

# MICROBIAL BIOREMEDIATION OF TOXIC AZO DYES: AN ECOLOGICALLY SUSTAINABLE APPROACH

## Abstract

A diverse spectrum of synthetic dyes produced by the industries endangers environmental conditions. Because of their stability, colour fastness and resistance to deterioration, existing effluent treatment technologies are incapable of entirely removing resistant azo dyes from effluents. Xenobiotic chemicals, such as azo dyes, are resistant to biodegradation. Furthermore, bacterial colour reduction and degradation of azo dyes is quite effective, resulting in total removal of these contaminants from wastewater. Additionally, this treatment strategy has shown promising results in industrial applications, making it a viable option for large-scale dye removal processes. In addition, the article discusses the factors influencing the bacterial decolorization efficiency, such as temperature, pH and dye concentration. Furthermore, it highlights the potential applications of bacterial azo dye degradation in industrial wastewater treatment and emphasizes the importance of further research in optimizing these processes for practical implementation.

**Keywords:** Decolorization, Degradation, Azo Dyes, Recalcitrant, Metabolites.

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## I. INTRODUCTION

Dyes are coloured substances (typically synthetic) that are widely utilised in the pharmaceutical, textile, cosmetics, leather, paint and food industries [1]. The dyeing industry is a major source of water pollution all over the world. While most commonly azo dyes are found in the various sectors such as the pharmaceutical, food, paper and printing, leather, and cosmetics [2-3]. Azo dyes are presumed to be simple to use, inexpensive, and capable of producing bright, vibrant colors [4]. Due to their numerous applications, colorfastness, and versatility, the textile manufacturing sectors rely heavily on azo dyes. [5]. The fast development of the textile dyeing and dyestuff manufacturing sectors has raised the amount and complexities of the effluent released into the environment. However, the sad reality is that the need for azo dyes is linked to a significant amount of outflow, primarily in the form of recalcitrant compounds, which pose a serious threat to all organisms present in an ecosystem due to the *pernicious* characteristic of these dyes and their intermediate metabolites, which are highly mutagenic and carcinogenic. Exposure to these harmful substances has also been linked to a higher probability of respiratory disorders such as asthma and chronic obstructive pulmonary disorder. Additionally, studies have shown that prolonged exposure to these chemicals can lead to hormonal imbalances and even contribute to the development of certain types of cancer, including lung and liver cancer [6]. The presence of these colors can have a detrimental effect on the accumulation of sludge, which makes it difficult to properly dispose of it and causes environmental damage. They affect the aquatic ecosystem by altering the pH of rivers, lowering oxygen levels, and reducing light penetration. These factors hinder photosynthesis [7-8]. The visual attraction of the water for reuse is likewise diminished by color. Farmers in developing countries are forced to use textile effluent, which frequently includes levels of pollutants from dyes, due to the fall in the availability of freshwater [9]. It is urgent to design an effective treatment for industry effluents due to the elevated levels of contaminants in wastewater, which constitute a serious threat to the environment.

## II. HISTORY OF DYES

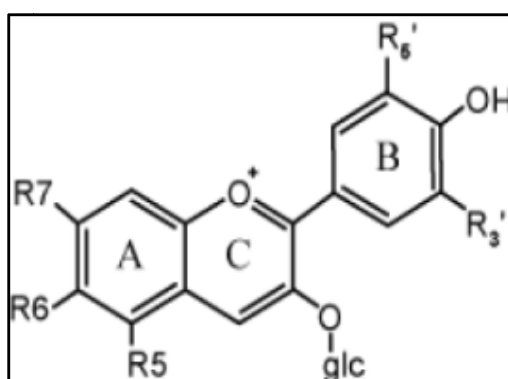
Since Ancient Egypt, humanity has been utilizing natural and manmade dyes to color clothing for more than 4000 years. Throughout history, go further into the history of dyeing and the materials used to create various colors [10]. Consider a world devoid of color, or more precisely, without colored apparel. There will be no black gowns. There will be no green headgear or red shirts. There is no color it's difficult to envision a wardrobe devoid of a rainbow of colors and tints. Fortunately, this was not a reality that our great ancestors faced. The first documented reference to cloth dyeing is from 2600 BC. Historically, dyes were created using natural pigments combined with water and oil and applied on the skin, jewelry, and clothes. Natural dyes were formerly utilized in caves in areas like Spain [11]. Today, 90% of clothes are synthetically colored. Synthetic dyes are employed so extensively in the production process that critics assert that the color of China's rivers may reveal the following season's current hue. It is thought that over 10,000 unique colorants are utilized in the industry today, with over 735 tonnes of synthetic dyes produced annually. Interest in green fashion or concern for workers reintroduced natural dyeing as a concept and art form [12]. The most widely used dyes in ancient times were discovered nearby. As a result, distinct colors often denoted distinct geographical areas. The hues were created by combining exotic flora, insects, and marine life. Indeed, the term "crimson" is derived from kermes, a

Mediterranean bug found on oak trees. Yellow was the most prevalent hue in the ancient world and could be seen in a variety of different places. Other hues, such as blue generated from indigo, have been discovered in plants native to India and Southeast Asia. However, it is the color purple that has had the most impact on history. It is the ideal introduction to the 545-year history of fabric and dyes, from its usage as a symbol of status (Roman emperors frequently wore purple) through the invention of synthetic colors and its role in establishing synthetic coloring over the world. Often, it was worth gold [11]. Purple could only be obtained by crushing thousands of Murex mollusk shells, which are located Mediterranean shore. As a result, it became, and continues to be, the costliest colors to mix. When Alexander the Great invaded Persia, he discovered purple-dyed garments and was so taken with the colors that they became synonymous with royalty [10].

### III. CLASSIFICATION OF DYES

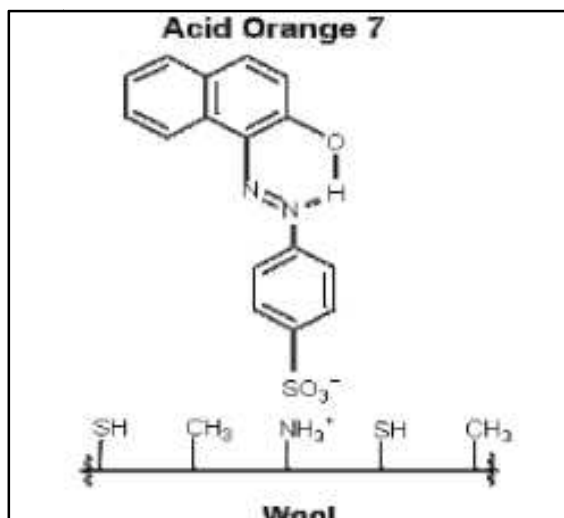
- 1. Natural Dye:** Pigments generated from natural sources are used to create natural colors. However, and they were formerly the dominant source of color, they have been largely displaced by dyes that are more reliable, less costly, more environmentally friendly versatile, and readily accessible. Still in use are natural dyes like hematoxylin, carmine, and orcein [13].

**Each dye is named according to the pattern: Natural + base color + number**



**Figure 1:** Natural dye

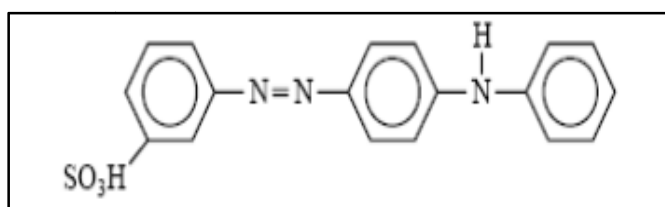
- 2. Synthetic Dye:** The term "synthetic dyes" refers to those that are synthesized from organic or inorganic substances. Direct, Acid, Basic, Dynamic dye, Madder, Related compounds, Vat, Sulphur and disperse dye all are examples of dyes that belong to this family. Synthetic dyes quickly supplanted natural dyes [13].



