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Azo Dyes Degradation Approaches and Challenges: An Overview

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ABSTRACT

Dyes play a very important role in our daily lives. The dye industries started manufacturing dyes with natural sources and then turned towards synthetic ones. Among these dyes, about 60% of the total dyes are azo dyes which are used in industries. They are mostly used as textile dyes because they are easy to synthesize, chemically stable and diverse in nature. Azo dyes come in about 3000 different variations and are employed in a variety of industries, including the textile, leather, paper, and pharmaceutical sectors. But sadly, the majority of azo dyes are poisonous and mutagenic to all living things. The effluent coming from the textile sector must therefore be removed and treated. The wastewater is often treated using a variety of physical and chemical methods, however, these methods have been shown to be inefficient, expensive, produce insufficient amounts of sludge, and have limited effectiveness. So, in order to treat and decolorize dyes and dye-containing effluents without further harming the environment or endangering life forms, It has practically universal dye degradability is economical and has also eliminated a number of drawbacks of the physicochemical method. The properties and classification of azo dyes, associated problems, biodegradation techniques, and possibilities are only a few of the subjects covered in this chapter's examination of recent research, advancements, and the body of existing information on them. Biological processes have received special attention as a remedy for the current problems with azo dyes.

Keywords: Synthesis, Biodegradation, Mutagenic, Azo Dyes, and Carcinogenic.

INTRODUCTION

Azo dyes are chemical molecules that have the functional group R-N=N-R', where R and R' are often acrylic groups. These are the dyes that are used the most in industries; azo dyes, which make up more than 60% of all dyes used in industries, account for over 70% of all dyes used. These are the most widely used synthetic colorants, which are crucial for creating textiles, prints, and papers, among other things (Benkhaya et al., 2020).

Origin

The textile dyeing industry has existed for more than 4000 years. For the last 150 years, it was the natural source from where dyes were obtained but it was in 1856 that brought a big change in the world of dyes. William Henry Perkin when trying to find a way to synthesize Quinine, a drug that cures malaria, produced a new generation of dyes (Benkhaya et al., 2020). W H Perkin synthesized mauveine or aniline purple, the first synthetic dye, from the chemicals that were derived from coal tar.

Methods of preparation

Despite the fact that azo dyes can be made in a variety of ways, they are always produced by coupling diazonium compounds with other substances like phenol, naphthols, arylamines, pyrazolones, etc. This coupling leads to hydroxyazo or aminoazo compounds or their tautomeric equivalents. The resulting dyes contain acts as a chromophore and the hydroxyl or amino groups as an auxochrome (RLM, 1971). Chromophores are the

molecules that absorb a certain wavelength of visible light and confer colour to the dyes while auxochrome is a functional group attached to the chromophore which is responsible for modifying the absorbing ability of chromophore by altering the wavelength or intensity of absorption.

Other techniques for making azo dyes include reducing nitro-aromatic derivatives in an alkaline medium reducing nitroso compounds with $AlLiH_4$, oxidising primary amines with potassium permanganate or lead tetra acetate, condensing hydrazine and quinones, and condensing primary amines with nitroso derivatives, among others (Benkhaya et al., 2020).

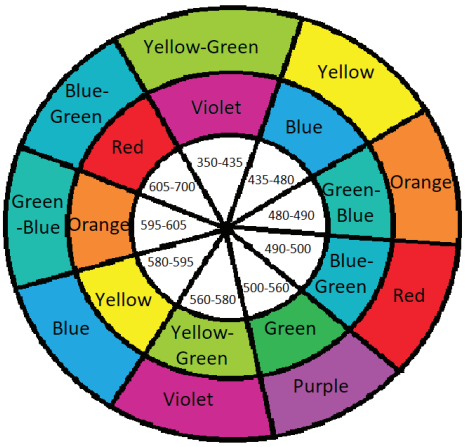


Figure 1: Relation between the wavelength of visible light and colour absorbed and colour observed in the case of organic dyes.

Dyes are characterized by their capacity of absorbing light radiation of the visible spectrum in the range from 380 to 750 nm. The white light is transformed into colored light by reflection or transmission or diffusion which is the result of the selective absorption of energy by chromophoric groups. The intensity of coloration depends on the chemical constitution linked to the dye (Benkhaya et al., 2020) Dyes are capable of absorbing light of a certain wavelength in the visible range and transmitting the light in the same range, the colour we see is the colour transmitted (Figure 1).

Chemical structure

An azo dye's backbone, the chromophoric group, the auxochromic group, and the solubilizing groups make up its basic chemical composition. The dye's colour is determined by the azo linkages and their corresponding chromophores and auxchromes (Benkhaya et al., 2020).

Various parts of azo dyes:

1. Chromophore: -N=N-
2. Auxochrome: -OH
3. Water-soluble group: $-SO_3H$

Classification of azo dyes

Classification of dyes according to the method used

A. Azo acid dye (anionic azo dyes)

Acid azo dyes with monoazo, diazo, or anthraquinone structures have low molecular masses. Due to their small sizes, these dyes produce vivid hues and have the capacity to diffuse quickly into complicated fabrics like leather. Additionally, they are known as penetrating dyes. These were given that moniker because an acidic phase was used in the dyeing process. Typically, these are employed for colouring and printing materials including wool, polyamide, silk, leather, paper, and food (Hunger, 2003).

Typically, sulphonate or carboxylate substituents are present in anionic dyes. While a dye containing the carboxyl group requires a relatively high pH to stay anionic because carboxylic acids are weak acids, a dye containing the sulphonate group remains anionic over the whole pH range. Since they stay anionic at low pH, acid dyes with sulphonate groups are chosen (Tehrani-Bagha and Holmberg et al., 2013).

B. Basic dye (cationic azo dye)

The coloured portion of cationic azo dyes typically has positive charge. They interact with the fiber's negative group to generate a salt that becomes securely linked to the fibre, causing the fibre to take on a dyed appearance. These are primarily used to colour polyacrylamide (acrylic) fibres and bleach cellulose. They are also, but to a lesser extent, used to colour leather, paper, plastics, and waxes (Hunger, 2003).

C. Direct azo dye

Direct azo dyes can directly dye the natural or regenerated cellulose or protein fibres without the use of mordents. The quality in fastness is not the best but its simple production method, reasonable price and ease of application make it the most used azo dye today. Direct dyes have a higher affinity towards cellulose and the dyeing of cellulosic fibre is done in neutral or weakly alkaline medium while proteins are dyed in a neutral or weakly acidic medium. For example Cotton is dyed using direct dye in a natural or alkaline process while paper is dyed in weak acidic process. Water is a soluble medium for direct dyes, and warmth increases their solubility. When dyeing fibres at a high temperature, the dye enters the fibre, creates a dye aggregate inside the fibre, and resists being rinsed off (Hunger,2003).It is a little difficult to dye leathers with the direct dye as the dye is not able to penetrate the leather due to their big molecular weight and lower permeability. So, a little ammonia is added before dyeing to promote penetration through the leather.

D. Mordant azo dye

Mordant dyes require a mordant for dyeing fibre. A mordant, also called dye fixative is a substance used to set dye on fabrics that form a coordination complex with dye which then gets attached to the fabric. Mordents are generally used to enhance colour. During the process, the material is pre-heated with metal salts which behave like a mordant and a metal complex is formed on the fibre that set dye on the fibre (Hunger, 2003). These dyes can be used with wool, wool blends, silk, cotton and certain modified cellulose fibres.

E. Vat azo dye

Vat dyes are reduced to a leuco form, which has a strong affinity for cotton and other cellulosic fibres, after being treated with alkaline liquor because they are insoluble in water. When the dye oxidizes, colour is produced (Christie, 2001; Vanhulle, 2004). Vat dyestuff cannot be used to dye wool since the procedure involves the use of caustic soda and a dye bath with a very high pH. Wools can, however, be coloured with indigo, a vat dye that can be used at room temperature, as well as other low-intensity vat dyes using soda ash as an extremely weak alkaline medium. The same method can also be applied with additional vat colours, such as Vat dye 10, Vat violet 13, and Vat orange 1. The first blue vat dye, indigo, was derived naturally from the leaves of plants in the Indigofera genus, primarily *Indigofera tinctoria*, but is now frequently made synthetically.

F. Reactive azo dye

Reactive Azo dyes have colour fastness in credible wash fastness properties which had a great impact on dye industry. A chemical reaction takes place between the reactive dye and the fabric where a covalent bond is formed between the two. A covalent link is created by an oxygen, sulphur, or nitrogen atom in a hydroxyl or thiol group on the polymer with a carbon atom of the dye (Christie, 2001). An alkaline medium is required for reaction initiation. The hydroxyl group of the cellulose polymer, for instance, is changed during the reaction into the reactive nucleophile O-, which subsequently combines with the dye to produce the covalent bond known as Cellulose-O-Dye. The alkaline ion in the solution and the dye molecules interact during the reaction to generate the hydroxyl group. Before the dyeing is finished, the hydrolyzed dye that had previously created must be removed because it is no longer capable of reacting with dye (Christie, 2001). The main flaw with reactive dyes is that, during the wash-off process, 10–40% of the dye gets rinsed out without ever reacting with the fibre. A step can be taken for improvement of the dyes by preparing the dyes with a bifunctional group i.e. two functional groups and efforts are been taken to design a dye with low sulphur content.

