




# Bioremediation: An Economical Approach for Treatment of Textile Dye Effluents

Khirabdhi Tanaya · Anamika Kumari ·  
Anil Kumar Singh · Durgeshwer Singh 

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**Abstract** Dye is a substance that imparts colour onto textiles, fabrics, paper, leather, etc. and is not altered by washing, heating, and light. Dyes were extracted from plants, animals, and minerals until synthetic dyes came to the market, as synthetic dyes were more stable, readily available, and inexpensive. Despite being extremely important to the economy, they have been among the most significant global polluters. Textile dye industries have become the chief source of water pollution, with their effluents increasing the turbidity of water and reducing photosynthesis and dissolved oxygen levels. This leads to significant damage to aquatic biodiversity, threatening the survival of many species. Synthetic dyes have carcinogenic, mutagenic, and genotoxic effects on animals and human beings, posing a severe health risk. The degradation of dyes is essential for ensuring the sustainability of the environment for future generations. The traditional physicochemical means of dye treatment are not convenient because of the high solubility in water, cost of method utilisation and

other disadvantages related to these techniques. To overcome the disadvantages of physicochemical treatment, biological methods or bioremediation can be used as an alternative. The objective of this review article is to study the mechanisms involved in the degradation of textile dyes by bacteria to obtain sustainable, economically and ecologically sound solutions for dye treatment. This paper will explain the various types of natural and synthetic dyes utilised in the textile industry, their chemistry, and how they affect water and soil ecosystems. The treatment of textile dye by various physicochemical methods and their advantages and disadvantages are also discussed. In bioremediation, the microorganisms utilize organic pollutants as a source of food or energy. Bioremediation uses biosorption and enzymatic activity for dye degradation, which does not disturb natural processes and is thus sustainable. The microorganisms secrete crucial extracellular and intracellular enzymes that carry out decolourisation and degradation through a series of events, which include hydrolysis, acidogenesis, and methanogenesis. We will discuss how aerobic and anaerobic microorganisms degrade these textile dyes through the process of biodegradation and bioaugmentation and how this technology provides a clean and eco-friendly method for removing textile dyes.

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K. Tanaya · A. Kumari · D. Singh (✉)  
Department of Botany, School of Life Sciences, Mahatma  
Gandhi Central University, Motihari, Bihar 845401, India  
e-mail: durgeshwersingh@mgcub.ac.in

A. K. Singh  
Department of Chemistry, School of Physical Sciences,  
Mahatma Gandhi Central University, Motihari,  
Bihar 845401, India

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## 1 Introduction

Dye is a substance that imparts colour that stains textiles, fabrics, paper, leather, etc. so that the stain is not altered by washing, heating, and light. Dyes have been a major commodity of commerce throughout history. The dyeing method is around four thousand years old; the evidence was provided by a dyed piece of cloth in Egyptian tombs. Dyes were extracted from plants (vegetables, lichens, berries, and roots), animals (insects and molluscs), and minerals (vermillion, Chrome yellow, Bistre) until synthetic dyes came to the market as synthetic dyes were more stable, readily available and inexpensive.

The first synthetic dye, named 'mauve', was discovered in 1856 by William H. Perkins in an attempt to achieve the synthesis of quinine, a drug for the treatment of malaria (Benkhaya et al., 2020). Mauve was made of phenazene derivatives components A and B. Component A consist of one *o*-toluidine, one *p*-toluidine, and two aniline molecules whereas component B consist two *o*-toluidine, one *p*-toluidine, and one aniline molecules (Nagendrappa, 2010). Mauve triggered the discovery of better dyes which led to a commercial revolution and these synthetic dyes were exploited for a long time. Soon synthetic dyes were eagerly accepted for usage in the textile industries.

In Latin, the word 'textile' is taken from *texere*, meaning weave. Globally, The textile industry generates around 1 trillion dollars and contributes about 7% of the total world exports and 27% of Global export income (Desore & Narula, 2018). Dyes and textiles showed a peak as an influential market in Asia-Pacific with 41.9% belonging to them which is then followed by North and South America, Western Europe, the Middle East, and other regions (Samsami et al., 2020). Historically, the origin of dyes in India dates to the Indus Valley Civilisation. With such a historical background and a series of advancements, India is one of the biggest producers of textiles and apparel (Kar, 2015). In India, the textile industry is the second largest in employing agriculture and is contributing around 11% to total Indian exports and is estimated to generate US\$ 190 billion by 2025–2026. Approximately 3400 textile units contribute ~4% of the Nation's Gross Domestic Product (GDP). After Bangladesh, India has become the second largest industrial hub for dyeing and clothing industries, i.e., contributing 14% to industrial production, 17% of the

country's exports, and 21% of employment (Sarkar et al., 2017). The diverse phases of manufacturing industrial textiles include pretreatment, dyeing, printing, and finishing operations (Madhav et al., 2018). These processes use a humongous amount of organic and inorganic compounds such as dyes, mordants, fixing agents, salts, etc., which can be potential pollutants (Uddin, 2021). Textile dyeing uses a large amount of water during wet processing, and various compounds that are not fixed persist causing coloured effluents (Madhushika et al., 2020). Despite being extremely important to the global and national economy, they have been proven to be one of the biggest global polluters because of the presence of toxic wastewater, which carries various types of organic and inorganic pollutants.

Incapable and careless processes of dyeing cause 15- 50% of unfixed dye molecules that did not bind to the fabrics and fibers to get mixed with various water bodies and produce water pollution (Singha et al., 2021). The dyes decrease the penetration of light through the water, which affects the growth of aquatic biomes by reducing the rate of photosynthesis, such as algae and other aquatic plants. The aquatic microorganisms in the presence of dye-containing wastewater showed reduced content of chlorophyll and carotenoids and decreased synthesis of protein and essential elements. In other aquatic animals, dye-containing wastewater causes mortality and cytotoxic effects on RBCs. They also interfere with enzymatic activities and decreases the level of carbohydrates, proteins, and lipids in animal tissues. Dyes consumed by fishes get metabolised and form noxious intermediates, which impact both fishes and their predators. When dye-retained fish is consumed, it may cause hypertension, cramps, fever, etc., in humans (Sharma et al., 2022). The untreated water, when used by farmers to irrigate their cultivation, detriments the quality of soil and germination rates of crops (Jiku et al., 2021). The plants grown in these agricultural fields also bioaccumulate the untreated waste such as dyes. The untreated wastewater causes alteration in the chemistry of soil and also interferes with the balance created by soil microflora. Plants show enhanced production of antioxidants and lipid peroxidase. Dye causes damage to roots, fronds, etc., and causes necrosis and chlorosis in plants. Textile dyes can be toxic, cause mutation, and are carcinogenic to humans and other animals. Consumption or breathing in textile dyes

can result in skin rashes and eye discomfort. Reactive dyes could cause many allergic reactions, such as asthma, rhinitis, dermatitis, and conjunctivitis. Harmful substances from textile industries cause hindrance in processes such as ovulation and spermatogenesis in mammals (Al-Tohamy et al., 2022). The azo dyes, which are metabolised by gut microbiota, form intermediates in the form of aromatic amines that are toxic and carcinogenic. Textile dyes also inhibit enzymes such as glutathione reductase. A study investigating the toxicity of textile dyes showed they are responsible for cancer in the human bladder (Tounsadi et al., 2020).

Textile dyes directly or indirectly affect all aspects of the environment, including air, soil, water, plants, ecosystems, etc., as they become recalcitrant and bioaccumulate in different life forms. Textile dyes cause disturbances to the tropic level of the food chain and disturb the ecological balance. Thus, the degradation of textile dyes has become one of the biggest concerns of today's world. Textile dyes can be degraded via many methods, including physical, chemical, and biological methods. However, physicochemical degradation has many drawbacks such as involvement of sophisticated equipments, higher quantity of energy, higher amount of chemicals, and the production of large amounts of sludges and byproducts. They also show a higher persistence of secondary pollutants and are not cost-effective and economical. To overcome these problems, biological methods provide an alternative to degrading the textile dye pollutants. The objective of this review article is to study the mechanisms involved in the degradation of textile dyes by bacteria to obtain sustainable, economically and ecologically sound solutions for dye treatment.

Biological Method, also known as bioremediation, utilises living organisms for degradation and decolourisation of textile dyes and is considered an economically reasonable, green, and cost-efficacious method of biodegradation. Mostly, Bioremediation does not produce secondary pollutants. Some bacteria that are found to decolourise dyes are *Bacillus subtilis*, *Pseudomonas sp.*, *Lactobacillus sp.*, *Staphylococcus sp.*, *Proteus sp.*, *Clostridium sp.*, *Escherichia coli* etc. Physicochemical methods use expensive equipment and chemicals, which makes it impossible for impoverished and small-scale industrial workers to use them. Due to this, they do not treat the effluent

properly, and hence increase the pollution and toxicity of the water. However, the degradation of dyes in effluent is essential for ensuring the sustainability of the environment for future generations. The knowledge regarding the cost-effectiveness of bioremediation will make such workers eager to use the method for the treatment of the effluent. This article provides a wide spectrum of knowledge about various types of bioremediation methods, which is an easy, economical, and green technique for the degradation of textile dye effluents. The microorganisms release various dye degrading enzymes like Azoreductase, Lignin Peroxidases, Laccases, Tyrosinase, Flavin Reductase and NADH-DCIP Reductase. The mechanism by which these enzymes degrade the dyes is also described in this review. This review can provide the foundation for knowledge regarding the usability of bioremediation. Bioremediation is an economical yet effective method for the degradation and decolourisation of dye.

## 2 Water Pollution and Textile Industry

Water consists of 71% of the earth's surface, of which only 2.5% is freshwater. Because of the growing pace of industrialization, wastewater from the industrial sector has caused havoc. Water pollution is referred to as pollution caused by human beings directly or indirectly, which causes deleterious effects on the aquatic environment. Rapid industrialization causes higher production of harmful pollutants such as dyes, heavy metals, aromatic hydrocarbons, etc. (Liong et al., 2021). Contamination of an aquatic environment is caused when a contaminant such as dye is increased above the natural background level. According to a report by the World Bank, dye processing and finishing results in 17% to 20% of water pollution (Sharma et al., 2021). Based on the colour index, around 10,000 types of dyes and 700,000 tonnes of dye are produced in a year (Samsami et al., 2020). Synthetic dyes have carcinogenic, mutagenic, and genotoxic effects, which cause damage to aquatic biota (Sharma et al., 2021). The surface water as well as groundwater vicinage to the textile industries, are heavily polluted and degraded gradually. Industrial effluents pose a threat to the aquatic life of rivers around the industries.

The primary damage attributed by the textile industry to the environment is by discarding untreated effluents into the water bodies that contain large amounts of non-biodegradable textile dyes. Dyes are durable to exposure to sunlight and water and have a half-life of 2–13 years in the environment (Copaciu et al., 2013). The dyes are organic compounds exhibiting high solubility in water making them hard to remove by conventional methods (Hassan & Carr, 2018). These dyes can modify the chemical and physical nature of the environment by deteriorating the water bodies and soil, which are dangerous for microorganisms, animals, and plants. Textile effluents have significant consequences on the Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Total Dissolved Solute (TDS), Total Suspended Solids (TSS), and pH of the aquatic reservoir. Aquatic vertebrates such as fishes and tadpoles take up these aquatic pollutants through their gills which causes homeostatic disorders that affect their life performances (Pereira & Alves, 2012). They cause acute ailments such as renal damage, hypertension, and cramps to the aquatic biota which uptake them in their diet (Samchetshabam et al., 2017). Textile effluents have an inhibitory effect on the green aquatic biome, they affect the rate of photosynthesis and biochemical parameters such as growth, pigment content, protein, minerals, and other nutrients. Dyes cause turbidity in waterbodies that leads to the formation of film that causes low penetration of sunlight. It adversely deteriorates phytoplankton, algae, and other aquatic organisms (Pereira & Alves, 2012). Water from polluted rivers, when used in irrigation, contaminates the soil and moves to the food chain via plants that are grown on those soils. The toxic compounds reach to the groundwater and plants grown on such soil, accumulate, posing risk to the consumers such as herbivores and human beings (Golui et al., 2022). The toxic compound in the soil also causes inhibition of plant growth by causing oxidative stress and dropping the rate of photosynthesis, protein content, and rate of carbon dioxide assimilation (Slama et al., 2021). Dyes can also cause acute toxic disorders to human health by means of respiratory disorders, skin sensitization, and allergic reactions such as rashes, redness, and circulatory problems.