**Figure 2:** Acid Orange 7

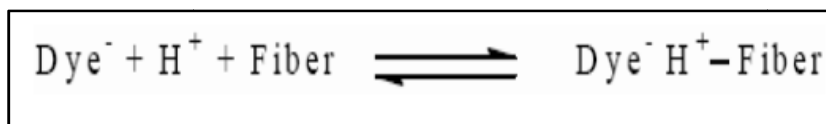
They were cost effective, introduced a variety of new colours, and intensified the qualities of colored substances. Dyes are classified based on their use during the dyeing process bath.

- Acid Dye:** Acid dyes are an anionic dye class that comprises one or more sulfonic acidic side chains as well as other acidic groupings concentrated hydrochloric Yellow 36 is just a member of the group [14].

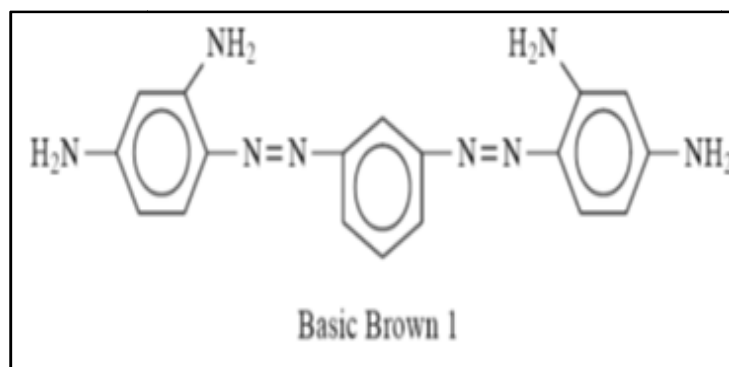


**Figure 3:** Acid Yello36

Acid dyes have little effect on cellulosic fibers. This category includes the majority of synthetic food colors. The process of dyeing is summarized as follows:

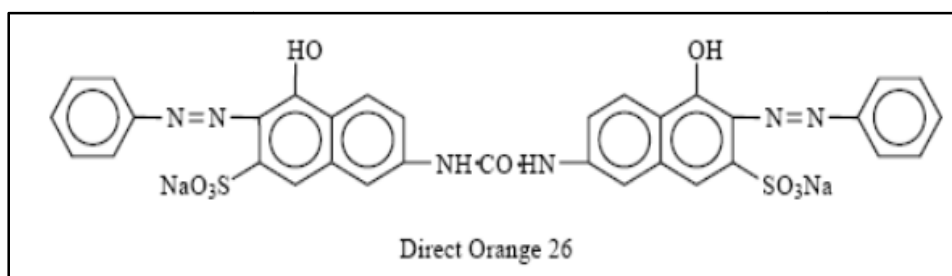


- Dye, Either Basic or Cationic:** This category of synthetic hues was the first to be derived from tar-coal compounds as textile dyes, they have mostly been supplanted by subsequent advances. In addition to discharge printing, they are also used for leather, paper, wood, and straw processing. They have recently been successfully used with a variety of prepared textiles, particularly acrylics. Basic dyes provide acrylic fabrics with vibrant colors that dry very quickly. That is used on fundamentally dyeable polyester varieties.



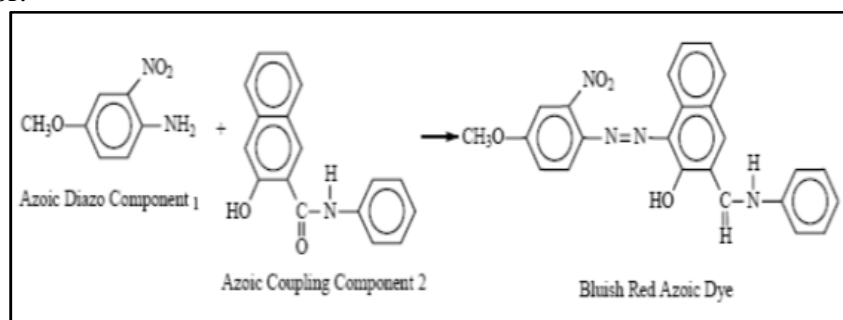
**Figure 4:** Basic Brown 1

5. **Direct Dye:** This is especially beneficial for materials that may form hydrogen bonds during the dyeing process. Immediate dyes, largely basic dyes, were lauded for their ability to dye cotton without the use of a binder or mordant. Typically, these dyes include an azo link – N=N – plus a molecular weight. Because sulfonic acid groups are present, they are water-soluble [15]. A well-known example is direct orange 26.



**Figure 5:** Direct Orange 26

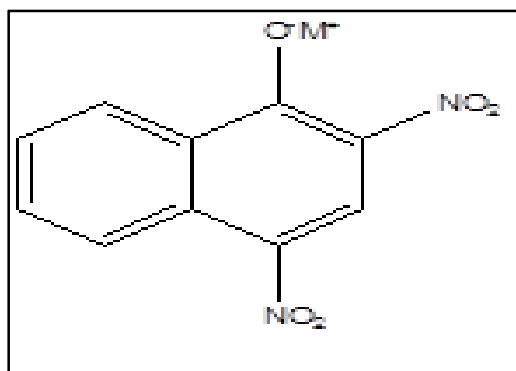
6. **Azoic Dye:** Typically, textile dyeing gets vivid red colors, since the bulk of other fast dye classes is lacking in outstanding red dyes. To generate Azoic dyes, which are often referred to as naphthol within the industry, are one kind of dye that is put into the cloth. The other half is next painted, when the two colors are combined to get the final hue. Unless veneers are correctly put and meticulously polished, they have a low resistance to rubbing and crazing [16]. The two components listed below are used to create a blue-red azoic color.



**Figure 6:** Components Used to Create a Blue-Red Azoic Color

7. **Nitro Dye:** Nitro is a type of polynitro phenol derivative with at least one orthogonal or perpendicular nitro group towards the hydroxyl group. That it is composed of at least two aromatic rings (benzene, naphthalene) [17].

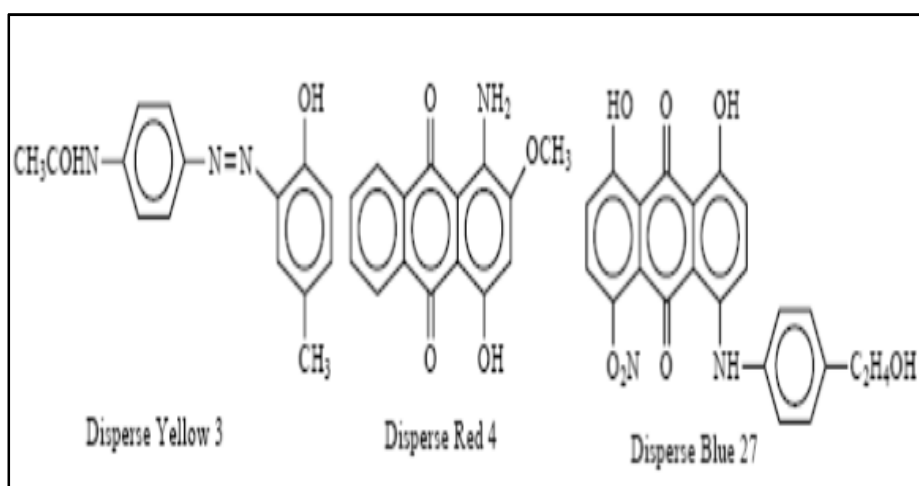
**Example:**



**Figure 7:** Maritus Yellow

8. **Disperse Dye:** Disperse dyes were initially created for coloring secondary hollow fibers. Due to their intractable nature in water, these colors must be crushed into a fine powder with dispersing agents present. A concentration of dye particles in a dye solution generates a combination of colors that Fibers absorb after that. This dye class is used to color polyester, acetate, triacetate and nylon fibers. Other great dispersion dyes are Dissipate Disperse Blue 27, Yellow 3, Distribute Red 4, or Disperse Red 4.