G. Disperse dye

Disperse dyes are best for colouring hydrophobic fibres like polyester since they have a low solubility in water (Christie, 2001). It was created to make it possible to dye hydrophobic thermoplastic fibres like nylon, polyester, acrylic, and other synthetics as well as acetate, triacetate, nylon, and triacetate. These are crystals with a low molecular weight and a high melting point (more than 150^0C). These dyes are non-ionic because they have a neutral electrical charge. There are no significant solubilizing groups, such as carboxyl or sulphonic groups. However, it's possible to find weakly solubilizing aliphatic or aromatic groups like -NH2, -NHR, or -OH. Due to the lack of non-ionizable groups, these dyes tend to sublime without decomposing, and as a result, the colour of dispersed dyes may fade during ironing.

H. Azo metal-complex dyes

Water-soluble metal-complex azo dyes exhibit a strong affinity for the majority of fibres. Complex commercial dyes containing chromium, nickel, cobalt, copper, and iron produce striking colours. When used on protein fibres, these dyes offer exceptional fastness for colouring (Christie, 2001; Hunger, 2003). These dyes often contain a monoazo group with other substituents like hydroxyl, carboxyl, or amino groups that form potent coordination complexes with transition metal ions. Sulphuric, formic, and acetic acid are pH regulators, sodium sulphate and ammonium acetate are electrolytes, and levelling agents are necessary when using metal-complex colours (mixture of anionic and non-ionic surfactants) (Chavan, 2011).

Classification of dyes by the number of azo groups

The azo dyes are divided into three groups based on the number of azo connections they contain in a single dye molecule: monoazo, diazo, and polyazo (Benkhaya et al., 2020).

I. Monoazo dyes

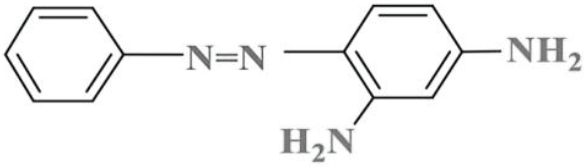
These dyes constitute single azo group in their compounds.

These can be represented as: Z-N=N-W, where Z and W can be:

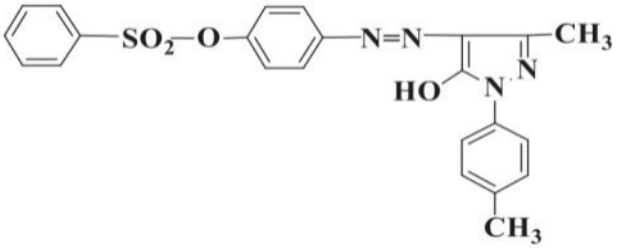
a) Benzene or heterocyclic derivatives
b) Benzene and naphthenic respectively
c) Both naphthalene

Dye in which Z and W can be benzene and heterocyclic derivatives

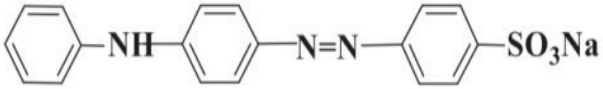
Example 1: Chrysoidine (ancient analog of this family) characterized by its orange colour which and used to dye cotton.



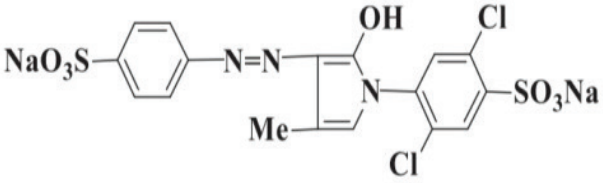
Example 2: Yellow basic dye characterized by better light fastness and washing process and used for colouring cellulosic fibre.



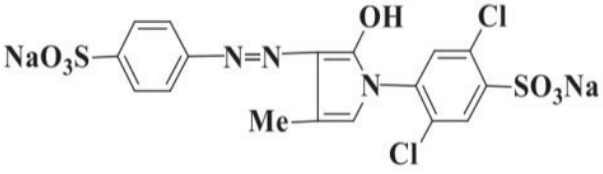
Example 3: It is a dispersed monoazo dye, used for dyeing cellulose acetate, polyamide, polyesters and polyacrylonitrile.



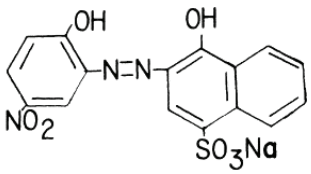
Example 4: Orange IV and yellow dye "-acidic monoazo dyes used to dye wool and nylon 2. Monoazo dye where Z is benzene and W is naphthenic derivative.



Example 5: Mordant brown 35: Manufactured by diazotization of 2-amino-4-nitrophenol and then coupling with 4-hydroxynaphthalene-1-sulphonic acid.



Example 6: Blue dye: derived from H acid ($C_{10}H_9NO_7S_2$) and used for dyeing wool.

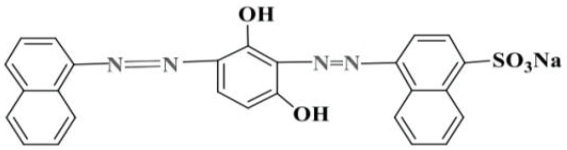


II. Diazo dyes

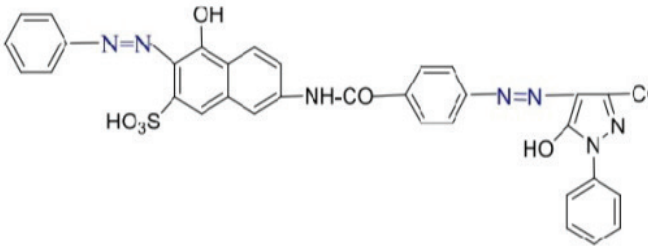
Diazo dyes contain two azo groups in their compound.

These are prepared by three methods:

1. **Primary Diazo:** Two moles of di azoic acid are coupled together in a process to create this type of colour. These are m-phenylenediamine and resorcinol compounds. They offer brown, green, matte blue and black pigmentation. For instance, brown dye.



2. Symmetrical secondary diazoic: Compared to acid and mordant dyes, these types have a lot more direct dyes. These are made from a diamine that has undergone two diazotization processes before being coupled in the same or a different way. For instance, blue direct dye.



3. Asymmetrical secondary diazoic: This type of dye is synthesised by coupling reaction of an amino azoic acid with a phenolic coupler. Example: Orange direct dye

III. Poly Azo Dyes

These complex dyes are distinguished by having more than two azo groups. These often fall under the category of direct dyes. These are typically used to dye leather, but they can also be used to dye cotton and other cellulose fibres in dark hues like red, brown, and deep black. Direct red dye, which is created by phosgenating the proper diazo dye, is the most popular dye of this sort. This is accomplished by combining N-acetyl-J after diazotizing 6-amino-3,4'-azo-dibenzenesulfonic acid. The hydrolysis of the acetyl group occurs before the phosgenation process. Another example of polyazo dye is the direct light-fast blue

Azo dyes and their Environmental Impact

Azo dyes: Uses and Background:

Numerous types of pollution have been produced over the past few decades as a result of population growth,

globalisation, urbanisation, and industrialisation. Among a variety of industries, the textile dyeing industries release a significant amount of waste water after dyeing that contains toxic chemicals that have a negative impact on the environment, soil fertility, aquatic organisms, and the ecosystem's integrity by altering pH, by increasing biological oxygen demand (BOD), and by increasing chemical oxygen demand (COD). These could have fatal consequences for both plants and animals, as well as genotoxicity, mutagenicity, and carcinogenicity (Sudha et al., 2014).

Azo dyes are the most often produced textile dyes due to their simpler synthesis, chemical stability, and wide range of colour options. One, two, or more azo links, phenyl, and naphthyl rings with substituted functional groups (which may be triazine amine, chloro, hydroxyl, methyl, nitro, or sulphonate) are typically present in azo colours (Sudha et al., 2014). According to research, between 10 and 15 percent of the dye used in the dyeing process leaks into the environment after becoming freed from the fibres (Singh et al., 2017).

Azo dies carcinogenic effect

The azo dye's ability to cause cancer may be attributable to the dye or a metabolite of the dye. The dye itself is the carcinogen for water-insoluble but solvent-soluble dyes like dispersion dyes and solvent dyes. In the case of a water-soluble dye, the carcinogenic component is the pigment's metabolite. The majority of natural dyes are non-toxic by nature, but they need mordants to intensify the colour. Typically, heavy metal salts of Al, Fe, Cu, Cr, and Sn are employed as mordants with dyes. As opposed to copper and chromium, alum and ferrous sulphates are regarded as being reasonably harmless. Alum and iron must still be used with caution. A child's deadly dose of alum depends on body weight, but it can be as little as 3g. The fatal dose of alum for an adult is 30g. The United States Food and Drug Administration (FDA) claims that while iron is not harmful to the environment when disposed of, it is dangerous if consumed in excess. Similarly, caution must be used when using copper and tin mordants. Chrome mordant, commonly known as a human carcinogen, is dangerous to both humans and the environment (Chavan, 2011).