### 3 Mechanism of Colour Production in Dyes

Unsaturated complex organic substances that absorb light give colour in the visible region and have chromophores that can absorb part of the visible spectrum are called dyes. The colour produced by the dye is due to the fraction of light that reflects from the fabric. Dyes consist of conjugated double bonds which have a chemical structure favourable for light absorption. They can be distinguished on the basis of the chemical groups that absorb light radiation in the visible spectrum (400–700 nm). Certain groups of atoms, which are called chromophores, are responsible for the alteration of white light into coloured light via selective absorption of light. Dye has the capability of absorption of certain luminous radiations, thus reflecting complementary colours (Benkhaya et al., 2020). A German Chemist, Otto Witt, in 1876, noticed that the colour of the dye is due to chromophores, which are groups containing multiple bonds. As the number of chromophores in the dye increases, the colour produced by the dye intensifies. Some examples of chromophores are nitroso group, acetylenic bond, carbonyl group, nitro group, ethylenic bond, etc. There is the presence of another group called auxochromes in dye molecules that are not directly responsible for colour production, but their mere presence deepens the colour produced. Some examples of auxochromes are -I, -OH, -COOH, -NH<sub>2</sub>, 1,3-Dinitronaphthalene, -NHR, -Br -NR<sub>2</sub>, -Cl (Kishor et al., 2021).

### 4 Dyes used in Textile Industries

Dyes are substances that interact with the introduced medium by dissolving and dispersing themselves to produce colours that can be classified as natural and synthetic dyes (Boudechiche et al., 2019). Colourful Dyes have become one of the basic commodities, which is being used in clothes, paints, foods, etc. Dyes have great chemical stability, are easy to synthesise, and produce a variety of colours. They have two properties: to produce colour and get fixed to solid substrates such as textiles (Benkhaya et al., 2020). Until the emergence of synthetic dye, all the dyes used were of natural origin and extracted from vegetables, trees, lichens, insects, molluscs, etc. Even after 1000 years of dye utilisation, few natural dyes

were found. Whereas, after the emergence of Mauve, scientists created about 7000 new synthetic dyes and in 1974, the world sales of these dyes reached £1500 million (Samsami et al., 2020).

Dye molecules are made up of two main constituents, the chromophore and the auxochromes. The chromophore is responsible for producing the colour by giving a specific hue to the dye. The chromophore includes functional groups such as methine, methane, carbonyl, aril, anthraquinone, azo, nitro, etc. The auxochrome is a group of atoms that accompanies the chromophore and enhances the water-soluble property of the dye molecules. They do not provide any colour directly; rather, their presence intensifies the hue produced by the chromophore. They also alter the ability of the chromophore to absorb light. Some of the groups that include auxochromes are hydroxyl, sulfonate, aldehyde, amine, carboxyl, and methyl mercaptan (Sweety, 2018).

### 5 Classification of Dyes

Dyes are classified on the basis of their structure (Chemical composition), source, and method of application (Gurses et al., 2016).

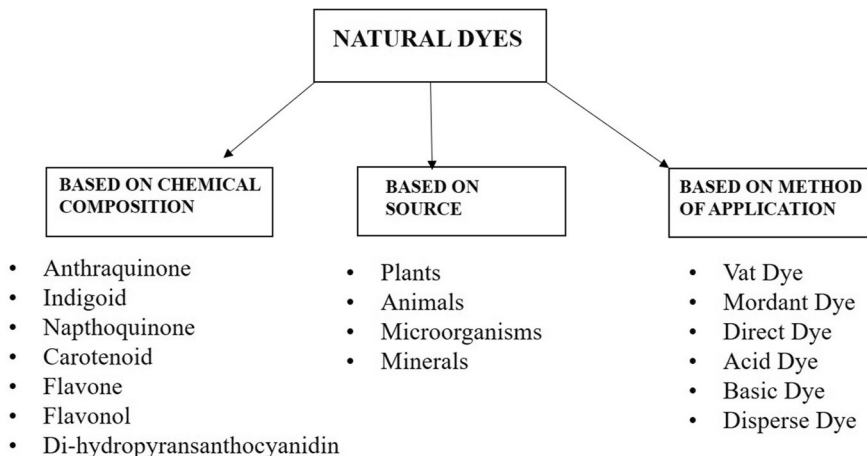
#### 5.1 Classification Based on Source

Based on the source, dyes are classified into Natural dyes and Synthetic dyes.

#### 5.1.1 Natural Dyes

Dyes that are extracted from natural sources and used to colour fabrics, textiles, paper, wood, etc., are called Natural dyes. Natural dyes can be extracted from animals such as molluscs and shellfishes; insects like beetles and lac insects; Plants such as Indigo and saffron; and minerals like ferrous sulphate, ochre and clay (Chengaiyah et al., 2010). Evidence of the presence of natural dyes, such as Indigo, Turmeric, Lach, Saffron, Safflower, madder, etc., has been found in Egyptian pyramids and mural art (Prabhu & Bhute, 2012). As these dyes are extracted naturally, they harmonise with nature and are not hazardous to health. They are non-carcinogenic, non-allergic, and non-toxic to the skin and they are biodegradable, renewable, and readily available (Verma & Gupta, 2017). Thus, Natural dye disposal needs less effort and minimum possibility of chemical reactions. Natural dyes can be classified in various ways as is shown in Fig. 1. Earlier, natural dyes were classified on the basis of alphabetical order. But now, they are being classified based on origin, source, and chemical composition. Natural dyes can be classified based on chemical composition into a wide variety of chemical classes such as indigoid, anthraquinone, flavone, naphthoquinone, carotenoid, flavonol, dihydropyran anthocyanidin, etc. They can also be classified into three groups based on sources from which the dye has been extracted i.e., plants, animals, microorganisms, and minerals. Table 1 shows the application and source of extraction of various natural dyes. Their classification is also based on the method of application into vat

**Fig. 1.** Classification of natural dyes



**Table 1** Natural dye, their source and application in textile industry

S. No	Dye/Pigment Name	Colour	Source of Dye	Application
<b>Plants</b>				
	Curcumin (Natural Yellow 3)	Yellow	Rhizome of <i>Curcuma longa</i> <i>linn</i>	Textile and Food Industry
	Crocin Crocetin	Yellow Orange	Dried stigma, upper styles of <i>Crocus sativus linn</i>	Food, Textile and Cosmetic Industry
	Carthamin	Cherry-Red	Flower of <i>Carthamus</i> <i>tinctorius L</i>	Textile Industry Especially in dyeing Wool
	Bixin (Natural Orange 4)	Radiant Yellow Orange	Extracts of <i>Bixa orellana</i>	Textile Industry (wool, silk, cotton linen) and Food Processing Industry
	Mallow	Purple Colour, Matte Green Yellow	Peel, fresh and dried fruits of <i>Punica granatum</i>	Textile Industry Especially in dyeing Cotton, Linen
	Indigo	Blue	Leaves of <i>Indigofera</i> <i>tinctorial</i>	Textile Industry
	Paprika Oleoresin Capsanthin	Intense Red	Fruits of <i>Capsicum annum L</i>	Textile Industry and Food Industry
	Lutein	Yellow to Orange	Dried and ground heads of flowers of <i>Tagetes erecta</i>	Textile Industry Especially in Cotton and Silk
	Lawson	Yellow Green to Orange	Fresh and Dried Leaves of <i>Lawsonia mermis Lam</i>	Textile Industry Especially in Silk and Wool
	$\beta$ carotene	Yellow	Fruits of <i>Mangifera indica</i>	Textile Industry Especially Cotton and Silk
	Rose Madder Alizarin	Brick Red	<i>Rubia cordifolia</i>	Textile Industry Blankets, Carpets
	Butein	Yellow and Bright Orange Red	Tesu flowers of <i>Butea</i> <i>monosperma</i>	Textile Industry
	Cutch	Brown	<i>Acacia catechu</i>	Textile Industry
	Al or Morinda	Red	<i>Morinda citrifolia</i>	Textile Industry
	Berberine	Orange Yellow to coral colour	<i>Berberis vulgaris</i>	Textile Industry especially wool, leather, silk
	Juglone	Black to Brown	Unripe fruits of <i>Juglans</i> <i>Nigra</i>	Textile Industry
	Woad	Blue	<i>Isatis tinctoria</i>	Textile Industry Especially cotton, wool, silk
	Awobanin	Blue	<i>Tsuyukusa cammelia</i> <i>communis</i>	Textile Industry Especially Silk
	Coreopsin, Sulfuretin	Mustard Yellow	Flowers of <i>Cosmos</i> <i>sulphurous</i>	Textile Industry Especially cotton
	Anthocyanin	Red	Flowers of <i>Canna indica</i>	Textile Industry Especially
	Salvianin	Red	Flowers of <i>Salvia splendes</i>	Textile Industry Especially
	Anthocyanin	Red	Flowers of <i>Rosa sinensis</i>	Textile Industry Especially
	Rutin-flavone, Quercetin	Green to Purple Pink	Flowers of <i>Plumeria rubra</i>	Textile Industry Especially
	Anthocyanin Dye	Pink Green to Purple	Flowers of <i>Ixora coccinea</i>	Textile Industry Especially
	Rutin, Quercetin	Dark Pink	Flowers of <i>Nerium oleander</i>	Textile Industry Especially
	Flavonoids	Pink, Yellow	Dried Fruit Pericarp of <i>Nephelium lappaceum</i>	Textile Industry Especially



**Table 1** (continued)

S. No	Dye/Pigment Name	Colour	Source of Dye	Application
<b>Animals</b>				
1	Cochineal Carminic Acid (Natural Red 4)	Pink, Crimson Red, Scarlet Red, Blue	Skin of dried female insect body of <i>Coccus cacti</i>	Textile Industry
2	Kermes	Red, Yellowish Red	Insect body of <i>Coccus ilicis</i>	Textile Industry
3	Lac Dye (Natural Red 25)	Red	Insect deposit of <i>Laccifera Lacca</i>	Textile Industry
4	Tekhelet	Bluish Purple	Extracts from sea snails <i>Stramonita haemastoma</i>	Wool
5	Tyrian Purple	Purple	Extracts from molluscs <i>Bolinus brandaris</i> <i>Hexaplex trunculus</i> <i>Stramonita haemastoma</i>	Textile Dye
<b>Microorganisms</b>				
1	Prodigiosin	Rose Pink	Pigments from wild type <i>Serratia marcescens</i>	Textile Industry Especially silk
2	Melanin	Black, Brown	<i>Streptomyces fildesensis</i>	Textile Industry
3	Rubrolone	Red	<i>Streptomyces echinoruber</i>	Food Industry
4	Anthraquinones	Brilliant olive green	<i>Alternaria alternata</i>	Textile Industry
5	Viridol	Yellow	<i>Trichoderma virens</i>	Textile Industry
6	Anthraquinone	Brown, grey	<i>Fusarium oxysporum</i>	Textile Industry Dyeing of wool and silk
7	Fungal Pyomelanin (Pyo <sub>Fun</sub> )	Brown	<i>Aspergillus niger</i>	Textile Industry Dyeing of Wool
8	Purpurogenone	Orange to yellow	<i>Penicillium purpurogenum</i>	Textile Industry Especially Cotton
9	Phycocyanin	Bright Blue	<i>Spirulina plarensis</i>	Textile Industry Especially Silk
<b>Minerals</b>				
1	Vermillion	Red	Powdered mineral cinnabar	Textile and Cosmetic Industry
2	Chrome Yellow	Strong Orange Yellow	Mineral Crocoite	Textile Industry Especially wool
3	Bistre	Brown	Coal Tar	Textile Industry Especially in wool, rugs, carpets

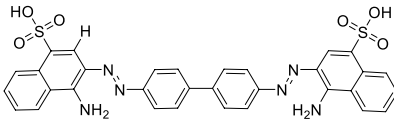
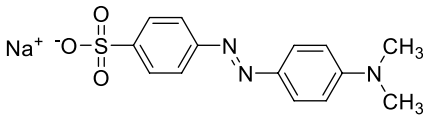
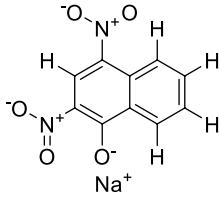
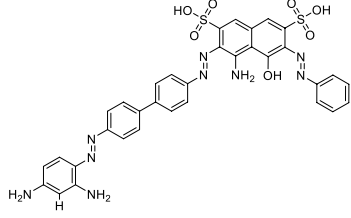
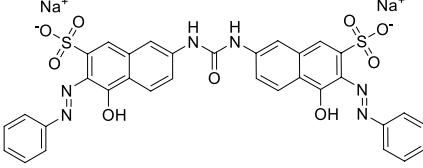
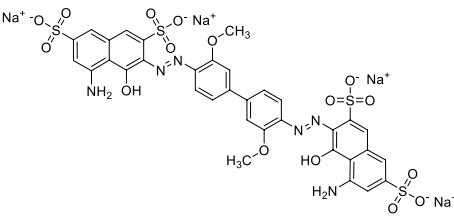
dye, mordant dye, direct dye, disperse dye, acid dye, and basic dye.