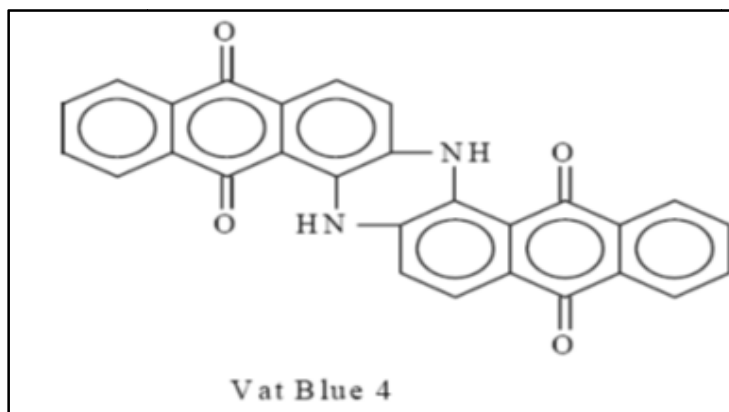
**Example:**



**Figure 8:** Disperse Dye

9. **Vat Dye:** Vat dyes are polycyclic chemicals with a complicated structure resembling quinones in their insoluble structure. The name "vat" originates from an ancient indigo dyeing method that requires lowering the blues to a light state. Indigo, carbazole and anthraquinone are utilized in the manufacturing of vat dyes. In addition, spot colors are

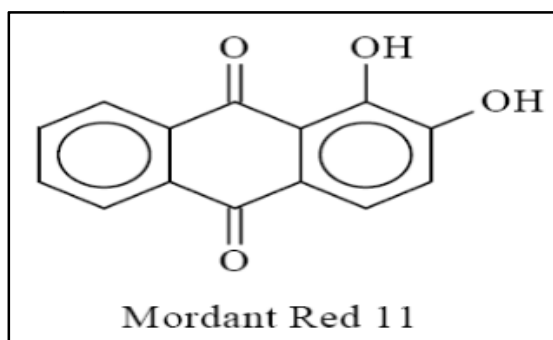
used in the process of continuous item coloring, commonly known as color application. These colorings are very washable and lightfast [18].



**Figure 9:** Vat Blue 4

**10. Mordant Dye:** The techniques of fabric dyeing don't directly color the fabric, but rather employ a mordant, a binding agent. The mordant functions as a binder in this example, between the dye and fiber. Certain colors react with metal salts to form insoluble color complexes (mordanting) (lakes). Within the fiber, the metallic precipitate develops, giving in exceptionally bright colors that are highly resistant to light and washing [19].

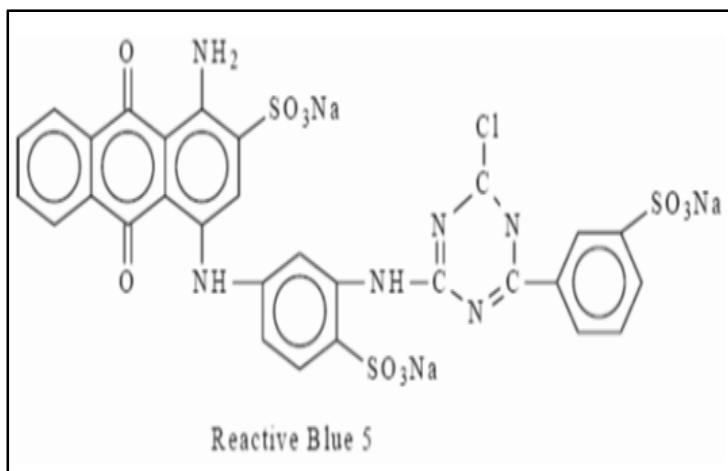
**Example:**



**Figure 10:** Mordant Dye

**11. Reactive Dye:** These pigments form a covalent bond with the cellulose fiber. This produces colored fabrics with exceptionally high textile dyes that have become very interacts immediately along with hydroxyl or carboxy group of fiber. As a result of the chemical reaction, the color dries rapidly on an extended shelf. In the dyeing business, reactive dyes come in a variety of varieties [20].

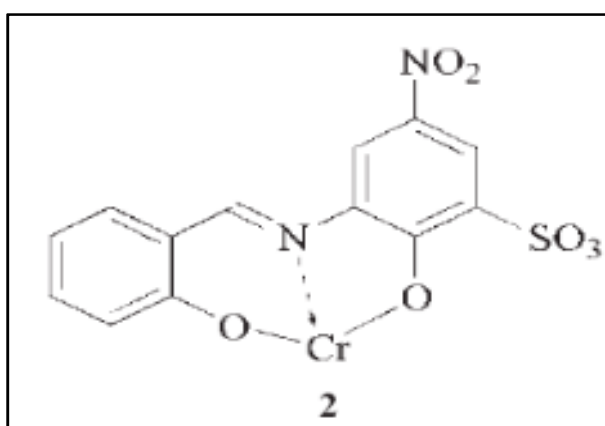
**Example:**



**Figure 11:** Reactive Blue 5

**12. Solvent Dye:** Those pigments are soluble in drinks, and chlorinated hydrocarbons, especially liquid ammonia, but resistant in water. By disintegrating particles in the substance, which in itself is undoubtedly a lipid or other non-polar solvent, they are administered. This classification and naming technique are utilized by the Color Index. The dye is labeled with the following information: solution + base hue + number. They have been applied to dye synthetic materials, plastics, gasoline, oils, and waxes [21].

**Example:**

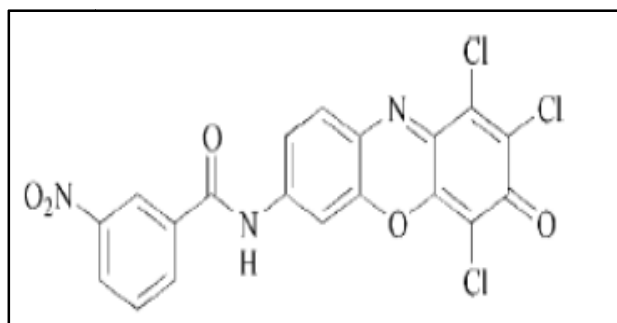


**Figure 12:** Solvent Yellow32

**13. Sulfur Dye:** Colors that are extremely resistant to washing but not to sunlight. A disadvantage of Sulphur dyes, particularly dark hues, is that they make the cloth fragile or impair its structure, making it brittle. They are inexpensive and have a high resistance to light, washing, and chemicals [22].