In addition to the mutagenic, carcinogenic and genotoxic nature of dyes, their presence in water bodies reduces penetration of sunlight to deep layers which affect the photosynthetic rate, deteriorates quality of water and lowers the solubility of gases too which adversely effects aquatic flora and fauna (Mervate et al., 2017). Textile dyes have ability to recalcitrant in aerobic environments, basically, in conventional treatment plant, which results in their bioaccumulation in sediments and soil which are then unfortunately released into public water supply systems.

The ecosystem's structure and function are impacted by the xenobiotic and recalcitrant properties of dyes (Lellis et al., 2019). They are highly refractory by nature due to the inclusion of one or more azo groups and sulphonic (-SO3-) electron-withdrawing groups (Mani et al., 2019). These dyes can also have an adverse effect on the immune system, which can result in respiratory sensitization, which can cause symptoms like itching, watery eyes, sneezing, and asthmatic symptoms like coughing and wheezing the next time the individual inhales a dye particle (Hassaan et al. 2017).

Azo dyes' effects on people and other animals

Numerous studies have been undertaken to demonstrate the toxicity of azo dyes since 1895 when higher rates of bladder cancer were noted in workers engaged in the dye production industry (Table 1) (Chavan, 2011).

Azo dye splits into its potentially cancer-causing ingredients, such as an aromatic amine and an aryl amine, when it is mineralized. The majority of dyes are water soluble and easily absorbed through the skin, increasing the risk of cancer and allergic responses. They are also an irritant to the dyes and extremely hazardous if ingested or inhaled. Workers who handle or work with reactive dyes are more prone to illness; they may develop contact dermatitis, allergic rhinitis, allergic conjunctivitis, occupational asthma, or other allergic reactions (Lellis et al., 2019).

When present in water at a concentration lower than 1 mg/lit, several synthetic dyes are extremely visible. The average dye concentration in wastewater from the processing of textiles is between 10 and 200 mg/lit. Most of these dyes are harmful to all life forms and can induce allergic reactions, skin dermatoses, and damage to the liver, lungs, vasculature, and even reproductive systems in both animals and people. Aquatic life is also at risk when sewage effluent is released into water bodies without being treated. The majority of these dyes and their post-degradation intermediates are poisonous, mutagenic, and carcinogenic to all life forms (Singh et al., 2017). The biomagnification caused by these dyes, which persist in the environment as pollutants and span the entire food chain, results in organisms at higher trophic levels having larger concentrations of these contaminants than their corresponding prey. It can affect humans negatively through the food chain, harming their organs and general health (Lellis et al., 2019).

Table 1: Effects of dyes and their intermediates.

S. No.	Dye or their degraded substituent's	Effects	Reference
1	Acid violet 7	It is a common textile azo dye that can cause chromosomal aberration, lipid peroxidation, and inhibition of the acetyl cholinesterase enzyme.	Singh et al., 2017
2	Aniline Yellow	It is also called 4-aminoazobenzene or 4-phenylazoaniline induces liver tumour in rats and causes epidermal tumour when applied on skin.	Chung et al., 2016
3	o-aminoazotoluene	It also goes by the name Solvent Yellow 3, and it causes tumours in the lungs, liver, gall bladder, and urine bladder of dogs and rats.	Chung et al., 2016
4	Azure-B	This colour can bind to the helix structures of DNA and duplex RNA, inhibiting a number of human enzymes in the process. It demonstrates its deadly effects by being a noteworthy reversible inhibitor of the central nervous system enzyme monoamino oxidase A (MAO-A), which regulates human behaviour. Additionally, it inhibits glutathione reductase, which is crucial for maintaining cellular redox equilibrium.	Lellis et al., 2019
5	Basic Red 9 dye	The dye may be hazardous to the environment and carcinogenic to humans. The dye typically degrades into cancer-causing aromatic amines under aerobic conditions, which when discharged into bodies of water can result in allergic dermatitis, skin irritation, mutagenesis, and cancer. The test on rats showed that the liver, bladder, mammary glands, and haematopoietic systems all had local sarcomas and tumours.	Lellis et al., 2019
6	Benzidine	It is a component of azo dye is linked to cancer of urinary bladder, genitourinary tract, pancreas, liver, gall bladder, large intestine, stomach, lymphopoiesis and renal cell in humans.	Chung et al., 2016
7	Crystal violet dye	A member of the cationic triphenylmethane group, which also causes aberrant metaphase accumulation and in vitro clastogenic effects in Chinese hamster ovules, is to blame for mitotic poisoning. Additionally, it encourages the growth of fish tumours and results in hepatocarcinoma, reticular cell sarcoma in a variety of organs including the vagina, uterus, ovary, and bladder, as well as calcified gland adenomas and ovarian atrophy in rats. In people, it results in chemical cystitis, skin and digestive tract irritation, respiratory failure, and renal failure.	Lellis et al., 2019
8	Direct Black 38	Urinary Bladder cancer in humans	Sudha et al., 2014
9	Direct Blue 15	Mutagenic	Sudha et al., 2014
10	Disperse red-1	It makes micronuclei more common in people. According to an experiment done on Salmonella spp., the dye causes DNA adducts, which in humans represent a mutagenic event that is characterised by cancer.	Jhadav et al., 2016; Lellis et al., 2019
11	Disperse orange-1	It effects lymphocytes and also induces DNA damage involving frame shift mutation and base pair substitution (according to Salmonella spp. Assay) and also has cytotoxic effect with apoptosis	Jhadav et al., 2016; Lellis et al.,2019
12	Disperse blue 291	causes DNA fragmentation, micronuclei to develop, is cytotoxic, mutagenic, and has genotypic effects on human hepatoma cells.	Jhadav et al., 2016
13	Malachite green	It is a multi-organ toxin that harms the heart, liver, spleen, kidney, and spleen in addition to reducing growth and fertility rates. When given to mice, malachite green induced apoptosis in the transitional epithelium of the thyroid and urinary bladder follicles.	Jhadav et al., 2016

14	3-methoxy-4-aminoazobenzene,	It is an aromatic amine that is a component of dye and has been proven to be a powerful rat hepatocarcinogen and a bacterial mutagen.	Singh et al., 2017
15	Methyl red	Mutagenic by nature and poisonous by nature. Its microbial breakdown byproduct, N, N-dimethyl-phenylenediamine (DMPD), is also a mutagenic aromatic amine.	Singh et al., 2017
16	Methyl yellow	Also known as Butter yellow, this food ingredient was removed from usage in 1918 after it was found to be a potent cancer agent.	Chung et al., 2016
17	M-methyl-4-aminoazobenzene (MAB)	Hepatocarcinogenic in nature to mice	Chung et al., 2016
18	Metalin Yellow	hepatotoxic to albino rats	Singh et al., 2017
19	Para-phenylene diamine (PPD)	PPD, also known as 1,4-diamino benzene or 1,4-phenylene diamine, is a poisonous aromatic amine that is a key ingredient in azo dyes. It can cause skin irritation, contact dermatitis, chemosis, lacrimation, exophthalmos, and irreversible blindness. Ingesting PPD products can result in respiratory distress as well as oedema on the face, tongue, neck, throat, and larynx. Additionally, it can occasionally result in rhabdomyolysis, acute tubular necrosis, vomiting, gastritis, hypertension, and vertigo.	Shah et al., 2014
20	Reactive dyes	Cause dermatitis, asthma, rhinitis, and mutagenicity as well as genotoxicity and carcinogenicity. Reactive brilliant red, for instance, prevents human serum albumin from functioning, while reactive black 5 reduces urease activity and the rate of arginine ammonification in terrestrial ecosystems.	Jhadav et al., 2016
21	Sudan-1 dye (Solvent Yellow14)	Cause mutagenicity, genotoxicity, carcinogenicity, dermatitis, asthma, and allergic rhinitis. For instance, reactive brilliant red impairs human serum albumin function, while reactive black 5 reduces urease activity and the rate of arginine ammonium synthesis in terrestrial ecosystems.	Lellis et al., 2019
22	Various other azo dyes such as: Allura Red Carmoisine Poncueau 4R Quilnoline Yellow Sunset Yellow Tartrazine	Harmful to children if used as additives in food and drinks	Singh et al., 2017

Impact of azo dyes on plants

These dyes have an adverse effect on soil microbes, plant germination, and growth when they are washed off of fabrics during processing and released into wastewater, which in underdeveloped countries is typically used to irrigate agricultural fields (Lellis et al., 2019).