### 5.1.2 Synthetic Dye

Dyes that are extracted from chemical sources are called synthetic dyes. Synthetic dyes are complex aromatic compounds which can be chemically synthesized and provide a wide range of colours. The colour produced by the dye is a collaborative effect of chromophores and auxochromes (Ayele et al., 2021). Before the development of synthetic dyes, dyes

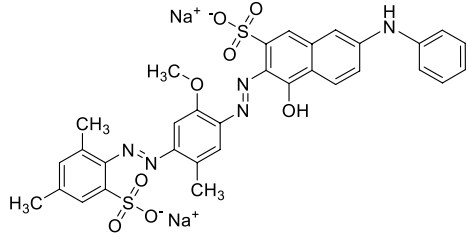
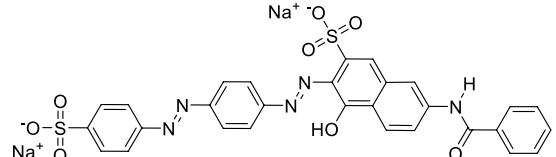
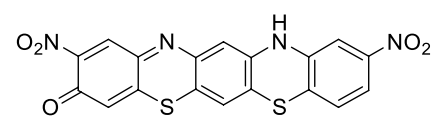
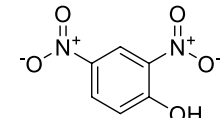
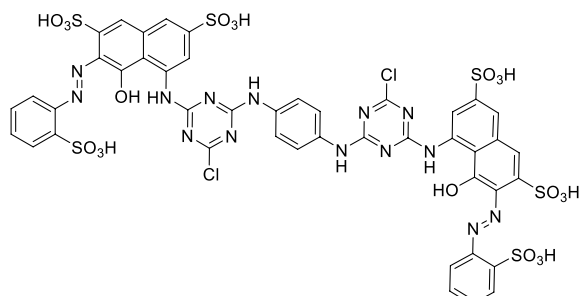
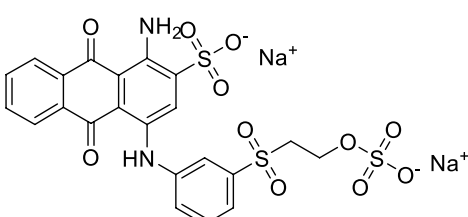
extracted from natural sources were extensively used. The emergence of 'Mauveine' led to the supremacy of synthetic dyes over natural dyes. It triggered the discovery of synthetic dyes, which were better than existing natural dyes in many aspects. They were easy to apply, cheaper than naturally obtained dyes, and were more persistent and gave the fabric a more vibrant colour. Ever since, these dyes have been commercially exploited and have evolved into a multi-million-dollar industry. Synthetic dyes are widely used in the textile, cosmetic, printing, and dyeing industries, as shown in Table 2. Synthetic dyes are

**Table 2** Synthetic dye, their chemical structure and application in textile industry

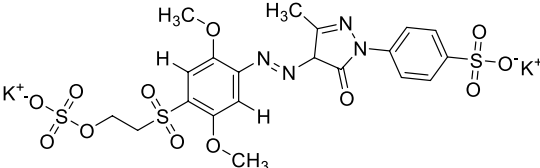
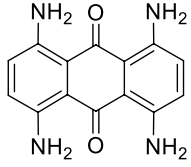
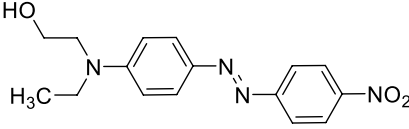
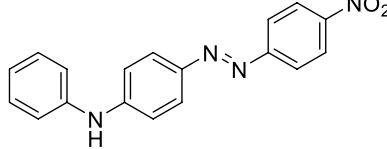
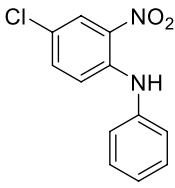
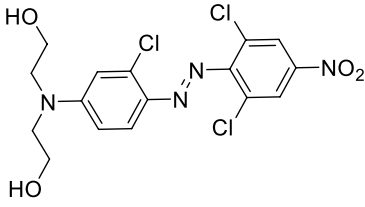
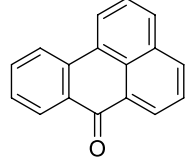
S. No.	Dye Name	Colour	Examples with chemical structure	Application
1.	Congo Red	Red		Textile Industry (Wool, Silk)
2.	Methyl Orange	Orange		Textile Industry (Wool, Silk)
3.	Martius Yellow	Yellow		Textile Industry (Cotton, Wool, Silk)
4.	Direct Black	Black		Textile Industry (Cotton, Wool, Silk)
5.	Direct Orange	Orange		Textile Industry (Cotton, Wool, Silk)
6.	Direct Blue	Blue		Textile Industry (Cotton, Wool, Silk)



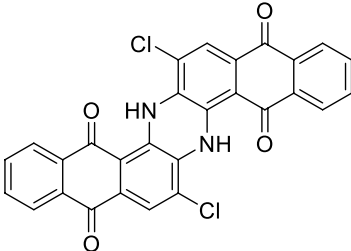
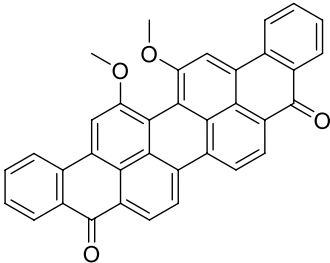
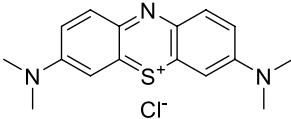
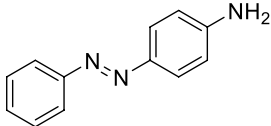
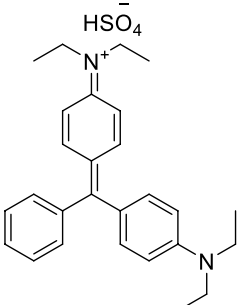
**Table 2** (continued)

7.	Direct Violet	Violet	 <p>The structure shows a central benzene ring with a methoxy group (-OCH<sub>3</sub>) and a methyl group (-CH<sub>3</sub>). It is connected via azo (-N=N-) groups to two other benzene rings. One of these rings has a methyl group and a sulfonate group (-SO<sub>3</sub><sup>-</sup>Na<sup>+</sup>). The other ring is part of a naphthalene system with a hydroxyl group (-OH) and a sulfonate group (-SO<sub>3</sub><sup>-</sup>Na<sup>+</sup>), and is further connected to a phenyl ring via an amino group (-NH-).</p>	Textile Industry (Cotton, Wool, Silk)
8.	Direct Red	Red	 <p>The structure features a central benzene ring with a sulfonate group (-SO<sub>3</sub><sup>-</sup>Na<sup>+</sup>) and an azo group (-N=N-). This is connected to another benzene ring with a hydroxyl group (-OH) and a sulfonate group (-SO<sub>3</sub><sup>-</sup>Na<sup>+</sup>). The latter is further connected to a naphthalene system with a hydroxyl group (-OH) and an amino group (-NH-), which is finally connected to a phenyl ring via a carbonyl group (-C(=O)-).</p>	Textile Industry (Cotton, Wool, Silk)
9.	Sulphur Black	Black	 <p>The structure is a complex polycyclic system consisting of two benzothiazine rings fused to a central benzene ring. It has nitro groups (-NO<sub>2</sub>) and a carbonyl group (=O) attached to the structure.</p>	Textile Industry (Cellulosic Fibers)
10.	Leuco Sulphur Black	Black	 <p>The structure is a benzene ring with a hydroxyl group (-OH) and two nitro groups (-NO<sub>2</sub>) at the 1 and 3 positions.</p>	Textile Industry (Cellulosic Fibers)
11.	Reactive Red	Red	 <p>The structure is a highly complex polycyclic system with multiple benzene rings, sulfonate groups (-SO<sub>3</sub>H), a hydroxyl group (-OH), a chlorine atom (-Cl), and a carbonyl group (=O) attached to various parts of the structure.</p>	Textile Industry (Wool, Cellulosic fibers)
12.	Reactive Blue	Blue	 <p>The structure is a complex polycyclic system with a central benzene ring and a naphthalene system. It features a sulfonate group (-SO<sub>3</sub><sup>-</sup>Na<sup>+</sup>), an amino group (-NH<sub>2</sub>), and a long chain containing a sulfonate group (-SO<sub>3</sub><sup>-</sup>Na<sup>+</sup>) and a sulfonamide group (-SO<sub>2</sub>-NH-).</p>	Textile Industry (Wool, Cellulosic fibers)

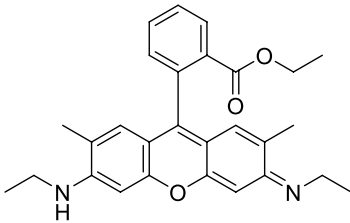
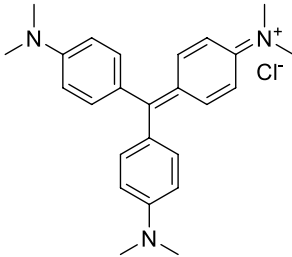
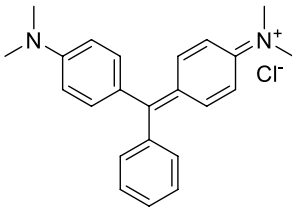
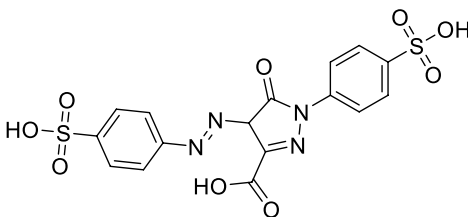
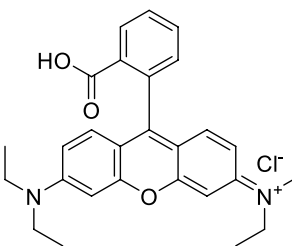
**Table 2** (continued)

13.	Reactive Yellow	Yellow		Textile Industry (Wool, Cellulosic fibers)
14.	Disperse Blue	Blue		Textile Industry (Polyester, Nylon)
15.	Disperse Red	Red		Textile Industry (Polyester, Nylon)
16.	Disperse Orange	Orange		Textile Industry (Polyester, Nylon)
17.	Disperse Yellow	Yellow		Textile Industry (Polyester, Nylon)
18.	Disperse Brown	Brown		Textile Industry (Polyester, Nylon)
19.	Benzanthrone	Violet		Textile Industry (Wool, Rayon)

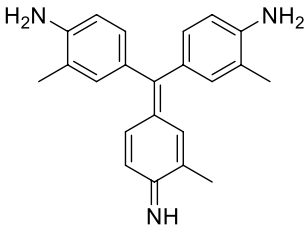
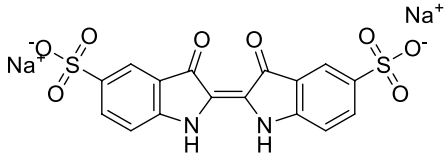
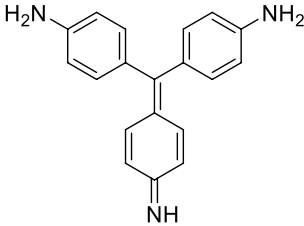
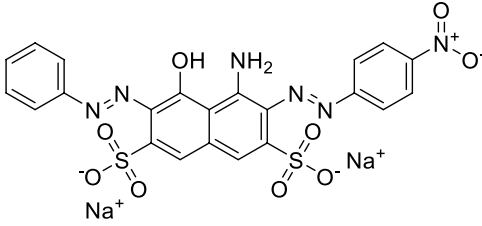
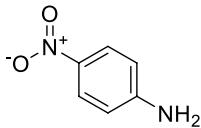
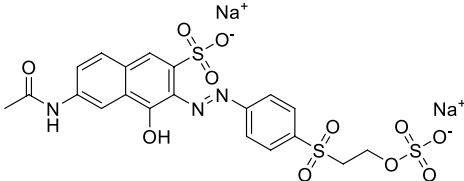
**Table 2** (continued)

20.	Vat Blue	Blue		Textile Industry (Wool, Rayon)
21.	Vat Green	Green		Textile Industry (Wool, Rayon)
22.	Methylene Blue	Blue		Textile Industry (Polyester, Wool, Silk, Modified Acrylic Nylon)
23.	Aniline Yellow	Yellow		Textile Industry (Polyester, Wool, Silk, Modified Acrylic Nylon)
24.	Brilliant Green	Green		Textile Industry (Polyester, Wool, Silk, Modified Acrylic Nylon)

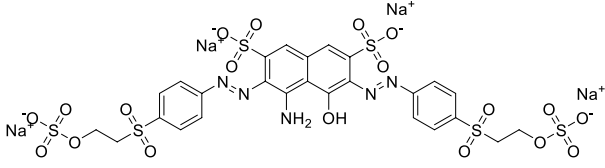
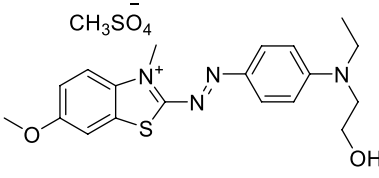
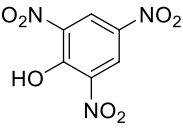
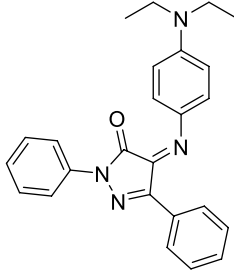
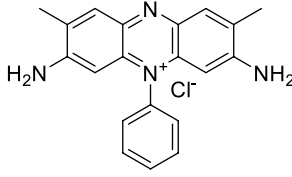
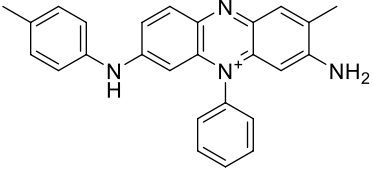
**Table 2** (continued)