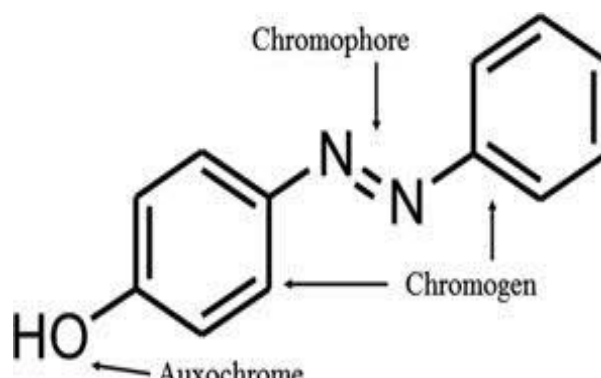
**Example:**



**Figure 13:** Sulfur Red 7

#### IV. AZO DYES

Azo dyes are a particularly common type of dye and are utilised extensively in the food, textile, printing, and cosmetic sectors. According to IUPAC, azo compounds are diazene (diamide) derivatives with the formula  $\text{HN}=\text{NH}$  with hydrocarbonyl groups replacing both hydrogen atoms. Two aryl groups are included in the more stable versions. Azo groups are what the  $\text{N}=\text{N}$  group is known as. The word "azo" is derived from the French word "azote," which is a Greek-derived name for nitrogen. The compound loses its hue when the azo bond is broken [23]. These dyes are made up of two key components [24]:



**Figure12:** Structure of a Typical Dye

- 1. Chromophore:** Chromophores, which are groups found in azo dyes, are what give dyes their colour because of their capacity to capture light in the near ultraviolet range. The dye's apparent hue is ultimately controlled by the absorption and reflection of both ultraviolet and visible light [25]. Common examples of chromophores are:  $-\text{N}=\text{O}$ ,  $\text{C}=\text{O}$ ,  $-\text{C}=\text{N}$ ,  $-\text{C}=\text{S}$ ,  $-\text{N}=\text{N}-$ ,  $-\text{NO}_2$ ,  $(\text{CH}=\text{CH})_n$ .
- 2. Auxochrome:** Through the use of strong chemical bonds created by certain groups known as auxochromes, these dyes can adhere to the fibers. They could have an acidic or basic composition. Examples include:  $\text{OH}$ ,  $\text{COOH}$ ,  $\text{SO}_3\text{H}$  (acidic),  $\text{NH}_2$ ,  $\text{NHR}$ , and  $\text{NR}_2$  (basic). A chromophore devoid of autochrome is unable to work as a dye. The majority of synthetic colorants, comprising between 60 to 70 % of all dyes manufactured, are azo dyes [26]. Azo dyes are characterized by the existence of reactive groups capable of

creating covalent bonds with textile substrates. The energy required to break these connections is equal to that required to degrade the support. Azo dyes are classified chemically and have certain structural features. On the one hand, it is demonstrated by synthesizing a huge proportion of azo dyes utilizing the most common method, which includes diazotizing a tertiary amine but does not involve coupling the resulting anion salt with a typically aromatic substrate. On the other hand, generating both symmetrical and asymmetric azo dyes [27].

## V. HAZARDOUS EFFECT OF AZO DYES

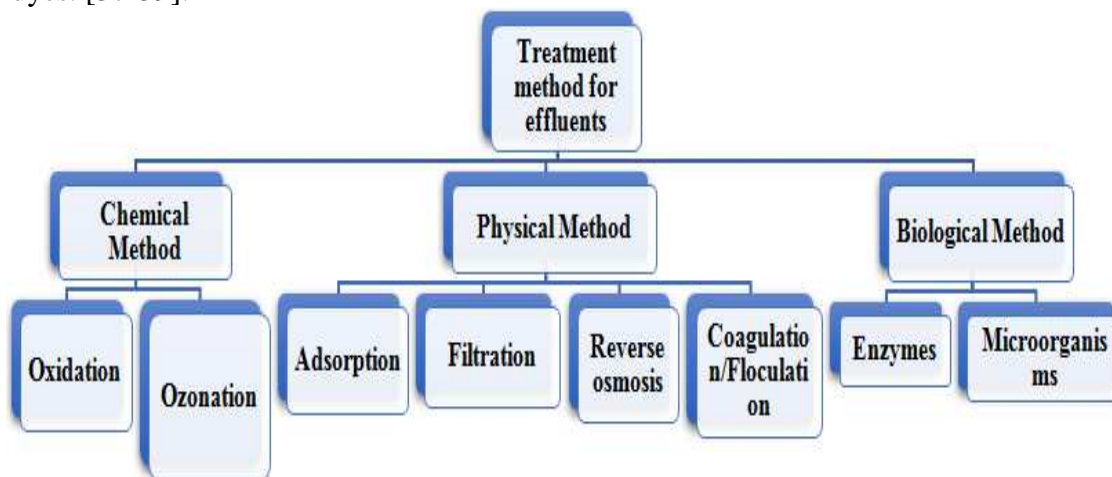
After dyes have colored things, Industries that use dyes often retain dyes from wastewater in industrial trash. These contaminants are therefore pushed into surrounding water bodies, transforming clear, white solid water in and out of contaminated, colored water. Many manufacturers should treat effluent containing color prior to releasing it into the ecosystem. Dye contamination of water is seen negatively by environmentalists and also by the general population. Owing to the dangers associated with these harmful compounds. Immediately after dyeing methods, color effluents with such occasions, high pH (acidic), and temperature are discharged. This occurrence will impact the mechanism for gas transfer and the self-purification of water bodies in the scientific environment. By damaging water sources, effluents constitute harm to the environment when they are vented into the atmosphere after use and restrict the use of water. Once dye effluents are blended with naturally occurring water sources, a foul odor is produced. odor is produced, in addition to generating an eye sore for humans. Starting with aquatic creatures and plants, textile effluents may also impact land-based life because they are less dense (0.8 kg/m<sup>3</sup>) than water (1.0 kg/m<sup>3</sup>), dye effluents may couple together., and total water dispersion rises with water sources. This limits the quantity of sunlight accessible for processes such as photosynthesis and respiration by aquatic organisms, thereby ending their life. The disposal of effluents into the environment degrades the ecosystem over time and contributes to human health concerns [28]. Once eye, both dogs and people may experience eye burns or irreversible eye damage. Chemicals like dye effluents that are deposited in water sources may evaporate into the environment, resulting in breathing difficulties or difficulty breathing upon ingestion. Consumption of colors may cause increased sweating, dizziness, methemoglobinemia, oral burns, nausea, and vomiting. Thus, cleaning wastewater containing harmful dye effluents is critical to avoiding severe effects on receiving waterways, animals, and people [29]. The environmental legislature has passed legislation prohibiting the presence of harmful colored effluent throughout bodies of water. The International Dye Industry Final Effluent Standards Of quality, which were amended from the minimal release of toxic Components, are required by this law for dye-using firms to make sure that the wastewater discharged from their activities fulfils these standards. Program (ZDHC) [30]. Textile wastewater has a high concentration of contaminants, including the level of oxygen (BOD), chemical oxygen uptake (COD), dyes, poisonous chemicals, and dissolved salts all contribute to the biological oxygen demand the terms TDS and TSS) Table 2 displays the international allowable limit for dye wastewater pollutant discharge. Dye effluent should be tested for a biological oxygen need (BOD), total nitrogen color, pH, and suspended particles, less than the maximum allowable quantity. Toxic contaminants should never be discharged into the environment owing to their horrifying repercussions [13]. Industries that create wastewater containing dyes should promptly begin correcting the harm they are inflicting on the ecosystem and dumping it. Enterprises that use dyes should use dye-effluent treatment solutions as opposed to harming

the environment. A few of the more ethical businesses that use dyes are strongly recommended to use treated dye waste in their dyeing processes rather than dumping this into the environment. It's a more cost-effective option than regularly obtaining and using water for coloring processes. Water in the quantities needed by the dyeing industry is expensive, and cost reductions are desired in almost every industry [21].