Numerous researchers conducted phytotoxicity studies on common agricultural crops in India, including *Triticumaestivum, Vignaradiata, Vigna mungo, Phaseolus mungo, Zea mays, Phaseolus vulgaris, Sorghum vulgare, Solanum vulgare, Hordeum vulgare, Oryzasativa, Cicer arietinum,* and *Sorghum bicolor, Phaseolus mungo, Ervum lens,* and *Triticumaestivum L*. When seeds were evaluated for phytotoxicity for malachite green and its degradation products, the length of the plumule and radicle were dramatically changed, indicating less toxicity towards plant seedling and growth. It was discovered that using 50mg/lit of Methyl Red, 2000mg/lit of Dispersed Brown 118, 100mg/lit of Black WWN, 1000mg/lit of Reactive Blue, and 100mg/lit of Direct Blue-1 completely suppressed plant seed germination (Pokharia et al., 2015). As the concentration of phenolic chemicals in dyes increased, a reduction in root and shoot length was seen (Jadhav et al., 2016). When wastewater from the dyeing process is discharged into bodies of water, it dramatically reduces light penetration intensity, which has a negative impact on plant photosynthesis and is hazardous to aquatic flora and wildlife (Hanan et al., 2008). The detrimental impact of azo dyes on plant growth may be caused by dye's inhibition of plant ATPase activity, which in turn prevents plants from evaluating and growing photosynthetic oxygen (Zhou et al., 2013).

The impact of untreated effluent from a viscose industry in South India on various maize cultivars has been documented. The level of dissolved oxygen is decreased by the high solid concentration in effluent, further limiting seed growth and development. The disruption in the osmotic relationship between the seed and the high concentration of material in the effluent may have prevented seed germination (Puvaneswari et al., 2006).

Biodegradation of azo dyes

Each year, the azo-dye wastewater produced by the textile industries is improperly dumped in water bodies in large quantities. Due to their toxicity and carcinogenicity, it raises serious concerns as it may alter the ecological balance and have negative health effects (Zhou et al., 2013). An estimated 280000t of textile dyes are emitted into textile wastewater each year on a global scale (Jhadav et al., 2016). The dye that is left unbound or unfixed during the dyeing process, when released, severely contaminates nearby soil and water bodies like rivers, streams, and groundwater. When released into water bodies, dye-based effluents contain a higher concentration of suspended particles, which raises BOD and COD levels (Uruj et al., 2015). The wastewater also contains additional organic stuff, a bright colour, a high pH variation, and a lot of suspended particulates (Tan et al, 2016). The visual appeal, transparency, and gas solubility of water can all be impacted by the presence of even very modest amounts of dye (10–50 mg/lit) (Kochher et al., 2020).

Techniques of disposal of azo dye

Different physical, chemical, and biological methods have been utilised to treat dyeing process effluent. Among these, biological technique is being recognized as promising technique because of its inexpensiveness, environment-friendly nature and sustainable properties (Zhou et al., 2013).

Various physicochemical methods like filtration, flocculation, coagulation, chemical oxidation, photodegradation, etc. are been employed to treat or remove azo dyes present in industrial effluent but they come out with limited effectiveness due to their low efficiency, incomplete removal of colour, high cost, large amount of sludge production and handling of the generated effluent (Saroj et al., 2015; Chandanshive et al., 2016).

In order to treat and decolorize dyes and dye-containing effluents without further stressing the environment or threatening life forms, biological systems, such as plants and microorganisms, have proven to be a viable and efficient option (Uruj et al., 2015). It has the ability to breakdown nearly all dye materials and has also addressed a number of physicochemical approaches' drawbacks (Singh et al., 2017).

Bioremediation

Bioremediation is a process that utilizes microorganisms and other biological system like plants or association of plant and rhizopheric microorganisms for reclamation of an already contaminated habitat by converting toxic contaminants into less hazardous or non-hazardous substances (Arora et al, 2018).

Decolorization

Decolorization, or the dissolution of the azo bonds, is the first stage of azo-dye degradation (Zhou et al., 2013). Numerous microorganisms, including bacteria, fungi, yeasts, actinomycetes, algae, and plants, have the ability to totally mineralize and decolorize different azo dyes (Saratale et al, 2011). Azo dyes are decolored and broken down using two procedures (Table 2).

Table 2: Bioremediation Decolourization Methods.

S.No	Method	Process
1	Absorption on the microbial biomass (Biosorption)	Biosorption is the term used to describe the uptake and accumulation of chemicals by microbial mass. For this, numerous microorganisms' biomass has been employed, including filamentous fungus, yeast, and bacteria. Because of the heteropolysaccharide sand lipids in their cell walls, which include a variety of functional groups (such as amino, hydroxyl, carboxyl, phosphate, and other charged groups), microorganisms are able to do this. This creates a powerful attraction between the cell wall and the dye.
2	Biodegradation of dyes by the living cells i.e. enzymatic degradation	Reductive (Azoreductase, FMN-dependent and independent reductase, NAD-dependent reductase, NADPH-DCIP reductase, etc.) and oxidative (polyphenol oxidase, manganese peroxidase, tyrosinase, N-demethylase, etc.) enzymes produced by microorganisms are the two groups of enzymes involved in the biodegradation. The enzymatic process offers a more advantageous alternative to the physicochemical approach of degradation since it generates less sludge and is more affordable. (Pradeep et al 2017).

Phytoremediation of azo dyes

Phytoremediation is a bioremediation process that uses various types of plants to destroy, stabilize, remove and detoxify contaminants present in soil, surface water, air or groundwater through their enzymes or microflora. Phytoremediation involves growing the respective plant in a contaminated matrix, soil or water, for a required growth period, to remove the contaminant and facilitate immobilization and detoxification or destruction of the pollutants. The plant uptake contaminant by root system which provide a large surface area for facilitating mobilization, clean up or detoxify contaminant within the tissues by various methods such as elimination, containment and degradation, etc. After that plant can be harvested, processed and disposed (Sureshvarr et al., 2010; Uruj et al., 2015) (Table 3, 4).

The following are some methods that plants can decolorize azo dyes and encourage bio-decolorization:

1. Rhizo-deposition releases 10% of the photosynthetic carbon that contributes to the proliferation of microorganisms in wastewater.
2. It has been noted that specific enzymes found in the root exudes, such as lignin peroxidase, manganese-dependent peroxidase, and laccase, can decolorize colours.
3. Anaerobic and aerobic environments are necessary for the decolorization of azo dyes; the rhizosphere is created by the roots pumping oxygen into it.
4. Plants may be able to remove colour from water by absorbing dye from sewage, which increases the contact of bacteria with azo dyes (Zhou et al., 2013).

Several plant species, including rye, bermuda grass, sorghum, fescue, legumes, sunflowers, Indian mustard, rapeseed plants, barley, hops, crucifers, nettles, and dandelions, have the capacity to accumulate waste like toxic azo dyes within them by absorbing them through roots and detoxifying and metabolising them internally (Sureshvarr et al., 2010). Plants not only detoxify toxins but also provide defence against wind and water erosion, preventing contaminants from spreading (Tan et al, 2016). Plants are good bioremediation options since they are simpler to manage and require fewer nutrients for growth. Plants with deep, fibrous roots and rapid growth are advantageous for phytoremediation (Tan et al., 2016).

Table 3: Depending on the mode of action and type of contaminant, phytoremediation is categorized in the following class.

S. No	Classes of Phytoremediation	Utilization	Plants involved
1	Phytodegradation	This process utilizes plants to take up, store and degrade the contaminants from soil, groundwater or wastewater within its tissues.	Phreatophyte trees (cottonwood,`1aspen), grasses (rye, bermuda, sorghum), legumes (clover, alfalfa).
2	Phytostimulation or rhizodegradation	This uses rhizopheric association of plant and microorganisms present in soil or sediments for degradation of the contaminant	Phenolic releasers (mulberry, apple), grasses with fibrous roots (rye, bermuda), aquatic plants for sediments
3	Phytovolatilisation	In this, plants take up the contaminant from the matrix, transform it and volatilize them into the atmosphere.	Phreatophyte trees (cottonwood, aspen), grasses (rye, bermuda, sorghum), legumes (clover, alfalfa).
4	Phytoextraction	In this, plants absorb the toxic contaminant from the soil or sediments, translocate and store it in their tissues of roots and shoot	Sun flower, Indian mustard, barley, hops, crucifers, nettles, etc.
5	Rhizofiltration	Roots absorb and store contaminants from aqueous growth medium.	
6	Phytostabilization	Involve plant mediated immobilization or binding of the contaminant with the soil matrix so that its bioavailability would be reduced.	Phreatophyte trees that transpires large amount of water, grasses for stabilizing soil erosion and dense root system to bind contaminants.