25.	Basic Red	Red		Textile Industry  (Polyester, Wool, Silk, Modified Acrylic Nylon)
26.	Crystal Violet	Violet/Blue		Textile Industry  (Polyester, Wool, Silk, Modified Acrylic Nylon)
27.	Malachite Green	Green		Textile Industry  (Silk), Leather and Paper Industry
28.	Acid Yellow	Yellow		Textile Industry  (Natural and Synthetic fibers)
29.	Rhodamine B	Opera Rose		Textile Industry  (Cotton, wool, silk), Paper and leather Industry

**Table 2** (continued)

30.	Basic violet	Purple		Textile Industry (Wool, silk, cotton)
31.	Indigo Carmine	Blue		Textile Industry (wool)
32.	Basic Fuchsin	Dark green Turns pink		Textile Industry (Cotton, Wool)
33.	Amido Black	Blue/ Black		Textile Industry (Denim Textile)
34.	4-nitroaniline	Bright Yellow		Textile Industry, food, leather, cosmetic, pharmaceutical Industry
35.	Remazol Orange	Orange		Textile Industry (Cotton, Rayon, Cellulosic fibers)

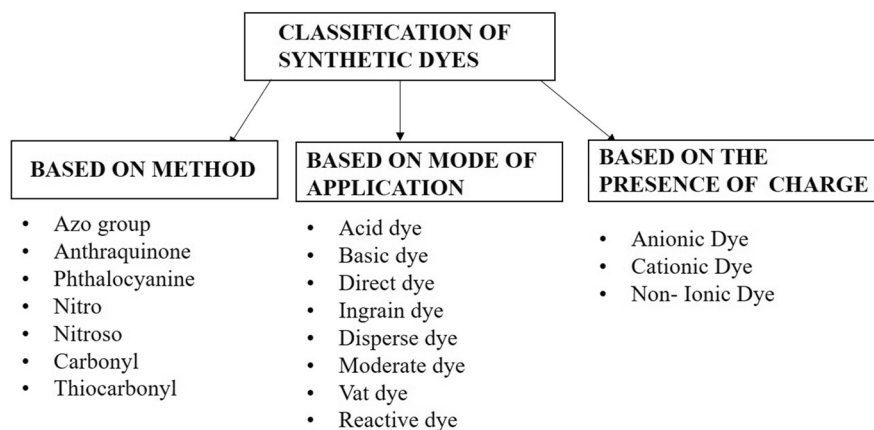
**Table 2** (continued)

36.	Remazol Black B	Black		Textile Industry (Cellulosic fibers)
37.	Basic Blue	Blue		Textile Industry (Wool, Silk, Linen, Hemp)
38.	Picric Acid	Yellow		Textile Industry (silk)
39.	Magenta	Pink		Textile Industry (Polyester, Cotton Blend)
40.	Safranine	Red		Textile Industry (Wool, Silk), Leather Industry
41.	Mauveine	Purple		Textile Industry (Silk)

more accepted for usage in the textile industry as they are more stable, readily available, and inexpensive.

Synthetic dyes can be classified based on various parameters which is shown in Fig. 2. They can be

classified based on chemical composition, i.e., chromophoric group in the chemical structure that includes azo group, anthraquinone, phthalocyanine, nitro, nitroso, carbonyl, thiocarbonyl etc. (Benkhaya et al., 2020).

**Fig. 2.** Classification of synthetic dye

They can also be categorized on the basis of their method of application, which includes acid dye, ingrain dye, basic dye, moderate dye, direct dye, disperse dye, vat dye, and reactive dye (Varjani et al., 2020). Synthetic dyes can be broadly classified into anionic, cationic, and non-ionic dyes on the basis of the charge on the chemical group. Anionic contains acid dyes, reactive dyes, and direct dyes whereas cationic dyes contain basic dyes (Forgacs et al., 2004). Non-ionic dyes contain azo dyes and disperse dyes (Raha et al., 2012).

## 5.2 Classification Based on Chemical Composition

Dyes are classified on the basis of the presence of chromophore and auxochrome. On the basis of chemical composition, they can be classified into Azo dyes, Triphenylmethane dyes, Phthalein dyes, Nitroso dyes, Nitro dyes, Indigoid dyes, Anthracenes, Carotenoids, etc. (Benkhaya et al., 2017; Kumar et al., 2021). Table 3 shows the classification of dyes on the basis of different classes of dyes, their chemical composition, and examples.

### 5.2.1 Azo Dyes

These groups of dyes belong to the biggest group of synthetic dyes. The chromophore is an aromatic system that is attached to the azo group, and its auxochrome is mainly amines and hydroxyl. These azo dyes can be classified based on the presence of the number of azo groups into monoazo dyes, diazo dyes, triazo dyes, etc. Examples of azo dyes may include Congo Red and Methyl Orange (Kumar et al., 2021). Azo dyes that contain sulphonate group are called

sulphonated azo dyes. Azo groups with enolizable groups and aromatic substituents are responsible for the variation of colours of dyes (Benkhaya et al., 2017). Azo dye has been proven to show lethal effects and genotoxicity and is carcinogenic and mutagenic in humans and animals. Benzidine, the component of azo dye was linked to causing cancer in the human bladder (Chung, 2016). Due to these harmful effects on humans and animals, Azo dyes are banned in several countries.

### 5.2.2 Triphenylmethane Dyes

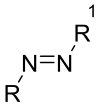
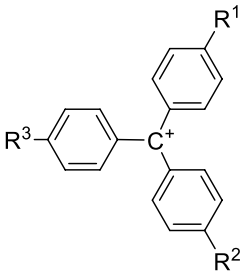
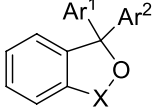
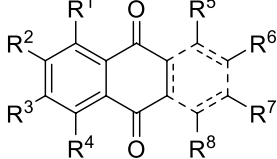
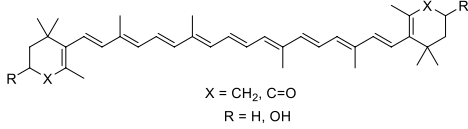
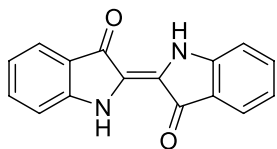
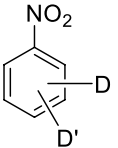
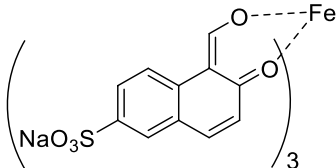
These dyes are derivatives of amines and hydroxyl groups in their rings. These compounds are usually colourless and are called leucobases, but upon oxidative reaction, they form carbinol bases which change from colourless form to coloured form when they react with acids. Examples of triphenylmethane dyes are Malachite green and crystal violet (Kumar et al., 2021). Some examples of triphenylmethane dyes, such as Crystal Violet and Malachite Green dyes can cause liver tumours and are carcinogenic and extremely cytotoxic. They were also reported to cause serious harmful effects on the digestive system and skin (Ouettar et al., 2023).

### 5.2.3 Phthalein Dyes

Phthalein dye do not dissolve in water but are soluble and impart colour in the presence of alcohol. They can be soluble in water by producing sodium or potassium salts. The lactone form of the compound remains colourless in an acid medium and the quinone phenolate form becomes highly coloured in an alkaline medium.



**Table 3** Classification of different types of dyes on the basis of chemical composition

Class of Chemical Composition	Core functional Group	Examples
Azo Dyes		Congo Red Methyl Orange
Triphenylmethane Dyes		Crystal Violet Malachite Green
Pthalein Dyes	 <p>X = CO, SO<sub>2</sub></p>	Phenolphthalein Thymolphthalein
Anthracene		Alizarin Lawson Juglone Morindone Lac dye
Carotenoid	 <p>X = CH<sub>2</sub>, C=O R = H, OH</p>	Bixin Crocin Capsanthin
Indigoid Dyes		Indigo
Nitro Dyes		Martius Yellow Picric Acid
Nitroso Dyes	<p>D, D' = electron donating groups</p> 	Naphthol green B

In basic pH, these dyes produced pink, magenta, purple, violet, blue, red, etc. colour. Some common examples of phthalein dyes are phenolphthalein, thymolphthalein, xylenolphthalein, naphtholphthalein etc. (Sabnis, 2010). Phthalein dyes are seen to cause irritation to the respiratory tract and, if they come in contact with the skin, may cause serious problems (Hassanien et al., 2022).

#### 5.2.4 Anthracenes

This class of dyes includes two major divisions of dyes such as anthraquinone and naphthoquinones. Both anthraquinone and naphthoquinones are groups of natural dyes. Anthraquinone produces yellow, pink and red pigments. Some chromogens that are part of anthraquinone compounds are Morindone, Laccic Acid, Kermisic Acid, Alizarin, etc. Naphthoquinone is a group of natural dyes that produces brown, pink and purple pigments. Some chromogens that are part of naphthoquinone are Juglone, Lawsone, Alkanin and Shikonin (Patel, 2011). Anthraquinone derivative has been reported to produce carcinogenic, mutagenic effects and possibly produce genotoxic mechanism of cancer induction.

#### 5.2.5 Carotenoids

They are the group of pigments that produce yellow and orange pigments and are extracted from photosynthetic organisms. They contain two main divisions namely, carotenes and xanthophylls. Carotenes such as  $\beta$ -carotene and canthaxanthin are orange or red orange-producing pigments that are found in algae and higher plants. Xanthophylls are oxygenated carotenes, which produce yellow and red coloured pigment found in photosynthetic organisms. Examples of Carotenoids may include Bixin, Crocetin, azafrin, capsanthin, lutein, etc. (Patel, 2011).

#### 5.2.6 Indigoid Dyes

Dye that belongs to the Indigoid family is called Indigo. These are dyes that can be extracted from natural as well as synthetic sources. Plants such as *Indigofera tinctoria*, *Wrightia tinctoria*, *Isatis tinctoria* and animals such as *Murex brandaris* (shellfish) contain dyes that belong to the Indigoid family. The plants contain compounds soluble in water such as glucoside

indican which, when hydrolysed form indoxyl that get oxidised in the presence of air to form indigotin used in dyeing processes. Indirubin, kaempferol, indirubin dyes also belong to this group of dyes (Patel, 2011). Dyes such as indigo carmine are said to be highly toxic and when they come in contact can cause skin irritation and permanent damage to the eye. They are also reported to be fatal as they are carcinogenic and cause toxic effects in developmental, reproductive, acute, and neurological stages. (Mittal et al., 2006).

#### 5.2.7 Nitro and Nitroso Dyes

Nitro and Nitroso dyes have small molecular structures, and they are being used minimally in the commercial dyeing of textiles such as wool, silk, etc. Structures of Nitro dyes consist of nitrogen dioxide ( $\text{NO}_2$ ), imino, hydroxyl etc., whereas nitroso dyes consists of  $-\text{N}=\text{O}$  linkages in the organic moiety (Maheshwari et al., 2021). These Nitro or nitroso dyes consist of an electron donating group via an aromatic system, which is conjugated to one or more nitro or nitroso group. These compounds cannot form dyes, but they can form metal complexes, which in turn produce dyes and pigments (Gurses et al., 2016). Nitroso dyes are the result of reaction between the phenol and nitroso groups, after which the nitroso group is attached to the ortho or para position of phenol (Chaudhary, 2020). Some examples of nitro and nitroso dyes are Martius Yellow, Picric Acid etc. Nitro and nitroso dye may cause inflammation in skin and can be dangerous.

### 5.3 Classification Based on the Method of Application

Dyes can be classified by the method of application to the substrate into various types. Some of the dyes that belong to this category are vat dye, acid dye, reactive dye, direct dye, disperse dye, basic dye etc.

#### 5.3.1 Vat Dye

Vat dyes are insoluble in water and need a special treatment done in wooden vessels to bring the compound to a solubilised form. This process is called vatting. The word 'vat' means vessels. The insoluble compound is converted in a soluble form due to chemical reduction in an alkaline solution. These dyes bind to the cellulosic molecules of the fabrics by hydrogen bonding or by van der Waals forces.

After diffusing into the fabrics, the dye gets oxidised and changes itself to water-insoluble forms, thus giving them extraordinary wash fastness. Dyes from anthraquinone and indigoid class of dyes usually belong to vat dyes. Indigo is one of the oldest naturally occurring vat dyes, which has been used to dye textiles for more than 4000 years (Gurses et al., 2016).

### 5.3.2 Reactive Dye

Reactive dyes are dyes which contain one or more reactive groups that form covalent bonds with fibres and become an integral part of it. These dyes have reactive groups that react with the hydroxy group of cellulosic fibres. They are preferred as they show an all-round performance, which includes ease of application, lower cost, extraordinary wet fastness, and a vibrant range of hues. The reactive groups are connected to the chromophore with the help of bridging groups like NH, CO, and SO<sub>2</sub> (Gurses et al., 2016). Reactive red and reactive blue are some examples of reactive dyes.