## VI. AZO DYES TREATMENT

- 1. Physical and Chemical Treatment of Dyes:** Finding methods to handle azo dyes is essential since they present a variety of concerns and hazards to human health and the environment. There have been several physical and chemical techniques investigated. Techniques like adsorption and filtration are examples of physical approaches. Adsorption is the process of removing chemicals from wastewater at its surface using materials like activated carbon [31]. Activated carbon is the preferred alternative for this type of treatment and has been for some time, but it is not frequently employed due to its expensive cost. Clay, banana peels, maize, corn cob and wheat straw are some of the substitutes that have been investigated [32-33]. Due to the difficult waste disposal of these less expensive options, there are some disadvantages. Filtration is another widely utilized physical technique. To remove suspended particles and other undesirable elements from water, membranes are frequently used. Though efficient, it also has some problems, including comparably high material and investment prices, membrane degradation or fouling, the creation of potentially hazardous sludge, and once again complex waste management. Coagulation-flocculation, electrochemical, fenton's reagent and ozone treatments are a few examples of the various chemicals or processes used in chemical treatment. In typical wastewater treatment facilities, techniques like flocculation and sedimentation are used [34]. Coagulation is the process of neutralizing suspended particles by using coagulants. When the solids have been neutralized, they may collide and produce microflocs. These microflocs can aggregate into larger flocs and sediment during flocculation, whereupon they can be gravitationally eliminate from the water. Additionally, the Fenton reaction enables the creation of hydroxyl radicals with the use of the Fenton reagent ( $H_2O_2$  and  $Fe^{2+}$  ions), which can eliminate hazardous contaminants in wastewater. Although the process as a whole is inexpensive, it can produce a lot of secondary waste in the form of sludge. The chromophores of dyes are oxidized and disassembled preferentially by ozonation, which uses reactive ozone ( $O_3$ ); however, because it is unstable, it is not a great option for wastewater treatment. Traditional methods for effluent treatment include these physical and chemical processes. However, as was already noted, there may be several disadvantages to these methods. Furthermore, dyes may be resistant or resistive to conventional procedures. [3].
- 2. Biological Treatment of Dyes:** Microorganisms possess a remarkable ability to adapt and evolve, allowing them to efficiently break down pollutants and detoxify contaminated areas. This unique capability makes them promising candidates for bioremediation strategies, offering a sustainable solution to environmental challenges. Bioremediation is the practice of using microorganisms in place of physical and chemical treatments [35]. Bioremediation has sparked a lot of attention in recent years. It is generally known that microorganisms can break down pesticides, change heavy metals into less toxic or immobilized forms, and decrease or mineralize azo dyes [36]. Utilizing microorganisms for dye degradation has several benefits. The colors are greatly degraded in a comparable

cost-effective manner, requiring less water and producing less sludge. Several investigations have been conducted to determine how bacteria decolorize and degrade azo dyes. [37-39].



**Figure 13:** Treatment Methods for the Removal of Dyes from Wastewater Effluent

## VII. EFFICIENCY OF DYE REMOVAL METHODS

The color removal procedure stated above has been explored and evaluated physiologically, chemically, and physically via multiple investigations. It should be noted that not all color removal procedures are 100% effective. Occasionally, the circumstances used for dye removal might affect the outcomes achieved after the operation [28].

**Physical dye removal techniques** remove between 86.8 and 99 percent of the dye, with adsorption being the most effective. The adsorption dye removal technique is an excellent approach for quickly degrading practically any dye or dye combination. As with enzyme degradation, the adsorption process may be repeated till it adsorbent is completely consumed. Due to the inherent efficiency of the technique, some adsorbents may be rather costly. This issue may be remedied by developing alternative adsorbents from low-cost raw materials. Given the efficiency of both enzyme degradation and adsorption-based dye removal procedures, it should be investigated for further dye removal technologies to combine both processes into a single dye removal methodology [40].

**Chemical dye removal techniques**, achieved the greatest proportion of dye removed varying between 88.8 and 99 percent. Additionally, except for new electrochemical dye destruction technologies, chemical dye removal processes are pH-dependent and often result in secondary pollutant creation. Due to the significant drawbacks associated with chemical dye removal procedures, these approaches should be avoided wherever feasible [28].

**Biological techniques** of dye removal are generally effective. Biological dye removal techniques remove between 76 and 100 percent of the dye, with enzyme degradation being the most effective. Enzymatic dye degradation is a suitable and dependable approach for dye removal. This technique is inexpensive, effective, non-toxic, and most importantly, reusable. Its sole downside is an unpredictable yield of enzymes; however, this may be readily remedied by selecting the appropriate source material and extraction process [41].

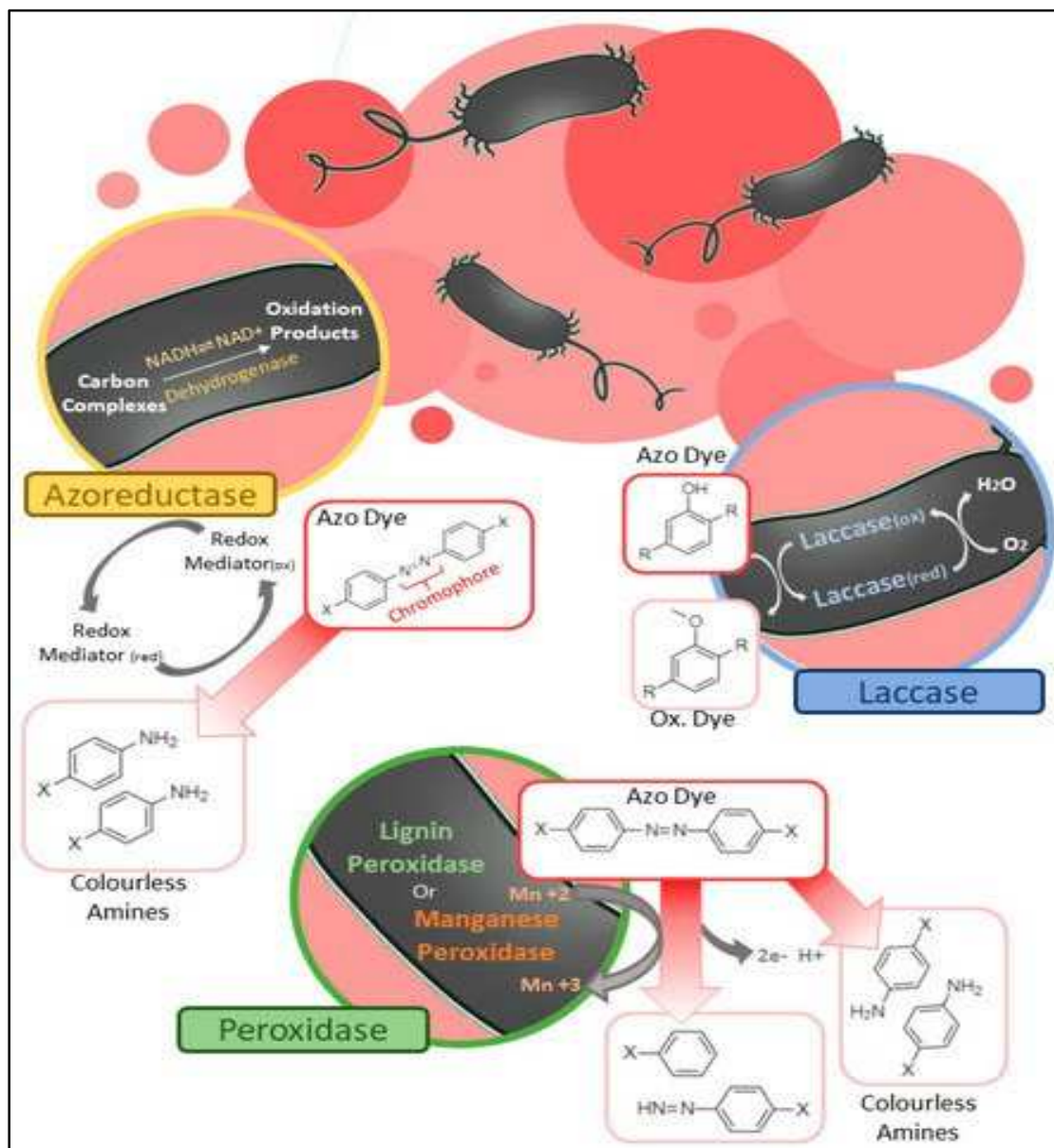
## VIII. BIOREMEDIATION

It is a process where organic wastes are biologically broken down under controlled circumstances, mainly with microbes, to break down environmental pollutants into less hazardous forms. Over the past decades, many microorganisms have been explored for degrading dyes including bacteria, filamentous fungi, yeasts, and algae [42]. The desirable attributes of a bacterial population for bioremediation include omnipotence, rapid development, facultative nature, and great adaptability [43]. There are various benefits to using biological methods to completely degrade textile wastewater, including (a) environmental friendliness; (b) cost-competitiveness; (c) reduced sludge generation; and (d) the production of non-toxic intermediates or total detoxification (e) less water is used compared to other oxidation techniques for dye degradation [44]. The adaptable nature of the chosen bacteria and the activity of the enzymes influence the success of biological approaches for degradation. As a result, multiple enzymes and microbes for colour degradation have been found and tested. An attractive feature of textile treatment of waste water is the identification of prospective microorganisms and their employment in degradation. Numerous microorganisms, including bacteria, fungi, and algae, can break down a variety of dyes contained in industrial wastewater [45]. Decolorization utilizing microbial cells is one of the most extensively utilized bioremediation processes. A more recent method that makes use of microbial enzymes promises to effectively decolorize effluent from the dyeing industry as well as degrade dye-contaminated ecosystems [46].