Table 4: Some of the plant species used for phytoremediation, their mode of action and the dye degraded or removed by them is given in the table below:

S. No.	Plant species	Mode of action	Dye degraded or removed	References
1	Brassica alba (Mustard)	Absorption and speciation of dye stuff within plant tissues.	Highest absorption efficiency for ethidium bromide	Uruj et al., 2015
2	Blumeamalcolmii (roots)	Assimilation and degradation of dye	Malachite green (up to 45%)	Uruj et al., 2015

S. No.	Plant species	Mode of action	Dye degraded or removed	References
3	Blumeamalcolmii	Enzymatic activities of plant [lignin peroxidase, DCIP (2,6-dichlorophenol-indophenol) reductase, tyrosinase, azoreductase and riboflavin reductase]	Direct Red 5B	Kagalkar et al., 2009
4	Banana (pulp)	Enzymatic activities of plant (polyphenol oxidase, peroxidase, laccase and lignin degradation enzymes).	Direct Red 5B (up to 90%) Direct Blue GLL (up to 80%), Reactive Navy Blue (up to 80%)	Khandare et al., 2013
5	Brassica juncea	Laccare activity by roots of B. juncea	Methyl orange	Telke et al., 2011
6	Eichhorniacrassipes	Absorption and speciation of dye stuff within plant tissues	99.5% removal efficiency for Black B and 95% for Red RB	Uruj et al., 2015
7	Eucalyptus sp.	Absorption and speciation of dye stuff within plant tissues	Compounds of azo dyes	Sureshvarr et al., 2010
8	Eichhorniacrassipes (Water hyacinth)	Absorption by roots	Methylene Blue (98.42%) Methyl Orange (66.8%)	Tan et al., 2016
9	Eichhornia spp.	Absorption by roots	Congo Red	Wanyonyi et al., 2014
10	Lemna minora	Accumulation of dyes	Methylene Blue	Priyanka and krishnaswamy, 2019
11	Nasturtium officinale	Enzymatic activities of plant (Superoxide dismutases and peroxidase)	Basic Red 46 and Acid Blue 92	Uruj et al., 2015
12	Phragmitesaustralis	Enzymatic activities of plant (Peroxidase)	Amarnath (93%), Amido Black (83%) and Acid Orange 7 and its aromatic amines (70%)	Uruj et al., 2015
13	Portulaca grandiflora	Enzymatic activities of plant [lignin peroxidase, DCIP (2,6-dichlorophenol-indophenol) reductase, tyrosinase, Riboflavin reductase]	Direct Red5B	Khandare et al., 2013
14	P. grandiflora	Enzymatic activities of plant [lignin peroxidase, DCIP (2,6-dichlorophenol-indophenol) reductase and tyrosinase]	Navy Blue HE2R	Uruj et al., 2015
15	Salviniamolesta	Enzymatic activities of plant (Oxidoreductase)	Rubine GFL	Chandanshive et al., 2016
16	Typhaangustifolia	Assimilation and degradation of dye	Reactive Red 141 (60%)	Priyanka and krishnaswamy, 2019; Uruj et al., 2015
17	TyphoniumFlagelliforme	Assimilation and degradation of dye	Brilliant Blue R (80%)	Uruj et al., 2015
18	Typhadomingensis	Enzyme activity of plant	Amarnath (83-92%)	Haddaji et al., 2019
19	Typhoniumflagelliforme	Enzyme activity of plant	Brilliant Blue R	Kagalkar et al., 2010
20	Typhadomingensis	Enzyme activity of plant (peroxidase)	Amido black	Haddaji et al., 2019

The toxic and carcinogenic dyes are converted by plants into simple and non-toxic metabolites by phytoremediation. Following *Blumeamalcomii's* phytoremediation of Direct Red 5B, three metabolites were found using HPLC (High-Performance Liquid Chromatography) and FTIR (Fourier Transform Infrared Spectroscopy): 3-amino-7-carboxyamino-4-hydroxy-naphthalene-2-sulfonic acid, 4-(4-amino-phenylazo)-benzene sulfonic acid, and 7-carboxyamino-naphthalene-2-sulfonic acid.

Bacterial degradation of azo dyes

Due to their viability, availability, high activity, and cost-effectiveness, bacteria are frequently utilised for the bioeradication of contaminants that contain colour. The breakdown of azo bonds, which is aided by the enzyme azoreductase produced by bacteria under anaerobic conditions, results in some toxic and cancer-causing aromatic amines, which are then transformed into non-toxic metabolites by the enzymatic activity of certain bacteria's hydroxylase and oxygenase (Mani et al., 2019). Aerobic processes alone are used by bacteria to break down aromatic chemicals into non-toxic molecules. Therefore, it is thought that bacteria could biodegrade azo dyes under both aerobic and anaerobic circumstances (Alabdrabam et al., 2014). Because each microbial strain and its enzymes are specific to a certain temperature and pH, the degree of dye decolorization varies with changes in temperature and pH (Kumar et al., 2018).

The biodegradation of azo dyes uses a variety of aerobic and anaerobic bacteria, including *Bacillus subtilis, Pseudomonas sp., Escherichia coli, Staphylococcus sp., Acinetobacter sp., Geobacillus, Lactobacillus, Rhizobium, Aeromonas* species, etc. Some strains of aerobic bacteria solely obtain their carbon and nitrogen from azo dyes, whereas others decrease the azo group via an oxygen-tolerant azo reductase (Sudha et al., 2014). *Bacillus cereus*, for instance, can destroy reactive orange 84, which is used to dye silk, cotton, other viscous fabrics, by up to 87%. Complete decolorization was seen within 24 hours after the MS Broth was supplemented with yeast extract (0.1%w/v) and reactive orange 84 (1000mg/lit) (Basutkar et al., 2019).

Because it serves as a rich source of carbon and nitrogen for bacteria, bacterial biomass is also regarded as a good biosorbant material for the biodegradation of textile dye. The mechanism involves a reaction between a dye molecule and either living or dead biomass (Lellis et al, 2019) (Table 5).

Table 5: Some bacterial strain with dye degraded.

S. No.	Bacterial strain	Name of the dye degraded	Reference
1	*Acinetobacter baumanii YNWH226*	Congo red (98.62% of dye with conc. 100mg/lit)	Mervate et al., 2017
2	*Acinetobacter calcoaceticus*	Direct Brown MR (88%)	Ghodke et al., 2009
3	*Aermonashydrophila*	Reactive Red 198 (60.2%) and Reactive Red 141 (100%)	Saratale et al., 2011
4	*Bacillus cereus*	Reactive orange 84 (87% at pH 8.0 and temp. 37°C	Basutkar et al., 2019
5	*Bacillus cereus MAM-B11*	Isma fast (95.3%) and Jakazol black (93.4%)	Mervate et al., 2017
6	*Bacillus cereus MAM-B22*	Isma fast (93.3%)	Mervate et al., 2017
7	*Bacillus fusiformis KMK5*	Disperse blue 99 and Acid Orange 10 (110%)	Kolekar et al., 2008
8	*Bacillus pumilus*	Reactive red 11 (96%), Reactive Blue 171 (95%) and Reactive Brilliant Blue (91%)	Kumar et al., 2011
9	*Bacillus subtilis*	Crystal Violet (by degradation mechanism)	Kochher et al., 2020
10	*Bacillus subtilis*	Congo Red (82.26% under static condition of incubation and 95.67% at pH 8 and temp. 37°C	Kumar et al., 2011
11	*Bacillus subtilis*	Acid Blue 113	Sudha et al., 2014
12	*Bacillus subtilis*	Fast Red (99%)	Kumar et al., 2011
13	*Bacillus wiehenstephanesis RI12*	Congo red (by biosorption)	Singh et al., 2017
14	*Corynebacterium glutamicum*	Reactive Red 4 (by biosorption)	Singh et al., 2017
15	*Desulfovibriodesulfuricans*	Reactive Orange 96 and Reactive red 120 (95%)	Saratale et al., 2011
16	*Enterobacter agglomerans*	Methyl Yellow	Shah et al., 2014
17	*Enterococcus faecalis YZ66*	Direct Red 81 (100%)	Singh et al., 2017
18	*Enterobacter sp.*	Reactive Red 195	Sudha et al., 2014

19	*Escherichia coli JM109*	Direct Blue 71 (110%)	Saratale et al., 2011
20	*Galactomyces sp.*	Amido Black (81.43% at pH 7-8 and temp. 30-37^{o}C)	Kumar et al., 2018
21	*Pseudomonas aeruginosa*	Reactive Blue (48%)	Mani et al., 2019
22	*Pseudomonas sp.*	Remazol Black (75%)	Mani et al., 2019
23	*Pseudomonas otidis WL-13*	Malachite green and Brilliant Green (more than 95%)	Mani et al., 2019
24	*Pseudomonas luteola*	Reactive Red 22 (conc. 200-600mg/lit)	Saratale et al., 2011
25	*Sphingomonasxenophaga*	Mordant Yellow 3	Alabdrabam et al., 2014

Fungal degradation of azo dyes

Numerous organic substances, including dyes and dye effluent, can be broken down by filamentous fungi, which are found all over the natural world. Due to the lignolytic enzymes on fungi, a wide variety of these substances can be broken down by them (Saratale et al., 2011).

Due to their non-specific enzyme system, white rot fungus produces lignin peroxidase, manganese peroxidase, and laccase (lignolytic enzymes) that can degrade aromatic components of colours (Jhadav et al., 2016). When nutrients (carbon, nitrogen, and sulphur) are insufficient, it has been discovered that fungus facilitates the decomposition of complex organic materials. Fungi subsequently utilise these organic molecules as a source of energy and nutrition needed for growth. Adsorption or enzymatic processes, which depend on a variety of variables including culture conditions (particularly those linked to nitrogen limitation), the carbon source, duration, pH, agitation, temperature, oxygen supply, additives, and salts, are both used by fungi to remove colour (Singh et al., 2017).