### 5.3.3 Disperse Dye

The term disperse dye is used for dyes which do not contain an ionizing group, have very low solubility, and are used to dye hydrophobic fabric. Disperse dyes are dyes that are not soluble in water and when used on the fabric, used in the form of aqueous dispersion with stabilizing agents such as cresol, phenol, benzoic acid etc. (Kumar et al., 2021). They have a very small molecular size. They are used to dye fabrics such as nylon, polyester, acrylic, cellulose acetate etc. Dyes that contain nitro, azo and anthraquinone groups come under disperse dyes (Gurses et al., 2016). They are used in large quantities, which in turn leads to the usage of large quantities of water. Thus, dyes that are unfixed generate huge amounts of wastewater (Benkhaya et al., 2017). Some examples of disperse dyes may include Celliton Fast Pink B and Celliton Fast Blue B (Kumar et al., 2021).

### 5.3.4 Acid Dye

Acid dyes contain sulfonated groups and carboxylic acid groups in their chemical composition. These dyes are classified as acid dyes as they need an aqueous

acidic solution of the dye to dye the fabric. The fabric is dyed in a solution of the dye that contains sodium salts of organic acids. The charged ions are present in the dye that show affinity toward the opposite charge present on the fabric. These dyes form ionic bonds and van der Waals forces. (Benkhaya et al., 2017; Kumar et al., 2021). They show efficient water solubility, vibrant colour, and fastness to light. These dyes are used as a colourant for fabrics such as wool, silk, polyamide, and blends of cotton, rayon, and polyester. Dyes from azo, anthraquinone, nitro, nitroso and triphenylmethane belong to the class of acid dyes (Gurses et al., 2016). Some examples of acid dyes are Acid Orange 7, Acid Brown 14 etc.

### 5.3.5 Basic Dye

Basic dyes are also called cationic dyes as they contain a positive charge due to the presence of ammonium cation. Basic dyes are soluble in water and give coloured cations when solubilised in water as the dye molecules get detached in an aqueous solution. The cations of the dyes are intrigued by the anionic sites on the fibres and form covalent bonds with them. They show vibrant colour hues but show low colour-fastness. These dyes are used on fabrics such as modified nylon, modified polyester, silk and wool (Gurses et al., 2016). These dyes contain chemical groups such as triphenylmethane group, azo group, xanthenes, and acridine dyes. Some of the examples of basic dye may include aniline Yellow, Butter Yellow, and Chrysodine G (Benkhaya et al., 2017; Kumar et al., 2021).

### 5.3.6 Direct Dye

Direct dyes are dyes which are used as a colourant and put into an alkaline or neutral bath directly without the use of mordant, and the dyeing procedure is simple. Direct dyes can also be called anionic dyes as they have a higher affinity for cellulosic fibres. Here, the fabric that has to be dyed is added to aqueous solution which contains the salt of the dye and simply the solution is heated to a boil for the fabric to be coloured. Direct dyes are water soluble, have high exhaustion value, high substantivity, and good colour reproducibility (Gurses et al., 2016). Direct dyes are also called substantive dyes as they show a higher substantivity than other dyes (Kumar et al., 2021). They are used to

dye cellulosic fibres such as cotton and other fabrics such as wool, silk and rayon. These dyes contain chemical groups such as polyazo, phthalocyanine, oxazines, anthraquinone etc. (Benkhaya et al., 2017; Gurses et al., 2016). Some examples of Direct dyes are Congo Red and Martius Yellow (Kumar et al., 2021).

## 6 Treatment of Effluents Containing Dyes

Pollution can happen due to many substances that may include chemical substances such as dyes, herbicides, and insecticides and may be caused by misusing the components of the environment such as air, water, etc. (Azanaw et al., 2022). Dyes can be widely used in several industries including tanneries, food, cosmetics, textile, etc. from small scale to large scale and its production reaches 1,000,000 tons globally. But Textile Industries play the most important role in pollution caused by dye in the ecosystem with a discharge of 7.5 metric tons produced annually. Pollution caused by effluents from textile industries can be water, air, or land pollution. The impartment of colour from dyes depends on two components, auxochrome, and chromophore which are non-biodegradable in nature and persistent, thus causing environmental hazards (Maheshwari et al., 2021). The huge quantity of water used in different stages of the dyeing process is the main reason for effluent generation. Thus, wastewater containing dyes should be eradicated before releasing the residual effluent into the environment. These effluents, when discharged into rivers etc., lead to alteration of Chemical Oxygen Demand (COD), Biological Oxygen Demand (BOD), Total Dissolved Solids (TDS), Total Suspended Solids (TSS), and pH, which causes detrimental effects on the aquatic biota and humans (Islam et al., 2023). Treatment of dye effluent is usually performed in three stages namely primary, secondary, and tertiary treatments. Primary treatment involves the removal of organic and inorganic wastes from textile effluents. Secondary treatment leads to the removal of dispersed solids, fine suspended solids, and some dissolved organics via volatilization, and incorporation into sludge. Further, the tertiary treatment is done to enhance the water quality so that the treated water can be released into natural waters. Tertiary treatment may include Physical, chemical, and Biological treatment of the effluent.

### 6.1 Physical Treatment of Dye effluent

Physical treatment is separating and removing external particulate matter based on physical and mechanical properties. Physical treatment techniques such as Filtration, Adsorption, Irradiation, Ion Exchange, etc.

#### 6.1.1 Filtration

Filtration is a technique in which the solid substances are separated from the solution via paper, textile metals, etc. They use a variety of filters made up of sand, cloth, and drum screens. Filtration can be of many types, including membrane filtration, ultrafiltration, and nanofiltration. The membrane filtration treatment method uses membranes of pore size 0.1–10  $\mu\text{m}$  for separation, which filters very fine particles (M et al., 2022). The dye solutes, being larger than the pores, get captured, which results in the formation of a dye-free solution. The ultrafiltration technique uses a filter of 0.1–0.001  $\mu\text{m}$  and can be used to filter out organic dyes. Nanofiltration is another technique that can be used for the separation of textile dye wastewater depending on the properties such as size and electrostatic repulsion and uses filters of a range of 0.5–0.2 nm. Reverse Osmosis technology can also be used for treating dye containing wastewater and provide a high quality of water. Filtration technique of wastewater treatment shows an efficiency of 90–100% in the separation of dye from dye containing wastewater (Azanaw et al., 2022). Polyamide nanomembrane is used to filter five different dyes; these include reactive orange 16, reactive blue 15, reactive black 5, reactive yellow 145, and reactive red 194 (Rashidi et al., 2015). Filtration is an effective method which is technologically simple and separates a wide range of pollutants. The problem with the filtration technique is they need regeneration and replacement more often as the filters get clogged and saturated rapidly.

#### 6.1.2 Adsorption

Adsorption is the process in which any material or substance is concentrated on the solid substance surfaces from its liquid surroundings (Ayele et al., 2021). Adsorption is used for separating pollutants mixed in water where the pollutants get attracted to the surface of adsorbents. The pollutants get adsorbed and stick to

the surface of the adsorbate by forming various chemical and physical bonds. The pollutants form various physical forces, such as Dipole–Dipole interactions, Polarity, Van der Waals forces, and, Hydrogen bonding which lead to the execution of Adsorption (Afroze & Sen, 2018). The adsorption process is the best physical treatment method as it can easily remove colour from effluents. Ferrierite, silica, alumina, and activated carbon are some of the examples of adsorbents. These adsorbents have the capability of completely depleting Chemical Oxygen Demand from textile Industry effluents (Azanaw et al., 2022). Commercially available Activated charcoal is the most efficient adsorbent to remove dye because of its properties such as surface area and high adsorbing capacity. Some biomass wastes can also be used as adsorbents for dye removal, such as rice husks, wheat residues, and ginger wastes (Adane et al., 2021). However, adsorption is a cost-intensive regeneration process (Singh et al., 2017). A natural absorbent bentonite was used to adsorb a cationic dye named Basic Red 46 (Paredes-Quevedo et al., 2021). Advantages of adsorption include minimum sludge requirements, high efficiency of detoxification and no usage of nutrients (Afroze & Sen, 2018). The disadvantages of adsorption are that it is a non-selective, non-destructive method that needs various types of adsorbents. These adsorbents need to be regenerated or replaced continuously. So, Adsorption is not cost-effective in case of certain industries such as pulp, paper and textile industries.

### 6.1.3 Ion Exchange

Ion Exchange treatment of dye-containing effluent is a simple, flexible, and highly efficient method. Separation of the effluent via the ion exchange method can be done by creating strong bonds between the resins of the reactor and the solutes (Al-Tohamy et al., 2022). This method can be used for the separation of cationic, and anionic pollutants and to soften hard water. Ion exchange method is used to remove 96.8% of Acid Orange 10 by using Amberlite IRA 400 resin (Marin et al., 2019). The ion exchange method shows high efficiency in the separation of water-soluble dyes from effluents and low efficiency in separating water-insoluble dyes such as disperse dyes (Adane et al., 2021). However, the application of the Ion Exchange Method is only on specific colourants (Singh et al., 2017). Ion exchange is a rapid and effective method that produces a high-quality

treated product that only works on certain types of pollutants. The problem with the ion exchange method is they are not cost-effective, have maintenance costs, and are time-consuming. Moreover, the matrix deteriorates on reaction with certain pollutants and time (Crini & Lichtfouse, 2019).

### 6.1.4 Irradiation

Irradiation refers to the technique that uses radiation derived from a monochromatic UV lamp with a wavelength under 237 nm. Irradiation is a simple technique that helps in the extraction of organic contaminants such as dyes from dye containing wastewater (Ayele et al., 2021). They show great efficiency in getting rid of coloured substances at very low volumes. However, they require high dissolved oxygen and are ineffective against dyes which are light-resistant (Singh et al., 2017).

## 6.2 Chemical Treatment of Dye Effluents

Chemical treatment of dye effluent can be referred as the treatment of wastewater by means of chemical processes. The chemicals present drive the removal of the contaminants present in the wastewater via separating with the help of added specific molecules. Chemical treatment methods can be applied for removing contaminants such as dyes and toxic metals from textile dye industries wastewater (Adane et al., 2021). Chemical treatment may include Coagulation, Advanced Oxidation Process, Fenton's Reagent, Ozonation, and Sodium hypochlorite (Madhav et al., 2018).

### 6.2.1 Coagulation

Coagulation or flocculation is one of the most efficient chemical treatment methods for wastewater removal. It is the process in which the separation of dye molecules from wastewater is done by changing the electrostatic charges of particles present in water. Coagulation of dye molecules happens in the presence of coagulants such as aluminium sulphate, alum, lime, ferric sulphate, or ferric chloride. Electrocoagulation is a type of coagulation where metal plates of aluminium or iron are used as electrodes to form highly charged metallic polymeric hydroxide species in the wastewater, which leads to coagulation (Azanaw et al., 2022). Coagulants such as Aluminium Sulphate or Alum (80 mg/L) are used for the

removal of Congo Red dye up to 84.37% (Li et al., 2024). The Advantages of coagulation are that it is easy to use and a better and more adequate method for separating insoluble contaminants. The disadvantage of coagulation may consist of the operational cost, sludge volume, higher unit cost, etc.

### 6.2.2 Oxidation

Oxidation is used for removing dyes from dye-containing wastewater. It uses Hydrogen Peroxide ( $H_2O_2$ ) as an oxidizing agent, which is activated by ultraviolet (UV) radiation. UV light leads to the production of plenty of hydroxyl radicals, which convert the organic and inorganic pollutants into carbon dioxide ( $CO_2$ ) and water ( $H_2O$ ) (Adane et al., 2021; Madhav et al., 2018). This method is effective for both soluble and insoluble dyes as it breaks down the aromatic ring present in the dyes, which leads to their removal from wastewater. Three azo dyes named Solophenyl Green, Erionyl Red, and Chromotrope were observed for degradation under UV wavelength at 253.7 nm (Silva et al., 2006). The advantages of oxidation are that it is an easy and effective method that causes efficient removal of dyes with no sludge production and reduces foul odour. It also degrades all types of organic pollutants (Kumari et al., 2023; Madhav et al., 2018). The Disadvantages are that they are costlier and need space for storage and transportation of the effluents.

### 6.2.3 Ozonation

Ozonation is the process where ozone ( $O_3$ ) is used to remove contaminants from water and leads to the expulsion of organic and inorganic pollutants such as dyes from effluents of textile dye industries. Ozone is highly unstable and thus is used as an oxidizing agent. Oxidation that happens due to the presence of ozone can break down phenols, chlorinated hydrocarbons, and other organic pollutants. It degrades colour and eliminates odour very efficiently from wastewater. The ozone treatment method is effective for azo dye removal, whereas they are not effective for the removal of disperse dye (Singh et al., 2017). Reactive red and Direct green were used to observe the degradation of dye by using a multi-walled carbon nanotube for photocatalytic ozonation (Mahmoodi, 2013). The major disadvantages of ozonation includes instability and has a very short

half-life of ozone; thus has a very limited efficiency on dyes (Crini & Lichtfouse, 2019).

