al.</em>, 2016</td>
</tr>
<tr>
<td><em>Fimbristylis dichotoma</em>, <em>Ammanniabaccifera</em>, and their co-plantation consortium</td>
<td>Lignin peroxidase (LiP), laccase, tyrosinase, riboflavin reductase, azo reductase, and DCIP reductase</td>
<td>Free</td>
<td>Methyl orange and textile effluents</td>
<td>(Kadamat <em>et al.</em>, 2018)</td>
</tr>
<tr>
<td><em>Tagetes patula</em></td>
<td>Lignin peroxidase (LiP), tyrosinase, laccase, and NADH–DCIP reductase</td>
<td>Free</td>
<td>Reactive Blue 160</td>
<td>(Patil <em>et al.</em>, 2013)</td>
</tr>
<tr>
<td><em>Brassica juncea</em></td>
<td>Laccase, NADH–DCIP reductase, and riboflavin reductase</td>
<td>Free</td>
<td>Present in Textile effluent</td>
<td>(Ghodake <em>et al.</em>, 2009)</td>
</tr>
<tr>
<td><em>Eichhornia crassipes</em> and <em>Salvinia natans</em></td>
<td>Polyphenol Oxidases</td>
<td>Free</td>
<td>Eriochrome Black T</td>
<td>(Rapó, Posta <em>et al.</em>, 2020)</td>
</tr>
<tr>
<td><em>Eichhornia crassipes</em> (Mart.) Solms and <em>Pistia stratiotes</em> L</td>
<td>Polyphenol Oxidases</td>
<td>Free</td>
<td>I Direct Blue 201, Cibacron Blue FR, Cibanone Gold Yellow RK, Vat Green FFB, and Moxilon Blue GRL</td>
<td>(Ekanayake, Udayanga <em>et al.</em>, 2021)</td>
</tr>
</tbody>
</table>

Biodegradation of Azo Dyes during practical phytoremediation are entirely different and constantly fluctuating. The use of a complete plant or part of the plant having various biotransformation enzymes is advantageous as it gives the high yield and low cost of production for the purification of the enzyme and the various enzymes will contribute towards the synergistic effect for the degradation of dye.

Various enzymes, including azo reductase, riboflavin reductase, and dichlorophenol indophenol (DCIP) reductase, were discovered to cause Direct Red 5B degradation in Bulmeamalcolmii tissue culture. (Kagalkar *et al.*, 2009; Kagalkar *et al.*, 2011). With diverse plant species, the enzyme status and dye degradation pathways are found to be highly variable. When utilized separately against dyes such as Brilliant Blue R, Scarlet RR, Red HE3B, Rubine GFL, and Navy Blue 2R, *Glandularia pulchella* demonstrated the varied level of activity of enzymes such as LiP, veratryl alcohol oxidase, tyrosinase, and DCIP reductase (Kabra *et al.*, 2013). A few examples of the use of whole plants or the part of plants for the biodegradation of dye are shown in Table 3.

### Conclusion and Future Prospective

Removal of azo dyes from industrial wastewater is a very difficult task due to their characteristic properties like stability in changing conditions and xenobiotic nature make them recalcitrant which alters the activities of flora and fauna and leads to environmental problems. It’s toxicity is determined by environmental conditions and the chemical qualities of the dyes. The symmetrical and asymmetrical breakage of the azo bond produces a variety of intermediates and end breakdown products which is challenging task to choose the optimal treatment approach for biodegradation and decolorization because of their complicated makeup. The physiochemical and chemical treatment methods have several limitations due to environmental impact and the cost input while biological treatment methods such as microbial and enzymatic degradation have significant potential. The enzymatic degradation is more eco-friendly and cost-effective as compared to other methods which does not produce sludge. Thus, in present review the plant-based enzyme (like Laccase, Polyphenoloxidase, and Peroxidases) degradation and decolorization of azodyes were explored and the use of phytoremediation have been taken into account. The laccases are part of the multicopper oxidase (MCO) protein family while peroxidase and polyphenol oxidase are oxidoreductive enzymes. Polyphenol oxidases act on a wide array of colored compounds and remove organic pollutants from wastewater whereas peroxidases are haemoproteins that catalyze the reduction reaction in the existence of $\text{H}_2\text{O}_2$. Laccases, polyphenol oxidases, azoreductases, and various peroxidases such as manganese peroxidase, lignin peroxidases, and decolorizing peroxidases are some of the most frequent enzymes isolated from plants that have the potential to biodegrade the dyes. The significance of enzymes