One of the main methods of decolorization displayed by fungi is the absorption of dyes to the microbial cell surface. Acid green 27, Acid violet 7, and Indigo carmine dye adsorption was seen in both living and dead Trametes versicolor. The interaction between the surface of biomass and the dye molecules, which allows binding and biosorption, is caused by the phosphate and carboxyl groups, which come from gluconic acid and are responsible for the negative charge, and the amino group, which comes from chitosan and is responsible for the positive charge (Lellis et al., 2019)(Table 6).

Table 6: Various fungal species and dye degraded by them

S. No.	Fungal species	Name of dye	References
1	Aspergillus niger	Congo Red (99%)	Singh et al., 2017
2	Aspergillus ochraceus NCIM-1146	Reactive blue 25	Sudha et al., 2014
3	Bjerkanderaadusta	Reactive Violet 5, Reactive black 5 and Reactive Red 198	Bumpus et al., 2003
4	Coriolus versicolor	Acid Orange II (85%)	Singh et al., 2017
5	Funaliatrogii	Astrazon Red FBL	Bumpus et al, 2003
6	Geotrichumsp	Reactive Black 5, Reactive Red 158 and Reactive Yellow 27	Sudha et al, 2014
7	Pleurotusostreatus	Methyl Orange	Bumpus et al., 2003
8	Pleurotuseryngii F032	Reactive Black 5	Singh et al., 2017
9	Phanaerochaetechrysoporium	Orange II	Shah et al, 2014
19	Phanaerochaetechrysoporium	Direct Red 180	Singh et al., 2017
11	Pleurotusostreatus	Congo Red	Mani et al., 2019
12	Trameteshirsuta	Amarnath and Remazol Black B	Bumpus et al., 2003
13	Trichosporonbeigelii	Navy Blue HER (100%)	Jamee et al., 2019
14	Trichoderma sp.	Malachite Green (100%)	Mani et al., 2019
15	Shewanella sp. NTOVI	Crystal Violet	Shah et al., 2014

Algal degradation of azo dyes

Algae are regarded as powerful biosorbents because they can grow in both fresh and salt water, have a sizable surface area, and have a high binding affinity. Because they require no nutrients, can be stored and utilised for a longer period of time, and can be renewed with the aid of organic solvents or surfactants, dead algal cells are favoured over living cells as biosorbents (Singh et al., 2017) (Table 7).

According to some researchers, azo dyes can be broken down into their respective aromatic amines by algae like Oscillatoria, Chlorella pyrenoidosa, and Chlorella vulgaris before being further broken down into simpler compounds or CO2. Because of this ability to degrade dye wastewater, these algae can be used to treat rivers and lakes (Zhou et al., 2013; Saratale et al., 2011).It was discovered that some algae could use aniline, a byproduct of azo dye degradation, and that some green algae from the Spirogyra genus may be used as viable biomaterials for the biodegradation of textile effluent (Mostafa et al., 2009).

Table 7: Some algal species and the dyes degraded by them.

S. No	Algal Species	Name of the dye degraded	Reference
1	Anabaena	Blue Drin dye (81%)	Dellamatrice et al., 2017
2	Chara vulgaris (macroalgae)	Congo red dye (95% at 33-40°C and pH 7-8)	Mahajan et al., 2013
3	Chlorella vulgaris	Methyl Red	Mostafa et al., 2009
4	Cosmariumsp	Triphenylmethane dye and Malachite green	Shah et al., 2014
5	Nostocmuscorum	Tartazine	Omar et al., 2008
6	Nostoclinckia	Methyl Red	Mostafa et al., 2009
7	Oscillatoriarubescens	Methyl Red	Mostafa et al., 2009
8	Phormidiumauumnale UTEX1580	Indigo	Dellamatrice et al., 2017
9	Phormidiumvalderianum	Acid Rd, Acid red 119 and Direct Black 155 (90%)	Dellamatrice et al., 2017
10	Scenedesmusbijugatus	Tartazine	Omar et al., 2008
11	Spirogyra rhizopus	Acid Red 247	Shah et al., 2014
12	Synechoccussp	Remazol Brilliant Blue, Indigo and sulphur Black	Dellamatrice et al., 2017

Biodegradation of azo dye using yeast

Fast-growing yeast can withstand unfavourable conditions like low pH, high salt content, and highly concentrated organic wastes, like that seen in dye effluent (Dias et al., 2010).Yeast may break down azo dyes using two different processes: enzymatic activity and absorption. The non-enzymatic process is the primary mechanism (Singh et al., 2017; Dias et al., 2010). The two non-enzymatic processes are biosorption and bioaccumulation. While biosorption refers to the passive uptake of chemicals by non-viable or viable biomass (in this case, dead yeast), bioaccumulation refers to the active uptake of substances and the subsequent biotransformation of dye through redox reactions by yeast metabolism (Dias et al., 2010).

The two steps of the yeast-mediated enzymatic processes of degradation are the reduction reaction and the oxidation reaction. The reduction reaction involves the lignolytic enzymes laccase, manganese dependent peroxidase, and lignin peroxidase (Dias et al., 2010) (Table 8).

Table 8: Yeasts that contribute to the breakdown of dyes.

S. No.	Yeast	Azo Dye degraded	References
1	*Candida albicans*	Direct Violet 51	Lellis et al., 2019
2	*Candida zeylanoides*	Methyl Orange	Dias et al., 2010
3	*Debaryomycespolymorphus*	Reactive Black 5, Reactive Yellow	Dias et al., 2010
3	*Khuyveromycesmarxianus*	Remazol Black B	Shah et al., 2014
4	*Trichosporonmultisporum*	Reactive Red 141	Dias et al., 2010
5	*Sachharomyces cerevisiae MTCC46*	Methylene Red	Shah et al., 2014

Degradation of azo dye using consortia

Single microbial strains or plant species can be used to biodegrade textile dyes, however, sometimes it is more difficult to biodegrade the metabolite that is created during destruction than it is to biodegrade the parent colour. Additionally, because each strain is distinct to a given colour, researchers are working to develop more effective processes due to the varying composition of wastewater produced by textile dyes. The cumulative activity of dye-degrading enzymes makes it feasible to completely degrade azo dye, indicating the importance of putting together microbial consortia. Consortia have several advantages over using a single strain, including the fact that

each degrading enzyme attacks the dye molecule from a different angle and that metabolites produced by one strain can be consumed by another for additional degradation, which in some cases results in the mineralization of azo dyes (Saroj et al., 2015)

Degradation of azo dye by plant bacteria consortium

A combination of microbes and plants can be used to remove the colour from an azo dye. Remployed a consortia of *Pseudomonas putida* bacteria and *Portulaca grandiflora* plants, it was discovered that the plant and bacterial consortium decolored the dye after 72 hours, while in vitro cultures of *P. grandiflora* and *P. putida* decolored the dye independently in 96 hours, decolorizing 92% and 81% of it, respectively, of the sulfonated diazo dye Direct Red 5B. In *P.* grandiflora roots, activity of several enzymes, including lignin peroxidase, DCIP (2,6-dichlorophenol-indophenol) reductase, tyrosinase, and riboflavin reductase, was seen during dye decolorization, whereas in P. putida, activity of laccase, veratryl alcohol oxidase, and 2,6-dichlorophenol in cells. In order to validate the breakdown of the dye, the metabolites were examined using UV-Vis spectroscopy, High Performance Liquid Chromatography, and Fourier Transform Infrared Spectroscopy. A phytotoxicity investigation also demonstrated that the metabolites were not hazardous. The removal of textile dyes from soil and wastewater can therefore be accomplished with the use of such a mixture in an effective and efficient manner (Khandare et al., 2011).

Khandare et al. (2011) used a consortium of the plants *Zinnia angustifolia* and *Exiguobacterium aestuarii* to increase the degradation of the dye Remazol Black B, and they discovered that it was more effective than using just the plant and bacteria separately. After biodegradation, the phytotoxicity of the metabolites was investigated and analysed using UV-Vis spectroscopy, High Performance Liquid Chromatography, and Fourier Transform Infrared Spectroscopy. The entire process was significantly influenced by enzyme activity. The activity of lignin peroxidase, laccase, DCIP reductase, and tyrosinase was significantly increased in the roots of *Z. angustifolia*, whereas veratryl alcohol oxidase, azoreductase, and DCIP reductase were discovered in *E. aestuarii*.

Degradation of azo dye by fungal consortium

In a related work, used a fugal consortium made up of *Penicillium oxalicum, Aspergillus niger,* and *Aspergillus flavus* to decolorize and degrade azo dye quickly and effectively. Acid Red 183, Direct Red 75, Acid Blue 161, Acid Red 88, Acid Blue 45, Reactive Black 5, and Direct Blue 15 were examined for degradation (Saroj et al., 2015). At lower concentrations, the consortium was shown to almost totally decolorize three dyes: Acid Red 183, Direct Red 75, and Direct Blue 15. It was discovered that the consortium is capable of decomposing dye-containing simulated textile effluent with a concentration range of 100–500 mg/lit. The main enzyme reported to be involved in this biodegradation is manganese peroxidase.