### 6.2.4 Fenton's Reagent Treatment

In Fenton's reagent treatment,  $H_2O_2$  is catalysed by ferrous ions and forms hydroxy radicals, which further can oxidize organic pollutants such as dyes into  $CO_2$ ,  $H_2O$ , and organic salts (Xu et al., 2020). They are an efficient method that can be used for the degradation of a wide variety of dyes. Procion Blue was degraded using iron oxide ( $Fe_2O_3$ ) nanoparticles which showed efficacy by degrading 83% of the dye (Tony & Mansour, 2019). One of the major drawbacks of Fenton's reagent Treatment is the production of sludge due to the coagulation of the reagent and dye molecules. They are also very expensive, thus not extensively used.

### 6.2.5 Sodium Hypochlorite Treatment

Sodium Hypochlorite attacks the chlorine in the amino group of dye molecules. This method is mainly specific for azo dye and cannot be used for the degradation of disperse dyes. But this method is not used as chlorine shows a deleterious effect on water and aromatic amines which are residues of azo dyes were released into the water which has a carcinogenic effect (Madhav et al., 2018). Disperse Blue 56 dye was degraded efficiently by using Sodium hypochlorite treatment and UV treatment (Zeng et al., 2009). Sodium hypochlorite treatment is extensively used for the degradation of azo dyes as it aids in the cleaving of Azo bonds. The drawback of sodium hypochlorite is that it cleaves azo bonds very efficiently, which results in the breakdown of azo dyes into aromatic amines and volatile compounds.

## 6.3 Disadvantages of Physicochemical Treatment

The physicochemical treatment is widely used but is not economical. Physical treatment methods of effluent can require a higher quantity of energy, and sophisticated equipment and also produce a large amount of sludge that needs safe dumping (Varjani et al., 2020). The chemical treatment method has high chemical requirements, high energy usage, production of by-products, generation of sludge, and persistence of secondary



pollutants. The secondary pollutants need another phase of treatment and safe dumping which doubles the cost, making the method costlier. To overcome these disadvantages of physicochemical treatment for dye degradation, a biological method or bioremediation approach can be used as an alternative for the treatment of dye.

#### 6.4 Biological Treatment of Dye Effluents

The biological method or bioremediation can be explained as the green treatment method, where organisms such as algae, bacteria, fungi, yeasts and plants are used for the degradation of organic pollutants such as dyes. Thus, bioremediation is basically the treatment method where the biological entities can degrade as well as take up the pollutants. This method of degradation is cost-effective, eco-friendly, and generates less to no residue or secondary pollutants. They have a low processing cost and minimal interference with the environment (Chen et al., 2021). Biological methods of treatment can be considered better than both chemical and physical methods of degradation because they are economically feasible and use a comparable amount of operating time. In case of dyes, the molecules are broken down into less toxic forms, which is due to the breakage of bond of chromophoric group, leading to degradation and decolourisation of synthetic dyes (Bhatia et al., 2017). The bacteria used for the bioremediation of dyes also utilize organic pollutants as a source of food or a source of energy (Ihsanullah et al., 2020). The usage of bacteria in bioremediation is advantageous over other microbes because of their ability to be easily culturable and the ability to multiply faster than other microorganisms. The two main mechanisms of bacteria that are used in bioremediation are either biosorption on the bacterial biomass or the ability of bacteria to catalyse the organic pollutant (Samsami et al., 2020). Both biotic as well as abiotic factors are responsible for the degradation of pollutants with the help of microorganisms. Some of the main factors are pH, temperature, nutrients, oxygen, the availability of pollutants to microbial population and isolation of the microbial population that has the ability to degrade the contaminant.

##### 6.4.1 Advantages and Disadvantages of Bioremediation

Advantages of bioremediation may include their cost-effectiveness, lesser effort, lesser labour charges and sustainability. Another advantage of bioremediation

is it can be performed on site, without disturbing the natural processes and without using harmful chemicals. Some of the disadvantages are it needs skilled labour, specific nutrients, proper environmental conditions for specific microbial growth and more time than other processes to degrade the organic pollutants (Ihsanullah et al., 2020).

## 7 Types of Bioremediations

Bioremediation can be divided and categorised into *ex-situ* bioremediation and *in-situ* bioremediation. *Ex-situ* remediation is when the organic pollutant is extracted from the pollution site and is transferred to a different site for biological treatment. *In-situ* bioremediation is when the organic pollutant is treated on-site without causing any disturbance. The *in-situ* technique is more cost-effective as compared to *ex-situ* bioremediation. Mainly, *in-situ* bioremediation includes intrinsic bioremediation, bioaugmentation and bio-stimulation.

Intrinsic bioremediation can be defined as the process of remediation where the degradation of polluted sites is done without the intrusion of human beings. In this technique, the bacteria that are present naturally are triggered to degrade organic pollutants present on site. The advantages of intrinsic bioremediation are that it is a naturally occurring process that does not include human interference, it is usually less expensive, and it needs comparably no labour (Sharma, 2020). The drawbacks of intrinsic bioremediation are that they need a longer period to bioremediate the pollutants. The long time taken by the pollutants to bioremediate causes problems such as until remediation, the pollutant stays exposed and affects human and animal populations. Intrinsic bioremediation couldn't reduce soil toxicity and remediate polyaromatic hydrocarbons (García-Delgado et al., 2015).

Biodegradation depends on many physical factors such as moisture, pH, temperature, nutrients, properties of soil, availability of oxygen etc. Thus, bio-stimulation implies the process where alteration and adjustment of these physical factors trigger the existing or indigenous bacteria to degrade the organic pollutants, such as dyes, etc., present onsite. Bio-stimulation is the process where electron donors, electron acceptors, nutrients and oxygen are added, causing alteration in environments which promote activity and increase the population



of indigenous bacteria. The advantage of biostimulation is that the degradation process is carried away by the naturally occurring bacteria, which are well adjusted to the environment. Challenges of biostimulation include delivery and distribution of additional nutrients, oxygen, phosphorus, etc. and any other modifications (Adams et al., 2015). Disadvantages of bio-stimulation are that the potency of the process depends on the environmental factors, they are immensely site specific and need scientific observation, and skilled labour. The biostimulation cannot be performed for pollutants that are riveted to the soil particles or are nonbiodegradable.

Bioaugmentation can be explained as a process where the addition of a specific bacterial strain or consortia, promotes the breakdown of specific organic compounds. The bioaugmentation process depends on the bacterial strain or consortia that are specifically designed for degrading the organic pollutants. The specific microbial strain or consortia that caters to bioaugmentation can be either pre-adaptive or genetically engineered by putting suitable genes for biodegradation into a vector (Herrero & Stuckey, 2015). Specific microorganisms can degrade specific organic compounds. Some can degrade alkanes and others can degrade aromatics and consortia of these two will have a greater capacity to degrade the combinations of organic pollutants (Adams et al., 2015). Bioaugmentation is done on sites that lack adequate microbial biomass or the requisite metabolic pathway to metabolize the organic pollutants (Tyagi et al., 2011). The benefit of bioaugmentation is that it is a cost-effective, sustainable, and green technology. Moreover, the efficiency of the bacteria to bioremediate is known. The efficiency of bioaugmentation decreases due to many environmental and non-environmental factors. The number of the desired, selected, and efficient bacteria decreases due to competition with indigenous bacteria, biotic stresses, and abiotic stresses. Abiotic stress includes stressful changes to the site, such as pH, water content, temperature, nutrition availability, and toxicity of the pollutants.

## 8 Microorganisms Involved in Dye Degradation

Various microorganisms can be employed for the degradation of textile dyes. Table 4 shows some common

microorganisms that can be used for biodegradation and decolourisation of dyes used in the textile industry. Forss and co-workers (2012) observed that microbes like *Bacteroidetes*, *Acinetobacter* and *Clostridium* were responsible for 80% decolourisation of Reactive Black 5 and Reactive Red 2 within 28h when put in a bioreactor. Cui et al. (2014) observed *Klebsiella sp.* Y3 showed 100% biodegradation of Methyl Red, Congo Red, Orange I and Methyl Orange within 48h at pH 4–9 at a temperature of 30–42°C. El Bouraie et al., in 2016 found a bacterial strain of *Aeromonas hydrophila* that could degrade Reactive Black 5 with an efficacy of 76% within 24h at pH 7.0 and temperature 35°C. Ito et al. (2018) isolated two groups of dye decolourising bacterial strains with about 40% efficiency. First belongs to the azo dye decolourising bacterial group which can completely degrade Congo red dye. The second group belongs to anthraquinone decolourising bacterial group which showed partial decolourisation of anthraquinone. Eskandari et al. (2019), selected two bacterial consortia, which consisted of bacteria from cold regions which are well adapted to cold named StSp and bacteria which were mesophilic named PsGo for decolourisation of Reactive Black 5. The bacterial consortium StSp decolourised the dye completely at 25–30°C and pH 9, whereas the bacterial consortium PsGo decolourised the dye at 37°C and pH 11 within 5 days. Roy and his co-workers succeeded in finding two potential bacterial strains of *Enterobacter spp.* that showed decolourisation of Malachite Green Dye without requiring a sole carbon source at pH 6.5 and temperature 35°C (Roy et al., 2020). Garg et al. (2020) studied decolourisation and degradation of reactive yellow 145 with the help of *Pseudomonas aeruginosa* and *Thiosphaera pantotropha* which showed decolourisation efficiency within 96h and 72h at pH 7.0. Dixit and Garg were able to purify Azo-reductase from *Klebsiella pneumoniae*, which was then expressed in *Escherichia coli*, further used to degrade sulphonated azo dye such as Methyl Orange which showed maximum activity at 40°C and pH 8.0 (Dixit & Garg, 2021). Thangaraj et al. (2021) found a bacterial strain of *Enterobacter hormaechei* with 98% possible efficiency of degrading azo dyes such as Reactive Red F3B and Reactive Yellow 145 at 37°C and pH 7 after 98h incubation. El-Rahim et al. (2021) performed a study on five bacterial strains to observe their biodegradation capability on fifteen different dyes such as Methyl red, Direct violet etc. The

**Table 4** Microorganisms involved in dye degradation

Bacterial strain	Dye Name	Degradation Efficiency (%)	References
<i>Staphylococcus sp.</i> K2204	Remazol Brilliant Blue	100%	(Velayutham et al., 2017)
<i>Bacillus megaterium</i> KY848339.1	Acid Red	91%	(Ewida et al., 2019)
<i>Pseudomonas aeruginosa</i> (RS1) and <i>Thiosphaera pantotropha</i> ATCC35512	Reactive Yellow 145	96%	(Garg et al., 2020)
<i>Micrococcus yunnanensis</i>	Methyl Orange	98%	(Carolin et al., 2021)
<i>Acinetobacter baumannii</i> JC359	Reactive Black 5 Reactive Red 120 Reactive Blue 19	98.8% 96% 96.2%	(Ameenudeen et al., 2021)
<i>Bacillus stratosphericus</i> SCA1007	Methyl Orange	100%	(Akansha et al., 2019)
<i>Aeromonas hydrophila</i>	Reactive Black 5	76%	(El Bouraie & El Din, 2016)
<i>Bacillus cereus</i>	Orange II	52.5%	(Garg & Tripathi, 2013)
<i>Lysinibacillus sp.</i> KMK-A	Reactive Orange M2R	98%	(Chaudhari et al., 2013)
<i>Pseudomonas extremorientalis</i> BU118	Congo Red	36–94%	(Neifar et al., 2016)
<i>Kocuria rosea</i> MTCC 1532	Methyl Orange	100%	(Parshetti et al., 2010)
<i>Alcaligenes faecalis</i> PMS-1	Reactive Orange 13	100%	(Shah et al., 2012)
<i>Micrococcus glutamicus</i> NCIM-2168	Reactive Green 19A	100%	(Saratale et al., 2009)
<i>Klebsiella sp.</i> VN-31	Reactive Yellow 107 Reactive Red 198 Reactive Black 5 and Direct Blue 71	94%	(Franciscon et al., 2009)
<i>Pseudomonas sp.</i> SUK1	Reactive Red 2	80%	(Kalyani et al., 2009)
<i>Lysinibacillus sp.</i> RGS	Remazol Red	100%	(Saratale et al., 2013)
<i>Shewanella sp.</i>	Disperse Orange 3, Acid Red 88, Direct Red 81, Reactive Black 5	~100%	(Khalid et al., 2008)
<i>Bacillus aryabhatai</i> DC100	Coomassie Brilliant Blue G250, Remazol Brilliant Blue R	100%	(Paz et al., 2017)
<i>Sphingobacterium sp.</i> ATM	Direct Blue GLL	100%	(Tamboli et al., 2010)
<i>Klebsiella sp.</i> strain Y3	Methyl Red	~100%	(Cui et al., 2014)

best results were obtained from *Bacillus subtilis*, where eight dyes were cleaved at an efficiency of 71.8–100%. However, the consortium of all five bacterial strains was not as efficient and showed lower rates of biodegradation. Tizazu et al. (2022) obtained halotolerant and thermo-alkaliphilic microbial consortia from Rift Valley, Shala Hot Spring, Ethiopia, which had the capability to decolourise 98% Reactive Red 141 under anaerobic conditions. The consortia contained Bacteroidetes, Proteobacteria, Chloroflexi and Halobacterota, which were identified via 16S rRNA sequencing. Fareed et al. (2022) observed efficient decolourisation and degradation of Reactive Orange 16 and Reactive Black 5 by indigenous bacterial strain *Bacillus cereus* ROC collected from Industrial effluents. Adenan et al. (2022) isolated *Streptomyces bacillaris* for decolourisation

and degradation of Triphenylmethane dyes such as, Cotton Blue, Crystal Violet, Methyl Violet and Malachite green with the help of either biosorption or biodegradation by enzymes manganese peroxidase (MnP), NADH-DCIP Reductase enzymes etc. Biosorption of these dye molecules is done by interacting with the cell surface by attaching to hydroxyl, phosphoryl, amino, and sulfonyl groups on the cell surface. Ligninolytic bacteria produce various enzymes such as laccase, lignin peroxidase, and manganese peroxidase that have the ability to decolourize dye. Rahayu et al. (2023) isolated four ligninolytic bacterial strains named L1, L7, L8, and L11 that showed significant results. The bacterial strain L8 showed the best result in the decolourisation of azo dyes (Methylene Blue, Congo Red), which was 82.79% at optimum pH and temperature. Based on

16S rDNA studies bacterial strain L11 has been identified as *Bacillus paramycoides*. The above research studies have confirmed that different groups of bacteria are capable of degrading synthetic dyes with very high efficiency over a wide range of dyes. Extracellular or intracellular enzymes secreted by these bacteria play an important role in the degradation.