**1. Bacterial Decolorization of Azo Dyes:** The use of microorganisms to treat dye wastewater is emerging as one of the most promising bioremediation strategies. Bacterial degradation of azo dyes is often driven by enzymatic biotransformation including reductive breaking of azo bonds using azoreductase and either NADH or NADPH as an electron donor [47]. Pure cultures refer to a population of bacteria consisting of a single strain, allowing for better control and understanding of their behaviour. This knowledge is crucial for optimizing dye decolorization processes and ensuring the safety of the end products. Additionally, the use of pure cultures facilitates the identification and isolation of specific bacterial strains with superior dye-degrading capabilities, leading to more efficient and effective bioremediation strategies. A significant study on the breakdown of dye-containing textile effluent has recently been conducted utilizing single bacterium cultures such as *Alcaligenes faecalis* PMS-1[48] and *Enterobacter* sp. FNCIM 5545 [49]. The colorless aromatic amines are less toxic and can be further metabolized by bacteria. Additionally, bacterial degradation of dyes can also occur under aerobic conditions through oxidative processes, involving the production of reactive oxygen species. Under aerobic conditions, these poisonous amines are reduced to simpler non-toxic forms by deamination or hydroxylation processes. As a result, interest is increasingly concentrated on facultative anaerobic bacteria, which can decolorize dyes at a high rate under anoxic circumstances while also detoxifying aromatic amines aerobically [50].

## IX. BACTERIAL DEGRADATION MECHANISMS OF AZO DYE

- 1. Biosorption:** Biosorption is associated with both adsorption and absorption processes, and microorganisms capable of adsorbing colors have been documented in the scholarly literature. Biosorption is intimately tied to the makeup of both dead and live cells, did refer to as bioavailability, were also capable of biosorption because of the cell wall's lipids and heteropolysaccharides, wherein the differing charged groups attach the colorants to the cell wall; thus, both dead or live cells, referred to that as bioavailability, were also capable of biosorbents. Based on a range of oh groups in microbes' cellular membranes as well as dye morphologies, a bacterium X adsorbs/absorbs dye A of the battery structure's lipids and heteropolysaccharides, wherein the differing charged groups bind the reactive dyes to the cell wall; thus, both dead and live cells are capable of biosorption, referred to as bioavailability. Given the variety of functional groups in microbe cell membranes and dye shapes, a bacterium X that adsorbs/absorbs dye A generated by a microbe Y may still not bind dye B produced by the same microbe Y. Pretreatment may alter the biosorption capability of cells., therefore improving the process and resulting in a more precise match for a certain demand. Acids, formaldehyde, but also bases are a few of the substances capable of inflicting these changes [51]. Dead cells are more favorable than live cells for use as biosorbents since they do not need nourishment, may be held for a longer period, and be regenerated with solvents or surfactants However, absorbing might not be the most efficient dyeing procedure removal so it generates large quantities of biomass enclosing dyes but also possibly other toxic byproducts that must be disposed of properly; does not entirely resolve the issue because it frequently somehow doesn't destroy a dye and instead seizes it in a matrix, the biomass[13].
- 2. Enzymatic Degradation:** The first step in decolorizing solutions containing azo dyes, whether trash, effluents, or environmental materials, is to diminish the azo bridge (-N = N-) inside the chromophore group. This stage may take place intrinsically or extrinsically and involves the transfer of four electrons in two steps: The dye transfers two electrons to its final electron acceptor in each step. Several enzyme families have previously been discovered [52]. These two categories are the most often discussed in the literature when it comes to decolorization responses. The overall method of action of these two enzyme groups, as well as the, is shown in Figure.



**Figure 14:** The Figure Illustrates Three Common Bacterial Enzymatic Degradation Processes for the Azo Chromophore Group

To begin, Yellow represents enzyme degradation by azoreductases, which in this instance use NADH as the principal reducing agent for the azo bond cleavage, leads to the formation of aromatic compounds that discolor liquid medium. The laccase-mediated photocatalytic oxidation cycle is cofactor-free and produces oxidized intermediates rather than potentially toxic amines. Green peroxidase enzymes such as lignin and titanium peroxidase, 2 of its most often utilized dye degradation enzymes are the last enzymes to be discussed. and, are shown, along with some potential products based on the breakage of their symmetric or asymmetric bonds [53]. Bacterial treatments for highly hazardous environmental contaminants are commercially feasible and inexpensive and they aid in the management of environmental toxins [54]. *Pseudomonas aeruginosa* was used by researcher to degrade the azo color methyl orange [55]. Numerous anaerobic and aerobic

bacterial strains have been identified in the literature, including *Micrococcus* sp., *Pseudomonas* sp., *Xenophilus* sp., *Dermaococcus* sp., *Acinetobacter* sp., *Geobacillus* sp., *Shewanella* sp., *Corneobacterium* sp., *Lactobacillus* sp., *Rhizobium* [56-58].

## **X. IMPACT OF AZO DYES AND THE TREATMENT METHODS**

After dyes have colored things, Dye-using enterprises often dispose of dye effluents as industrial trash. These toxins are subsequently forced into nearby bodies of water, transforming clear, colorless liquid into polluted, colored water. Water dye pollution is seen unfavorably by environmentalists as well as the general public. Color effluents with a very high temperature and pH (acidic) are now and then released immediately after dyeing methods. This occurrence will have an impact on the gas permeation mechanism as well as the self-purification method of water bodies in the scientific environment. When effluents are emitted into the environment after use, they pollute water sources and endanger the ecosystem, restricting the use of water [59]. Once dye effluents are blended with naturally occurring water sources, a foul odor is produced. If dye effluent seeps into woodlands or fields and clogs soil pores, soil production will be reduced. With time, water quality deteriorates, creating a breeding ground for viruses, and leaving it unsafe for routine use or ingestion. Animals that use water from such a source will face a shortage of drinkable water. Villager's Disposal of effluents into the environment degrades the ecosystem over time and contributes to human health concerns. Once Inflammation may occur if dye effluents come into skin contact [60]. Chemicals like dye effluents that are deposited in water sources may evaporate into the environment, resulting in breathing difficulties or difficulty breathing upon ingestion. Consumption of colors may cause increased sweating, dizziness, methemoglobinemia, oral burns, nausea, and vomiting. Throughout bodies of water. This law requires dye-using businesses to ensure that Prior Aerobic and anaerobic techniques are employed to discharge dye effluents into the environment. Because of its low cost and ease of execution, this process was adopted as the industry standard for color removal. This treatment is inadequate to remove all dangerous particles from textile effluent, which is why whey-colored water remains in the environment. Microbe adsorption, algal degradation, enzyme degradation, antifungal cultures, and microbial cultures, including pure and mixed cultures, are all effective biological colour removal methods [61]. To remove the dye, chemical dye removal treatments use chemistry or chemical concepts. Advanced oxidation, electrical destruction, organic Fenton reaction removal, oxidation, ozonation, photochemical oxidation, and UV irradiation are all typical chemical colour removal procedures. Physical colour removal procedures are often simple and employ the mass transition process. Physical dye removal procedures include adsorbent, coagulation or death, ion exchange, irradiation, membrane filtration, nano- or ultra-filtration, and reverse osmosis. Microbes are used in biological processes for decolorization and degradation. The majority of chemical and physical colour removal methods are more expensive than biological colour removal therapies [62].