Degraded products were isolated and examined using UV-Vis and FTIR spectroscopy after biodegradation. The development of the yeast *S. cerevisiae* was used as the basis for a toxicity test based on the inhibitory effects of azo dye and their metabolites following degradation, which demonstrated an almost complete reduction in toxicity (Table 9).

Table 9: A number of additional microbial consortiums and dyes that decomposed on their own.

S. No.	Consortium	Species involved	Name of the dyes	Reference
1	Fungal	*Bacillus sp., Alcaligenes so. And Aeromonas sp*.	Acid Violet 17(86%) Acid Blue-15(85%) Crystal Violet (82%) Malachite Green (82%) Brilliant Green (85%)	Sharma et al., 2004
2	Bacterial	*Bacillus flexus NBN2, Bacillus cereus AGP03, Bacillus cytotoxicusNVH, Bacillus sp. L10*	Direct Blue 151 (97.57%) Direct Red 31 (95.25%)	Lalnun-hlimi et al., 2016; Mani et al., 2019
3	Fungal-Bacterial	*Aspergillusochra-ceus NCIM-1146 (fungi)* and *Pseu-domonas sp. SUK1 (bacteria)*	Rubine GFL (95%)	Lade et al., 2012
4	Bacterial-Fungal	*Pseuodomonas sp. SUK1* (bacteria) and *Aspergillus ochraceus NCIM-1146* (fungi)	Reactive Navy Blue HE2R (90%)	Kadam et al., 2011
5	Fungal-bacterial	White rot fungus and *Pseudomonas aeuroginosa*	Direct Fast Scarlet 4BS	Mani et al., 2019

Future prospects

Due to the risky and harmful nature of azo dyes, numerous studies and tests have been conducted to identify the best alternative. Many nations, including India, are now engaged in similar activities, but using microbes for biodegradation would likely be a superior alternative

in the future. It is still being researched how best to use microorganisms including bacteria, algae, fungi, and plants for bioremediation. To improve any microbe's ability to degrade materials, more research on microorganisms is necessary. Various microorganisms have been isolated that are capable of degrading azo dyes and are been used for the same purpose and it is required to study and isolate more microorganisms with high capability and enhanced properties. Research should be conducted to find various cheaper supplementary sources for degradation. The complexity of the complete degradation of azo dyes indicates the requirement for more research. Considerable progress has been marked in molecular cloning and characterization of azoreductase from different microorganisms (Chen et al., 2006). Numerous genetically altered microbes have been created and are currently being employed to biodegrade azo dyes. To completely degrade dyes, genetically engineered microbes (GEMs) or genetically modified organisms (GMOs) are crucial.

These GEMs have been successfully used in experiments to demonstrate their strong ability to cause the degradation of a variety of contaminants under predetermined conditions (Joutey et al., 2013). Genes from one species can be transferred to another, or genes can be altered, to create GMOs. GMOs including *Sphingomonas desiccabilis, Escherichi coli, Bacillus idriensis, Pseudomonas putida, Mycobacterium marinum, etc.,* which were transplanted into other species, were created using functional genes from numerous bacterial strains. To detect the expression of genetically modified genes, a variety of instruments and methods are available, including single-stranded conformation polymorphism, randomly amplified polymorphic DNA (RAPD), polymerase chain reaction (PCR), etc. By introducing the azoreductase gene from *Bacillus latrosporus RRK1 to E. Coli* DH5a and plasmid paZR-SS125, numerous genetically modified organisms were created, including Escherichia coli SS125 for the breakdown of Remazol Red dye.Another illustration is the genetically altered *E. coli JM109* (pGEX-AZR) strain, which was created to decolorize the dye Direct Blue. This strain was created by inserting the azoreductase gene into the expression vector pGEX4T-1, which was then transformed into *E. coli JM109* under the control of the lac operon.

The Himalayas cut across Uttarakhand, a state in northern India, and it is a popular destination for Hindu pilgrims. Due to the numerous Hindu temples and pilgrimage sites spread throughout the state, it is also known as Devbhumi. With a population of 101.17 lakh and a total area of 53,483 km2, it contains 1802 heavy and medium enterprises, 25,294 small-scale industries, and 54,047 handicraft units in all. The effluent was examined for the presence of a number of heavy metals, including Zn, Cd, Mn, Cu, Pb, Ni, and Fe, as well as for physicochemical parameters like pH, turbidity, colour, suspended solids, alkalinity, hardness, chloride, and sulphate, as well as for biochemical and chemical oxygen demand (BOD and COD). The effluent was a dark brownish-black colour with a pungent smell, and the concentration of COD was a very high 2461.5448.45mg/lit. Sulphate concentration of 6620.83 7.22 mg/lit was found. According to this study, effluent from the textile sector was severely contaminated and needed to be cleansed before being released into the environment. Thus, before being released, the polluted dye effluent needs to be treated, which can be accomplished by the biological processes mentioned above.

Plant parts such as the root, rhizomes, stem, bark, leaves, flowers, fruits, seeds, or even the entire plant are used to make dyes. In rare situations, resins and gum are also used to make dyes. *Curcuma domestica, Rubiacordifolia (roots), Acacia catechu, Aesculusindica (stem and bark), Dahlia, Hibiscus, Mirabilis (floral), Acacia nilotica, Emblica officinalis (fruit),* etc. are a few of the species that were gathered. There are over 4048 angiosperm and gymnosperm species in Uttarakhand, distributed among 1198 taxa and 192 families. Of these, 116 or so species are unique to Uttarakhand, meaning that their geographic range is constrained to the state's borders.

Being such a rich state in flora, it is advantageous for the residents of Uttarakhand to have access to a number of dye production facilities and shift away from the production of colours from synthetic sources, particularly the azo dyes.In order to develop more advanced efficient, effective, and affordable options for the biodegradation of azo dyes, it is necessary to thoroughly investigate these organisms that are capable of degrading colours and their functions with reference to azo dyes. Future research should focus on the decline in the limiting factors that are influencing the bioremediation process. Various biological pathways involved in degradation of dyes by these microorganisms should be considered to achieve enhanced properties. There are many microorganisms and GMOs which utilize the dye but result in the production of less toxic metabolites which should be studied and considered.

Declaration: *We also declare that all ethical guidelines have been followed during this work and there is no conflict of interest among authors.*

REFERENCES

Alabdrabam, W.M. and Bayati, M. (2014). A review on azo

dye biodegradation. *Int.J.Environ.Eng.Nat. Resour.* **1:**179-189.

Arora, N., Fatima, T., Mishra, I., Verma, M., Mishra, J. and Mishra, V. (2018). Environmental sustainability: Challenges and viable solution. *Environmental Sustainability*. **1(11):** 309-340.

Basutkar, M.R., Mala, M., Shubhashchandra, M. and Shivannavar, C.T. (2019). Decolourization of Reactive Orange 84 Azo dye by *Bacillus cereus* CMGS-5. *Int.J.Adv.Res.Biol.Sci*. **6(5):** 98-109.

Benkhaya, S., Rabet, S., Harfi, A.E. (2020). A review on classifications, recent synthesis and applications of textile dyes, Inorganic chemistry communication. *Inorganic Chemistry Communications*. **115:**107891.

Bumpus, J.A. (2020). Biodegradation of azo dyes by fungi. *Biotech.* **10(9)**:384.

Chandanshive, V.V., Rane, N.R., Avinash, R., Swapnil, M., Jeon,B. and Govindwar, S.P. (2016). Efficient decolourization and detoxification of textile industry effluent by Salviniamolesta in lagoon treatment. *Environ Res*. **150:**88-96.

Chavan, R.B. (2011). Handbook of textile and industrial dyein. *Textiles*. **1:**446-465

Chen, H. (2006). Recent advances in azo dye degradating enzyme research. *Current protein and peptide science*. **7(2):**101-111.

Christie, R.M. (2001). Colour Chemistry. *The Royal Society of Chemistry*. 978-0-85404-573-0

Chung, K.T. (2016). A review on Azo dye and human health. *Journal of Environmental Science and Health*. **34(4):**1236602.

Dellamatrice, P.M., Alberto, L., Moraes, B., Fiore, F.M. and Monteiro, R. (2017). Degradation of textile dyes by cynabacteria. *Braz J Microbiol.* **48(1):**25–31.

Dias, A.A., Lucas, M.S., Sampaio A., Peres, J.A. (2010). Decolorization of azo dyes by yeast. *Biodegradation of azo dye*. 183-193.

Ghodke, G.S., Tekle, A.A., Jhadav, J.P. and Govindwar, S.P. (2009). Potential of Brasssicajuncea in order to treat textile effluent contained site. *International Journal of Phytoremediation.* **11(14):**297-312.

Haddaji, D., Zeineb, G., Hamed, K.B. and Bousselmi, L. (2019). A re-circulating horizontal flow constructed wetland for the treatment of synthetic azo dye at high concentrations. *Environmental Science and Pollution Research*. **26:**13489–13501.

Hassaan, M.A. and Nemr, A. (2017). Health and Environmental impact of dyes. *American Journal of Environmental Science and Engineering*. **1(3):**64-67.