## 9 Biological Method of Dye Degradation

The method of removal/degradation of dye achieved by various activities of biological organisms such as bacteria is called the biological method. The residue of organic pollutants such as dye is either converted into atmospheric gases or into bacterial biomass. Bioremediation can degrade and decolourise dyes, thus producing non-polluting or less-polluting compounds. Bioremediation can be done using either aerobic bacteria or anaerobic bacteria. The final product of degradation by aerobic bacteria is usually CO<sub>2</sub> and the bacterial biomass, whereas by using anaerobic bacteria, the final product is methane (CH<sub>4</sub>), CO<sub>2</sub>, and bacterial biomass (Ghangrekar & Behera, 2014). The other method that results in dye decolourisation is biosorption, where the organic pollutant such as dye gets adsorbed, absorbed, or accumulated on the surface of the bacterial biomass. Biosorption causes the decolourisation of dye from wastewater (Moyo et al., 2022; Valli Nachiyar et al., 2023).

### 9.1 Anaerobic Method

The process via which the anaerobic bacteria are involved in the degradation of dye is called the anaerobic method of treatment. Here, the bacteria are added by the method of bioaugmentation. The microbes secrete crucial extracellular and intracellular enzymes that carry out decolourisation and degradation. The dye decolourisation and degradation treatment go through a series of events which include hydrolysis, acidogenesis, and methanogenesis. The starting point and the most important mechanism is hydrolysis which is carried out by the extracellular enzymatic secretion. The hydrolysis stage uses the extracellular enzymes secreted by microorganisms such as lipases for fatty acids, proteases for amino acids and proteins, cellulases for carbohydrates, etc. (Madondo et al., 2023). The basic building blocks are further hydrolysed with the help of bacterial

intracellular enzymes to form acetic acid, propionic, butyric and valeric acids etc. This process is also called acidogenesis. The products of hydrolysis and acidogenesis are converted to methane and carbon dioxide via the process of methanogenesis (Altowayti et al., 2022). In methanogenesis, the methanogenic bacteria breaks down the organic compounds to the simplest form (Madondo et al., 2023). Azoreductase enzyme, one of the intracellular enzymes in anaerobic bacteria is held responsible for the degradation and decolourisation of dye (Ikram et al., 2021). The sulfo and hydroxy group which is present in the ortho position of azo dye, makes the azo group more desirable for the degradation by azoreductase. AzoR gene isolated from *Klebsiella oxytoca*, produced oxygen insensitive azoreductase enzyme which was greatly efficient in the degradation of Methyl Red azo dye (Hua & Yu, 2019). Anaerobic treatment decomposes pollutants in the absence of oxygen and converts the pollutants into smaller and harmless products such as carbon dioxide, methane, and water. The methane produced can be utilised as a renewable source of energy which is considered as the best advantage of anaerobic treatment. Anaerobic treatment also utilizes less energy, sludge yield, and investment when compared to aerobic treatment. Disadvantages of anaerobic treatment are it produces a bad odour due to the presence of methane or hydrogen sulphide and has an efficiency lesser than aerobic in nutrient removal.

### 9.2 Aerobic Method

The treatment where aerobic bacteria degrade and decolourise dye in the presence of molecular oxygen (O<sub>2</sub>), by oxidising complex organic pollutants and in turn leads to production of carbon dioxide, simpler organic matter and bacterial cell biomass (Ahammad et al., 2020). The aerobic bacteria cannot use dyes as carbon sources. Thus, organic carbon sources are vital for this mechanism (Pandey et al., 2007). Bacteria have ability to break -N=N- bonds present in dye molecules via reduction and then use the byproducts as the sole Carbon source. Different species of aerobic bacteria such as *Pseudomonas nitroreducens*, *Vibrio logei*, *Bacillus sp.*, *Xanthomonas sp.* and *Sphingomonas sp.* could utilise dyes such as Acid Orange, Methyl Red, and Acid Red as organic Carbon and Nitrogen source. These bacteria contain oxidoreductase enzymes, which cause asymmetrical

and symmetrical cleavage of different dye molecules by processes such as deamination, hydroxylation, and desulfonation (Khandare & Govindwar, 2015). Peroxidase enzyme isolated from *Pseudomonas sp.* SUK1 was able to induce initial asymmetric cleavage of Reactive Red 2 dye (Kalyani et al., 2009). Aerobic treatment has many advantages. They produce less odour as compared to anaerobic treatment as aerobic treatment does not produce methane or hydrogen sulfide gas. They also are more efficient in removing nutrients from the pollutants. The disadvantage of aerobic treatment is it consumes a high amount of energy and has more maintenance costs. Aerobic treatment also produces undigested solid wastes. Thus, extra cost is used to remove those undigested solid wastes.

### 9.3 Anaerobic–Aerobic Method

Anaerobic Bacteria have the ability to break down dye molecules but form recalcitrant and carcinogenic secondary pollutants such as aromatic amines from azo reduction. Whereas aerobic bacteria need organic carbon and nitrogen sources for their activity and are not efficient in breaking dye molecules. The aerobic bacteria utilize secondary pollutants produced by anaerobic bacteria as carbon and nitrogen sources and produce residues that are environment friendly. Thus, the combination of anaerobic and aerobic bacteria is proven to be very efficient in the cleavage of dye molecules and is a cost-effective and green method of degradation (Khandare & Govindwar, 2015). Degradation of textile dyes by the combination of anaerobic–aerobic mechanisms was observed where Disperse Blue 79 (DB79) was cleaved in the anaerobic phase and produced aromatic amines which were then broken down in the aerobic phase and produced non-toxic residue (Melgoza et al., 2004).

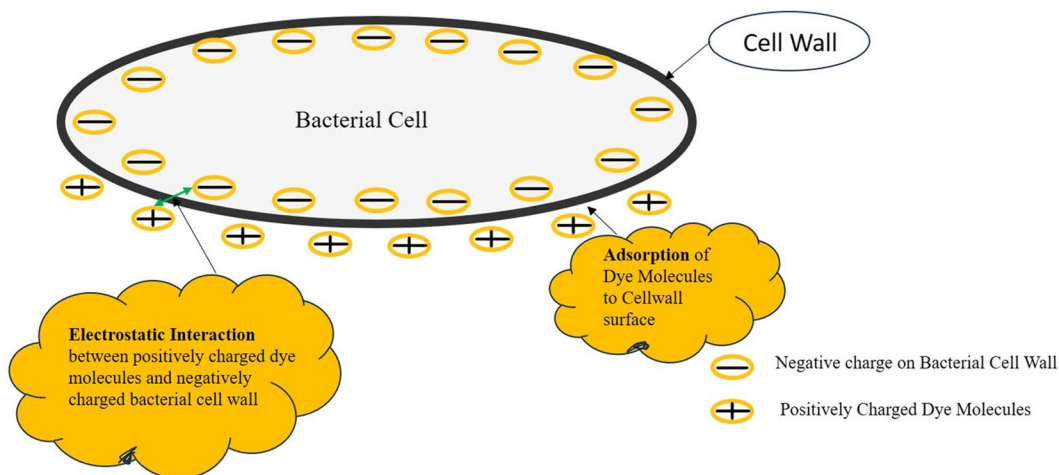
## 10 Biological Mechanism of Dye Degradation

The mechanism of dye degradation and decolourisation depends on the environmental conditions such as aerobic and anaerobic conditions. Different species of aerobic and anaerobic bacteria show the capability of decolourisation and degradation. Some examples of bacteria that could decolourise and degrade dyes are *Bacillus subtilis*, *Pseudomonas sp.*, *Lactobacillus sp.*,

*Staphylococcus sp.*, *Enterococcus sp.*, *Morganella sp.*, *Corynebacterium sp.*, *Xenophilus sp.*, *Rhodobacter sp.*, *Clostridium sp.*, *Rhizobium sp.*, *Micrococcus sp.*, *Dermaococcus sp.*, *Shewanella sp.*, *Klebsiella sp.*, *Proteus sp.*, *Alishewanella sp.*, *Aeromonas sp.*, *Acinetobacter sp.*, *Alcaligenes sp.*, *Escherichia coli* (Ajaz et al., 2019).

Biosorption happens when the dye compound binds itself to the bacterial cell wall by means of various interactions such as electrostatic interaction, chemical adsorption, chelation, complexation, and micro-precipitation. The mechanism of biosorption is illustrated in Fig. 3. The positively charged dye compounds tend to have an affinity towards negatively charged bacterial cell walls via electrostatic interactions. Gram-positive bacteria have a thicker cell wall due to the presence of peptidoglycan, teichoic acid, etc.; thus, they are efficacious in trapping complex compounds (Bharathi et al., 2022). Biosorption can have both advantages as well as disadvantages. Advantages include cost-effectiveness, mechanical strength, durability, revival and reuse of adsorbent, and the affinity of specific dyes to binding with specific microbial biomass. Disadvantages include biosorption cannot be operated on all dyes (Elgarahy et al., 2021; Sivarajasekar & Rajoo, 2015).

The mechanisms that take part in decolourisation and degradation by the biological method are based on either enzymatic processes, chemical reduction by organisms that have the capability to reduce dyes, or by integrating both (Pandey et al., 2007). The main mechanism adopted by anaerobic and aerobic bacteria for biodegradation is the involvement of enzymes secreted by the organism intracellularly or extracellularly shown in Fig. 4. Extracellular enzymes secreted by bacteria attach to the surface of dye compounds, resulting in the formation of smaller fragments that either get used as carbon sources to give up CO<sub>2</sub> or can be incorporated into cells and then degraded via classical pathways of the enzyme inside of cells (Mohanan et al., 2020). The fragmented compounds when taken up or incorporated into cells are catalyzed by intracellular enzymes. Intracellular enzymes catalyze the recalcitrant complex bonds via reduction or oxidation processes. Hazardous coloured compounds get converted into decolourised compounds by breaking the chromophoric bonds by utilizing various enzymes and energy in the form of Nicotinamide Adenine Dinucleotide Phosphate Hydrogen (NADPH), Nicotinamide Adenine

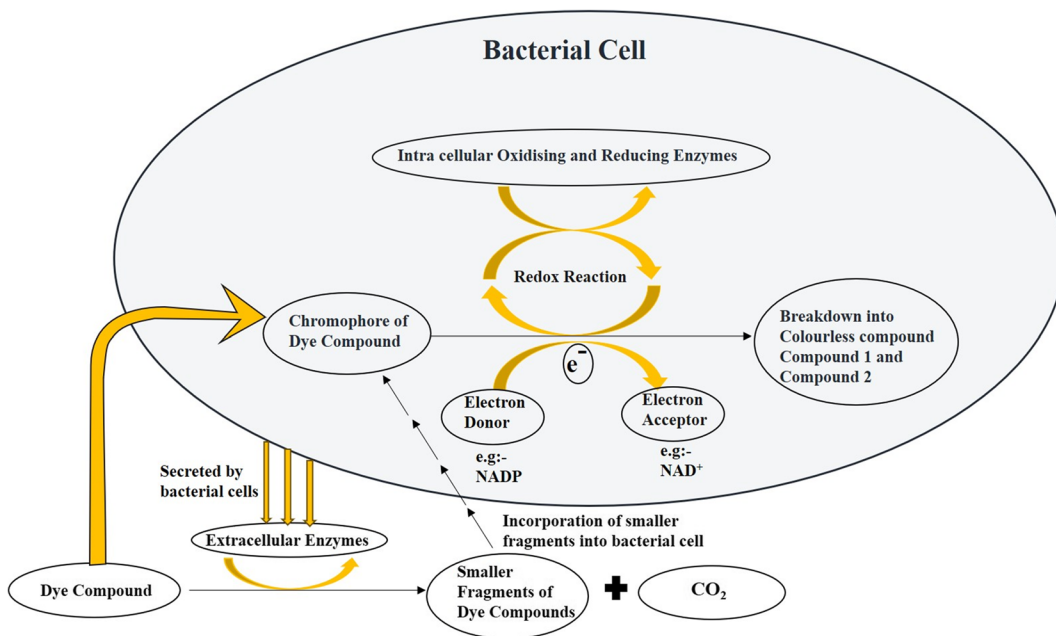


**Fig. 3.** Mechanism of biosorption

Dinucleotide Hydrogen (NADH) and Adenosine Triphosphate (ATP), (Bharathi et al., 2022). Oxidizing enzymes could degrade textile dyes whereas reducing enzymes could break dye complex. Some examples of oxidizing enzyme and reducing enzymes are lignin peroxidase, laccase, tyrosinase and azo reductase, flavin reductases, and Dichlorophenolindophenol (DCIP) reductase respectively (Khandare & Govindwar, 2015).