**Table 3**

<table>
<thead>
<tr>
<th>Source</th>
<th>Enzymes present in plant extract</th>
<th>Free/immobilized</th>
<th>Dyes</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Typha angustifolia</em> and <em>Paspalum scrobiculatum</em></td>
<td>Lignin peroxidase (LiP), veratryl alcohol oxidase (VAO), laccase, DCIP reductase, tyrosinase, azo reductase, and riboflavin reductase</td>
<td>Free</td>
<td>Rubine GFL, Green HE4B, Scarlet RR, and Congo Red</td>
<td>Chandanshiv <em>et al.</em>, 2016</td>
</tr>
<tr>
<td><em>Fimbristylis dichotoma</em>, <em>Ammanniabaccifera</em>, and their co-plantation consortium</td>
<td>Lignin peroxidase (LiP), laccase, tyrosinase, riboflavin reductase, azo reductase, and DCIP reductase</td>
<td>Free</td>
<td>Methyl orange and textile effluents</td>
<td>(Kadamat <em>et al.</em>, 2018)</td>
</tr>
<tr>
<td><em>Tagetes patula</em></td>
<td>Lignin peroxidase (LiP), tyrosinase, laccase, and NADH–DCIP reductase</td>
<td>Free</td>
<td>Reactive Blue 160</td>
<td>(Patil <em>et al.</em>, 2013)</td>
</tr>
<tr>
<td><em>Brassica juncea</em></td>
<td>Laccase, NADH–DCIP reductase, and riboflavin reductase</td>
<td>Free</td>
<td>Present in Textile effluent</td>
<td>(Ghodake <em>et al.</em>, 2009)</td>
</tr>
<tr>
<td><em>Eichhornia crassipes</em> and <em>Salvinia natans</em></td>
<td>Polyphenol Oxidases</td>
<td>Free</td>
<td>Eriochrome Black T</td>
<td>(Rapó, Posta <em>et al.</em>, 2020)</td>
</tr>
<tr>
<td><em>Eichhornia crassipes</em> (Mart.) Solms and <em>Pistia stratiotes</em> L</td>
<td>Polyphenol Oxidases</td>
<td>Free</td>
<td>I Direct Blue 201, Cibacron Blue FR, Cibanone Gold Yellow RK, Vat Green FFB, and Moxilon Blue GRL</td>
<td>(Ekanayake, Udayanga <em>et al.</em>, 2021)</td>
</tr>
</tbody>
</table>
isolated from plants in the treatment of industrial wastewater is undeniable. Thus, understanding the mechanism behind the biodegradation of dyes would open the path for researchers to develop novel effective treatment options. In conclusion, plant-based enzymatic treatment could only be practiced when the research is made possible to explore the role of different plant species in biodegradation.

The mechanism behind the biodegradation of dyes would open the path for researchers to develop novel effective treatment options in future. Plant-based enzymatic treatment could only be practiced when the research is made possible to explore the role of different plant species in biodegradation while utilization of genetic engineering, immobilization of free enzyme on suitable support, nano-technology and evolutionary approaches may be used to modify enzyme-based systems with improved stability, catalytic features and optimal performance etc which provide better understanding in development of robust dye water treatment in near future.

References


Biodegradation of Azo Dyes

Husain, Q. and Kulshrestha, Y. 2009. Removal of colored compounds from textile carpet industrial effluents by using immobilized turnip (Brassica rapa) and tomato (Lycopersicon esculentum) peroxidases. Water Science Technology.


Biodegradation of Azo Dyes


Biodegradation of Azo Dyes