## **XI. FACTORS AFFECTING BACTERIAL DECOLORIZATION**

Because varied structures of synthetic dyes utilized fiber processing, the effluents generated have a highly variable chemical makeup, which includes organics, minerals, sulphur compounds, salts other hazardous chemicals. Temperature, dye structure, pH, dye concentration, agitation level, oxygen content, the addition of carbon and nitrogen sources,



electron donors, and redox conciliators are all physicochemical operational elements in biological treatment processes that have a direct impact on azo dyes' ability to decolorize bacteria [63]. Before developing a technique that is more successful, quicker, and practical, it is necessary to assess the impact of each variable on the elimination of the dye from azo dyes by bacteria.

- 1. Temperature Effects:** The temperature of microorganisms is determined directly by their environment since the microbe adjusts to temperature fluctuations biochemically or enzymatically. As a result, temp for all activities involved in microbial life, including soils and water remediation. Numerous studies on the activation energy needed for microbial dye removal of azo dyes have shown that limited temperature ranges are necessary for dye removal of azo dyes by highly complex bacterial consortiums residing in active sludge. Furthermore, surface temperatures in infectious disease outbreaks outcome in such a sudden change in the energy gap. The impact of temperature on growth rate, biomass yield, and response process have also been studied. The incidence of dye decolorization was observed to grow up to the ideal temperature and then drop to some extent [64]. At high heat, this loss throughout viability may well be necessary for cell death through conformational changes of an azo duct lipase. A quicker rate of dye degradation can be obtained by providing the bacterial culture an optimal temperature, which is commonly quoted as 30-40°C for most bacteria. However, it has been evidenced that the dihydrofolate reductase enzyme has been extremely thermostable and therefore can remain active for brief periods time at temperatures as high as 60 °C in some whole bacteria preparations [65].
- 2. pH Effects:** Furthermore, the pH level has an impact on decolorization. The pH has a significant influence on the efficacy of dye decolorization, and the ideal pH range for decolorization is in between 6.0 and 8.0. The rate of decolorization is fastest at optimal pH and instantly declines at excessively acidic pH. The pH effects are assumed to be related to dye molecules transit through cellular membranes, which is regarded to be the rate-limiting step in the reduction procedure. Due to the formation of aromatic ammonium, azo bond dissociation might very well result in an increase in pH. In general, changing the pH to around 7.0 but also 9.5 has only a minor impact on the dye removal process. Chang discovered that increasing the pH from 5.0 to 7.0 increased the rate of dye degradation by about 2.5-fold, but have become pH-insensitive between 7.0 and 9.5. In a pH range of 5 to 9 [65]. Jadhav discovered that conglomerate decolorized Alizarin G [66]. Furthermore, regained *Citrobacter* sp. CK3 was effective in waxing Reactive Red 190 at both pH 4 and pH 8. pH 12 environments. This pH tolerance is significant because it qualifies decolorizing bacteria for the biotreatment of dyeing plant effluents in practice [51].
- 3. Dye Concentration Effects:** Literature review indicates raising the detection limit progressively reduces the decolorization pace, the more often associated with the dyes' toxicity to independent bacteria and/or insufficient substrate concentration, but also dye molecules with different structures meant to block the active sites of azoreductase. Similar findings were obtained when microorganisms decolorized numerous reactive azo dyes; however, low dye concentrations may have been missed by enzymes released by dye degrading bacteria. High dye concentration was poisonous to bacteria and also affects dye breakdown by inhibiting enzyme active sites [67]. Furthermore, it has been proven

that at higher dye concentrations, reformat azo dyes with sulfonic acid ( $\text{SO}_3\text{H}$ ) units on their aromatic rings effectively inhibit bacterial growth. Increased dye concentration reduces dye decolorization and degradation [68]. Numerous research has been conducted to study the relationship between surface selective decolorization rates or equilibrium conversion from a kinetic standpoint [69].

- 4. The Influence of Dye Structure:** Different chemical structures, including such isoforms or the presence of different ligands, have a significant influence on dye removal capabilities as measured by renewability and decrease. It's been reported that colorants with simple structures and low molecular weights start by removing color more quickly, whereas basically with electron donating such as  $-\text{SO}_3\text{H}$  but also  $-\text{SO}_2\text{NH}_2$  in the position 3 of phenyl ring comparative to the ester linkage, as well as dyes with a high molecular weight, remove color more slowly. Likewise, it has been said that monoazo dyes fade the color faster than diazo and triazo dyes. In addition, it was shown that the development of the enzyme (azoreductase) was linked to the structure of certain dyes. When azo dyes undergo conversion to anion ions, a rapid one-electron transfer process is followed by an extended two-electron transfer reaction to produce the stable dianion. As a result, azo dyes with a higher electronic density might be incompatible with the third electron transfer necessary to form the dianion, resulting in limited or no decolorization capacity. According to Schneider, the electron-drawing moieties on the phenyl ring aid in colour removal. Suzuki investigated the aerobic biodegradability of 25 sulfonated azo dyes utilising their chemical structures and discovered that the steric influence of the chemical structure had a significant impact on the effectiveness of colour removal. [70-71].
- 5. Oxygen and Agitation Effects:** In anaerobes (methanogenic), facultatively anaerobic, and aerobic settings, distinct trophic groups of organisms decolorize azo dyes. It has been reported that feeding a carbon source such as glucose, starch, acetate, or ethanol to a bacterial culture under anaerobic conditions, as well as because more complex substrates, such as whey but also tapioca, could affect the color removal process, the majority of the azo dye reduction to amines occurs during active bacterial growth. When bacterial suspension strains like *Pseudomonas luteola*, *Clostridium ncm*, *Aeruginosa* sp. SUK1, and *Mycobacterium glutamicus* NCIM-2168 were used, and similar results were obtained. While anaerobic conditions are expected to enhance reductive enzyme activity, the oxidoreductase degradative of azo dyes requires a small amount of oxygen as well. As per various studies, bacterial degeneration of azo dyes tends to involve both oxidative and catalytic processes. and reductive enzymes. According to these previous results, aeration and agitation, both increasing the oxygen content in the solution, must be avoided for successful color removal [72].
- 6. Supplementation with Carbon and Nitrogen Sources has a Number of Beneficial Effects:** Azo dyes are carbon poor, therefore biodegradation of dyes in the absence of obtaining additional carbon and nitrogen sources is difficult. In most circumstances, azo dye decolorization requires complex organic sources like yeast extract, tryptone, or a combination of complex biological sources or carbohydrates. When azo dyes are decolorized via azo bond reduction, it has been found that reducing equivalents from the activated carbon prepared is transmitted to the dye. These volatile organic acids and alcohols play a crucial role in the overall balance of anaerobic consortia, as they can be further metabolized by other bacteria to produce methane, hydrogen sulfide, or additional

organic compounds. The competition for these substrates among different bacterial groups ultimately determines the metabolic pathways and end products in anaerobic environments. Several experiments have been conducted to investigate how azo dyes decolorize in the presence of additional carbon sources. The addition of carbon sources appeared to be less successful in encouraging decolorization in these cases, most likely due to the cells' proclivity towards absorption of additional carbon sources rather than the use of the dye element as a carbon source. On the other hand, the inclusion of organic nitrogen sources such as phytase, beef extract, and urea, as well as yeast extract, may regenerate NADH, which acts as an electrophile for microorganisms to decrease reactive colours, resulting in successful decolorization. Several researchers incorporated lignocellulosic waste material as an effective decolorizer to make the method economically viable as well as applicable [72].