Enzymes used for degradation are capable of lowering the activation energy, thus making the reaction rapid and effective (Saravanan et al., 2021).

**Lignin Peroxidase (LiP)** also called as 1, 2-bis(3,4-dimethoxyphenyl) propane-1,3-diol: hydrogen-peroxide oxidoreductase is a 38–47 KDa enzyme leading to lignin oxidative breakdown. The first step in the breakdown process is the utilization of H<sub>2</sub>O<sub>2</sub> and other substrates



**Fig. 4.** Enzymatic mechanism of degradation and decolorisation



as electron sources, which leads to the breakdown of the substrate and the completion of the catalytic cycle. Secondary metabolites are utilized as cofactors while interacting with lignin present in Pollutants. LiP breaks down lignin by forming a cleavage at the  $\beta$ -O-4 linkage, which consists of around 50% of all the linkages. Lignin Peroxidase contains two glycosylating sites, two sites for binding of Calcium ions ( $\text{Ca}^{2+}$ ) and four sites of disulfide bonds which stabilise their structure (Singh et al., 2024). Dye compounds can be oxidized by this enzyme at their methanol or heme iron position by using hydrogen peroxidase as an electron donor to form radical cation (Khandare & Govindwar, 2015). Peroxidase breaks the dye compound bond as they have a long chain electron transition mechanism (Bharathi et al., 2022). A strain named *Bacillus sp.* React 3 was observed for its capability of biodegradation of Methylene Blue dye. LiP produced by them, along with their capability of biosorption, were able to biodegrade 99.5% Methylene Blue in 48 h (Pham et al., 2022).

**Laccases** or multi-copper oxidases are a group of multi-copper containing enzymes (60–75 KDa) that show efficient degradation capability via dye oxidation (Bharathi et al., 2022). During the dye oxidation reaction, four copper atoms become essential, and at the end, oxygen becomes the last receptor of the electron (Agarwal et al., 2022). Laccase utilises the aggregated form of four copper ions (de Gonzalo et al., 2016) and dioxygen as an electron acceptor in the oxidation reaction of dye molecules, resulting in the formation of free radicals, which further engages in demethylation, depolymerization, polymerisation and quinone formation (Khandare & Govindwar, 2015). Laccase can be found in bacteria such as *Streptomyces lavendulae*, *Streptomyces cyaneus*, *Marinomonas mediterranea*, and *Pseudomonas sps.* etc. (Kanagaraj et al., 2015). Enzyme laccase found in *Pseudomonas desmolyticum* NCIM 2112 showed 100% decolourisation of dyes such as Green HE4B, Direct Blue-6, and Red HE7B (Kalme et al., 2009). A novel laccase LacHa isolated from *Halomonas alkaliantartica* and expressed in *E.coli* at optimum temperature and pH was able to decolourise dyes such as indigo carmine and two other azo dyes at a rate of 90.28–100% (Wang et al., 2023).

**Tyrosinase** also known as Polyphenol oxidase, is a group of copper-dependent enzymes which has a vital role in pigmentation of all organisms. Tyrosinases induce hydroxylation at the ortho position of phenolic substrates such as tyrosine and catechol and lead to an

oxidation reaction which results in the formation of quinones such as dopaquinone and *o*-benzoquinone by reducing oxygen to water. The active site of this enzyme is associated with different substrates of the reaction (Phenolic compounds and oxygen). It converts from one oxidation stage to another into three different states (oxy form, met form, deoxy form). Tyrosinase is an oxy state attached to oxygen and hydrolyses monophenols to diphenols, converting them into met form. Then, met form, the most stable form of tyrosinase, converts itself into deoxy form by oxidising diphenols to quinones. The deoxy form binds to oxygen and converts itself to oxy form. Tyrosinase is very efficient in breaking down phenolic compounds and dye molecules (Faccio et al., 2012). A strain of bacterium named *Pseudomonas aeruginosa strain* BCH at optimum temperature and pH was able to decolourise Direct Orange 39 by secreting enzymes such as DCIP reductase, Lignin peroxidases and tyrosinase (Jadhav et al., 2010). Enzyme tyrosinase rTYR found in *Bacillus aryabhatai* showed remarkable efficiency in decolourising several dyes and degradation of dye-containing effluents (Wang et al., 2021).

**Azoreductase** is the most prominent oxidoreductase enzyme for the biodegradation of azo dyes. The breakdown of azo dye is carried out by reductively cleaving azo bonds ( $-\text{N}=\text{N}-$ ) by utilising electron donors such as  $\text{FADH}_2$ , NADH, etc. This mechanism also involves two stages known as the two-cycle ping-pong mechanism, where each stage leads to the transfer of two electrons to the ultimate electron acceptor. The azo dyes are converted into transitional metabolites such as aromatic amines. These aromatic amines are degraded further by aerobic or anaerobic processes. These enzymes are classified on the basis of their position in the cell such as cytoplasmic or membrane-bound (Ajaz et al., 2020). In the case of aerobic degradation, azo reductase induces a reduction reaction by utilizing molecular oxygen and reducing equivalents such as flavin adenine dinucleotide dihydrogen ( $\text{FADH}_2$ ), flavin mononucleotide (FMN), NADH, NADPH, etc. Azoreductase enzymes found in various organisms were either monomeric or homodimeric. Flavin-free azoreductase was monomeric, whereas FMN-dependent AzoR from *E. coli* and AzrA from *Bacillus sps.* existed in homodimeric form (Misal & Gawai, 2018). Azoreductase gene K (AzK) was isolated from *Klebsiella pneumoniae* in which the enzyme azoreductase shows efficiency in breaking down Remazol red R (RRR) and Acid Blue 29 (AB-29) (Mustafa et al., 2023).

**Flavin Reductase** also known as riboflavin reductase, is a class of enzyme that is responsible for the breakdown of the -HC-NH- bond, which acts as a donor with NADP<sup>+</sup> or NAD<sup>+</sup> as an electron acceptor. They can also be known as Flavin-mononucleotide (FMN) reductase, NADPH-flavin oxidoreductase, NADPH-dependent FMN reductase, NADPH<sub>2</sub> dehydrogenase etc. They utilize reduced riboflavin and NADP<sup>+</sup> as substrates and form three products namely riboflavin, NADPH, and H<sup>+</sup> (Khandare & Govindwar, 2015). This enzyme goes through two cycles of NADPH-dependent reduction of FMN to FMNH, which results in the conversion of dye compounds to hydrazine and then converts them to amines. This mechanism is also known as the Ping-pong Bi-Bi mechanism. Flavin Reductase enzyme is specific to a broad spectrum of substrates in regards to the structure and dimensions of the dye compounds. They can be proved as a potent group of enzymes as they are not specific to any dye group (Mahmood et al., 2016). Enzyme Flavin Reductase found from *Sphingobacterium sp.* ATM could decolourise and degrade textile dye Direct Blue GLL (Tamboli et al., 2010). *Aeromonas hydrophila* SK16 produced Enzyme Riboflavin Reductase which is responsible for the degradation of Reactive Red 180, Reactive Black 5, Remazol Red (Thanavel et al., 2018).

**The NADH-DCIP Reductase** is a reducing enzyme that is responsible for decolourisation and detoxification of dyes. Nicotinamide adenine dinucleotide-dichlorophenolindophenol is a prominent enzyme biomarker for the degradation of dyes. They can also detoxify many xenobiotic molecules (Haque et al., 2021). NADH-DCIP reductase has a role in reductive cleaving of -N=N-bonds (Al-Tohamy et al., 2020). The enzyme NADH-DCIP leads to the reduction of DCIP by utilising an electron donor (Selvaraj et al., 2021). These are responsible for the reduction of blue-coloured DCIP to colourless solution by utilizing NADH as an electron donor. NADH-DCIP reductase found in *Bacillus sp.* VUS was responsible for the complete decolourisation of orange T4LL after 24-h incubation (Khandare & Govindwar, 2015).

## 11 Future Prospective

Synthetic dyes used in textile industries have caused havoc by polluting water bodies. These non-biodegradable dyes cause harmful effects on the environment. Thus,

the degradation of such dyes is very essential for the sustainability of the environment. Degradation of dyes can be accomplished by physical, chemical, and biological methods. Physical and Chemical methods are extortionate, laborious, and may cause secondary pollution, whereas the bioremediation approach is environment friendly, needs less labour, and is cost-effective. Bioremediation done by bacteria is advantageous over other living organisms as they are easily cultured and multiply at a faster rate. Bioremediation uses biosorption and enzymatic activity for dye degradation, which doesn't disturb natural processes and thus is sustainable. However, more research should be performed to screen and identify bacterial strain that shows higher efficiency of dye degradation. Genetic engineering can play a very important role in the field of bioremediation in the future. Genetic engineering focuses on the discovery of genes encoding enzymes responsible for the biodegradation of various pollutants, including dyes. This empowered researchers to design bacteria with significantly advanced capabilities, enabling them to target and destroy specific pollutants efficiently. Genetically engineered bacteria can be deployed in contaminated areas through bioaugmentation or biostimulation strategies to increase the efficiency of existing bioremediation processes. Genetically engineered bacteria also strengthen the environmental resilience of bacteria, which provides a robust solution to environmental challenges. Microbial consortia are also playing an important role in the field of bioremediation. The consortium of desirable bacterial strains has shown better efficiency than individual strains. It is important to understand the structure, composition, dynamics, and interactions within consortia to optimize bioremediation strategies so that environmental cleanup can be achieved in the most effective manner. Thus, research should be done on the development of effective dye degrading bacterial consortia and to observe their efficiency. One major concern also associated with bioremediation is their controlled growth in contaminated sites. So, research should also be done to restrict the uncontrolled growth mechanism of efficient bacteria. Some bacterial strains are efficient dye degradants, yet are slow growing, then they can be genetically modified to become fast-growing and thus degrade dye more rapidly. Awareness about bioremediation processes is not widely spread. Research is being held on bioremediation of dyes on a laboratory scale, but it should be commercialized and should be used on an industrial scale for the awareness to be spread.



## 12 Conclusions

Bioremediation technology removes pollutants using microorganisms. At present, many microorganisms are used to degrade textile dyes. Some of these researched bacteria are *Acinetobacter sp.*, *Aeromonas sp.*, *Bacillus sp.*, *Clostridium sp.*, *Escherichia sp.*, *Klebsiella sp.*, *Lactobacillus sp.*, *Micrococcus sp.*, *Proteus sp.*, *Pseudomonas sp.*, *Shewanella sp.* and *Staphylococcus sp.* Numerous research studies have confirmed that these bacteria are capable of up to 100% dye degradation of a broad range of dyes. Extracellular or intracellular enzymes of various bacterial origins, such as azoreductase, flavin reductase, laccases, lignin peroxidase, NADH-DCIP reductase and tyrosinase are responsible for the degradation of the dye. While genetically modified bacterial strains offer significant potential to advance bioremediation strategies and aid in the clean-up of contaminated sites, their deployment also brings up important considerations regarding biosafety and environmental impact. These considerations underline the need for careful planning and responsible use of this technology. Bioremediation can take more time than physical and chemical degradation, but it is more efficient, cost-effective, and less laborious than them. It needs skilled labour; people will have to be made aware of the process, and they can be trained. Bioremediation technology needs specific nutrients, pH, temperature, and proper environmental conditions, so the process can be a little tedious. However, trained and skilled labourers can maintain the proper environment, which is very advantageous in the long run. In conclusion, bioremediation is a simple, safe, fast, and environment-friendly technique for removing pollutants including dye. However, through new research techniques for developing genetically modified bacteria, consortia development and optimising the favourable environment for bioaugmentation can enhance the biodegradation capability of bacteria, which will make bioremediation an efficient method for the degradation of dye in the future.

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**Data Availability** The authors state that the data supporting the findings of this study is available within the article and can be obtained from the corresponding author upon reasonable request.

## Declarations

**Ethical Approval** Not applicable.

**Consent to Participate** Not applicable.

**Consent for Publication** The paper is submitted with the consent of all authors.

**Competing Interests** All authors declare no competing interests.

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