Hunger, K. (2003). Industrial Dyes: Chemistry, Properties, Applications. *Weinheim, Cambridge*. 14-35.

Jamee, R. and Siddique, R. (2019). Biodegradation of synthetic dyes of textile effluent by microorganisms: an environmentally and economically sustainable approach. *European Journal of Microbiology and Immunology.* **9(4):**1-5.

Jhadav, I. and Jadhav, K. (2016). Microorganism based treatment of azo dyes. *Journal of Environmental Science and Technology*. **9(2):**188-197.

Joutey, N.T., Bahafid, W., Ghachtouli, N. (2013). Biodegradation: Involved microorganisms and genetically engineered microorganisms. *Biodegradation - Life of Science.* 290-320

Kadam, A.A., Telke, A.A., Jagtap, S.S. and Govindwar S.P. (2011). Decolorization of adsorbed textile dyes by developed consortium of *Pseudomonas* sp. SUK1 ans *Aspergillus ochraceus* NCIM-1146 under solid state fermentation. *Journal of Hazardous Material*. **189:**486-494.

Kagalkar, A.N., Jagtap, U.B., Jadhav, J.P., Bapat, V.A. and Govindwar S.P. (2009). Biotechnological strategies for phytoremediation of the sulphonated azo dye Direct Red 5B using Blumeamalcolmii Hook. *Bioresour Technol*. **100(18):**4104-10.

Kagalkar, A.N., Jagtap, U.B., Jadhav, J.P., Govindwar S.P. and Bapat, V.A. (2010). Studies on phytoremediation potentiality of Typhonium flagelliforme for thedegradation of Brilliant Blue R. *Planta*. **232(1):**271-285.

Khandare, R.V., Kabra, A.N. Awate, A.V. and Govindwar, S.P. (2013). Synergistic degradation of diazo dye Direct Red 5B by *Portulaca grandiflora* and *Pseudomonas putida*. *Int.J.Environ.Sci. Technol*. **10:**1039–1050.

Khandare, R.V., Rane, N.R., Waghmode, T.R. and Govindwar, S.P. (2011). Bacterial assisted phytoremediation for enhanced degradation of highly sulfonated diazo dye. *Environmental Science and Pollution Research*.**19(5):**1709-18.

Kochher, S., Kumar, S. and Kumar, J. (2020). Microbial decolorization of crystal violet by *Bacillus subtilis*. *IOSR Journal of Environmental Science, Toxicology and Food Technology*. **14(2):**29-34.

Kolekar, Y.M., Pawar, S.P., Gawai, K.R., Lokhande, P.D., Shuoche, Y.S. and Kodam, K.M. (2008). Decolourization and degradation of Disperse Blue 79 and Acid Orange 10 by *Bacillus fusiformis* KMK5 isolated from the textile dye-contaminated soil. *Bioresour Technol*. **99(18):**8999-9003.

Kumar, A. and Sawhney, R. (2011). Identification of *Bacillus subtilis* subsp. subtilis, "RA-29" a congo red degrader using 16S rDna sequencing. *Researcher*. **3(12):**18-22.

Kumar, A., Sharma, R. and Sawhney, R. (2018). Enzyme mediated Amido Black decolourization by soil-borne RS-II strain isolated from an industrial town. *Nature and Science*. **9(11):**125-129.

Lade, H.S., Waghmode, T.R., Kadam A.A. and Govindwar S.P.

(2012). Enhanced biodegradation and detoxification of disperse azo dye Rubine GFL and textile industry effluent by defined fungal-bacterial consortium. *International Biodeterioration & Biodegradation*. 72:94-107.

Lalnunhlimi, S., Wamy, V.K. (2016). Decolourization of azo dyes (Direct Blue 151 and Direct Red 31) by moderately alkaliphilic bacterial consortium. Braz.J.Microbiol. **47(1):**39-46.

Lellis, B., Fávaro-Polonio, C. Z., Pamphile, J. A., & Polonio, J. C. (2019). Effects of textile dyes on health and the environment and bioremediation potential of living organisms. Biotechnology Research and Innovation, 3(2), 275-290.

Mahajan, P. and Kaushal, J. (2013). Degradation of Congo Red in aqueous solution by using Phytoremediation potential of Chara vulgaris. *Chitkara Chemistry Review.* **1(1):**67-75.

Mani, A.K. and Hammed, S.A. (2019). A review on Improved Bacterial-Fungal consortium as an alternative approach for enhanced decolourisation and degradation of azo dyes. *Nature Environment and Pollution Technology*. **18(1):**49-64.

Mervate, A., Abo-State, Y.E., Hebatallah, H. (2017). Decolorization of Congo red dye by bacterial isolates. *J.Eco.Heal.Env*. **5(2):**41-48.

Mostafa, M., Gharieb, M.M. and Souod G.W. (2009). Biodegradation of dyes by some green algae and cyanobacteria. *International Biodeterioration & Biodegradation*. **63(6):**699-704.

Omar, H.H. (2008). Algal decolourizaion and degradation of monoazo and diazo dyes. *Pakistan Journal of Biological Sciences.* **11(10):**1310-1316.

Pokharia, A. and Ahluwalia S.S. (2015). A review on Toxicological effect of textile dyes and their metabolites. *Progress and Communication in Sciences*.**1:**1-11.

Priyanka, J.V. and Krishnaswamy, V.G. (2019). Phytoremediation of mixed reactive azo dyes in contaminated water by Ceratophylumdemersum and its toxicity analysis on other life forms. *Journal of Environmental Sciences*. **1(2):**16-25.

Puvaneswari, N., Muthukrishnan, J. and Gunasekaran, P. (2006). A review on toxicity assessment and microbial degradation of azgo dyes. *Indian Journal of Experimental Biology*. **44:** 618-626.

RLM, A. (1971). The Chemistry of Azo Dyes, in colour chemistry, Studies in modern chemistry. *Springer Boston*. 21-36.

Saratale, R.G., Saratale, G.D., Chang, J.S. and Govindwar S.P. (2011). A review on Bacterial decolourization and degradation of azo dyes. *Journal of the Taiwan Institute of Chemical Engineers*. **42(1):**138-157.

Saroj, S., Dubey, S., Agarwal, P., Prasad, R. and Singh R.P. (2015). Evaluation of the efficiency of a fungal consortium for degradation of azo dyes and simulated textile dye effluent. *Sustainable Water Resources Management*. **1**:233–243.

Shah, K. (2014). Biodegradation of azo dye compounds. *Toxicol Int.* **21(2):**160–166.

Sharma, D.K., Saini, H.S., Singh, M., Chimni, S.S. and Chadha, B.S. (2004). Isolation and characterization of microorganisms capable of decolorizing various triphenylmethane dyes. *Journal of Basic Microbiology*. **44:**59-65.

Singh, P.K. and Singh, R.L. (2017). A review on Bio-removal of azo dyes. *International Journal of Applied Sciences and Biotechnology.* **5(2):**108.

Singh, R.P., Singh, P.K and Singh R.L. (2017). Present status of Biodegradation of textile dyes. Current Trends in Biomedical Engineering & Biosciences. *Juniper Publishers Inc*. 3(4):66-68.

Sudha, M., Saranya, A., Selvakumar, G. and Sivakumar, N. (2014). A review article on Microbial degradation of azo dyes. *Int.J.Curr.Microbiol.App.Sci*. **3(2):**670-690.

Sureshvarr, K., Bharathiraja, B., Jayakumar, M. and Jayamuthunagai, J.L. (2010). Removal of azo dye compounds from paper industries wastes using phytoremediation methodology. **8:**687-700.

Tan, K., Morad, N. and Oo, J.I. (2016). Phytoremediation of Methylene Blue and Methyl Orange usng *Eichhornia crassipes*. *International Journal of Environmental Science and Development*. **7(10):**724-728.

Tehrani-Bagha, A.R., Holmberg, K. (2013). Solubilization of Hydrophobic Dyes in Surfactant Solutions. *Materials*. **6**:580-608

Telke, A.A., Kagalkar, A.N., Jagtap, U.B., Desai, N.S., Bapat, V.A., and Govindwar S.P. (2011). Biochemical characterization of laccase from hairy root culture of *Brasiccajuncea L*. and role of redox mediator to enhance its potential for the decolourization of textile dyes. *Planta*. **234(6):**1137-1149.

Uruj, T and Khan, U.H. (2015). Phytoremediation: Potential flora for synthetic dyestuff metabolism. *Journal of King Saud University Science.* 28(2):119-130.

Vanhulle, S. (2004). SOPHIED Novel Sustainable Bioprocesses for European Colour Industries. *Environmental Science and Technology.* **42(2):**584-589.

Wanyonyi, W.C. and Onyari, J.M. (2014). Adsorption of Congo Red dye from aqueous solution using roots of Eichhorniacrassipes: Kinetics and Equilibrium studies. *Energy Procedia.* **50:**862-869.

Zhou, X. and Xiang, X. (2013). Effect of different plants on azo-dye wastewater bio-decolorization. *Procedia Environmental Sciences.* 18:540-546.