- 7. Electron Donor's Effects:** The Textile wastewater contains azo dyes or other organic substances that do not offer enough substrate for anaerobic bacteria to thrive, preventing complete decolorization. To improve the anaerobic decolorization efficacy, a source of the exogenous substrate (electron donor) is required. While there are several electron donors or acceptors of, ubiquitous nature, it is vital to examine their effects on bacterial azo reduction. It has been shown that the Electron donors such as sodium acetate, potassium formate, potassium succinate, sodium carbonate, but also sodium pyruvate help *Bacillus* sp. ADR decolorizes C.I. Reactive Orange 16 and several azo dyes. According to some, anaerobic bacterial azo reduction is a metabolic procedure in which electron donors are oxidized and electrons are transferred to acceptors via a This electron transport chain is a multicomponent system. Dehydrogenases, cytochromes, but also menaquinones are also important electron parts in the azo reduction pathway. The electron process has been observed to be hampered in the involvement of electron donors, possibly due to donors' competing for electrons. According to thermodynamic research, the multiple electron-donating half-reactions have distinct characteristics as a result of their changeable reaction rates, which are most likely caused by temperature differences governed by electron donors. In addition, it has been observed that catalysts such as fructose or lactate ions trigger the reductive breakdown of azo bonds. Additionally, formate is an extremely effective electron acceptor for the anaerobic environment-driven electron transfer pathway to the dyes, which might be a result of the presence of the formate peroxidase enzyme. Methanol merits particular note among these substrates owing to its extensive use as a fee electron donor in treating wastewater. It has been shown that cell lysis products may operate as electron donors to anaerobic azo decline, with active cells metabolizing those lysis products. These results add to our knowledge of microbial anaerobic azo reduction and have significance for alkaloid wastewater treatment systems. Furthermore, determining the physiological electron acceptor for each bio colour removal step is critical, because donors not only initiate the reduced mechanism, but also activate the diminished enzymatic system [13].

## XII. DECOLORIZATION OF AZO DYES BY THE FUNGI

The metabolism of a fungal colony may adapt to changing environmental circumstances. Fungus cannot survive without this capacity. Internal and external enzymes utilized in metabolic activity. These enzymes can destroy a wide range of colors found in textile effluent. These enzymes are lignin peroxidase (LiP), manganese peroxidase (MP),

manganese peroxidase (MnP), and laccase [73-74]. To remove azo dyes, white rot fungal cultures have been applied in the most of cases. Degradation was seen in combination with COD removal in the white rot fungus *Corioloopsis* sp., *Penicillium simplicissimum*, and *Pleurotus eryngii*. Nonetheless, the breakdown of colours in textile wastewater by white-rot fungus has significant limitations, including a protracted lag phase and the need for nitrogen-restricted conditions, variable enzyme production, and a high reactor size owing to the longer holding period for full degradation. The biggest issue with employing fungus is that the system is unstable; bacteria will emerge after 20-30 days, and the fungi would ceased to regulate the system and eliminate the dye [75].

### **XIII. DECOLORIZATION OF AZO DYES BY YEAST**

Yeast cultures have also been demonstrated significantly in the decolorization of azo dyes. *Kluyveromyces marxianus* IMB3 remove color from Remazol Black-B dye under aerobic conditions, and 98% decolorization was obtained at 37 °C in the pH range of 3.0-5.5. When *Kluyveromyces marxianus* IMB3 was incubated under anaerobic conditions, there was little or no colour loss. Further investigation, which included monitoring decolorization at high temperatures and low pH (to restrict growth) and using a tenfold dense inoculum, revealed that decolorization was induced by biosorption rather than a metabolic mechanism [76]. *Pseudozyma rugulosa* Y-48 and *Candida krusei* G-1 removed 99% of a reactive azo dye, Reactive Brilliant Red K-2BP (200 mg/L), and 22-98% of (50 mg/L) in 24 hours. *Candida oleophila* decolorized up to 200 mg/L of Reactive Black 5 dye in 24 hours at 26 °C in the presence of 5 g/L glucose without significant dye adsorption to yeast cells [77].

### **XIV. DECOLORIZATION OF AZO DYES BY ALGAE**

Algae are everywhere, and they're becoming increasingly popular in the field of textile wastewater degradation. Several algae species have been successfully used to cleanse coloured wastewater. Algae degrade colours by three distinct mechanisms: (1) dye consumption for growth, (2) dye transformation to non-colored intermediates such as CO<sub>2</sub> and H<sub>2</sub>O, and (3) chromophores adsorption on algae. The mediated decolorization of acid red 27 resulted in the formation of less phytotoxic aromatic amines. Furthermore, algal waste can be utilised instead of more expensive materials such as activated carbon for colour removal via biosorption [78].

### **XV. PHYTOTOXICITY ASSESSMENT**

There is a growing demand for resources to meet the demands of an expanding population. This leads to industrialization and, as a result, increased pressure on existing natural resources. The rising urban population, as well as the consequent industrialisation, consumes a lot of water and generates a lot of wastewater or waste effluent. The problems with getting rid of these effluents are far worse. As part of the push towards industrialisation, industrial sites were established all throughout the subcontinent. Industries demand special attention to environmental concerns due to their important economic contribution to the country. The careless and indiscriminate dumping of industrial effluents into natural rivers and on land is producing severe problems. One of the most important techniques for controlling land degradation and improving environmental conditions is to use these industrial effluents in tree plantings. Uneven rainfall distribution, long dry intervals, soil

water stress, and nutrient deficiency are all significant restrictions to the development of planted tree seedlings in arid areas where higher-quality water has become less common. The application of effluent in tree plantations is a viable alternative because of the necessity to dispose of wastewater both safely and economically and for water conservation. For the microbial toxicity research, a 2 mm zone of inhibition was detected with the control Methyl Orange dye, whereas the degraded metabolite did not limit growth. A phytotoxicity study was performed on *Vigna radiata* seeds in relation to dye and its degradation compounds. Several investigations have been conducted on the various microorganisms that are capable of eliminating azo dye from their surroundings with varying degrees of efficiency. An extensive array of microorganisms must be examined for their activity before choosing one that can remove the dye. The majority of the time, source microorganisms are collected from polluted soil or water, as microorganisms that live in contaminated areas [79].

## **XVI. CONSORTIA IN THE DECOLORIZATION OF AZO DYE**

Several investigations have been conducted on the various microorganisms that are capable of eliminating azo dye from their surroundings with varying degrees of efficiency. An extensive array of microorganisms must be examined for their activity before choosing one that can remove the dye. The majority of the time, source microorganisms are collected from polluted soil or water, as microorganisms that live in contaminated areas [80].

## **XVII. CHARACTERIZATION OF DYE METABOLITES**

The study of the metabolic metabolites of azo dyes is critical for understanding the environmental destiny of the dyes and revealing the likely process of dye decolorization. In order to determine the breakdown products created when azo dyes biodegrade, several analytical techniques are used. Some of these approaches are detailed in the following section.

- 1. UV-Visible Spectroscopy:** The principal approach for detecting dye decolorization is due to absorption or biodegradation is UV-vis spectroscopy [81]. The distinct peaks in a molecule's absorption spectra regularly diminish in contrast to one another, accompanied by a deep colour of bacterial cells due to dye loss because of adsorption. In contrast, if the dye remediation occurs by biodegradation, the initial absorbance peaks in the visible area either disappear entirely or additional peaks in the UV region form, but the cells preserve their initial colour [82]. The real colour of the dyes and industrial effluent combination, irrespective of hue, is assessed using ADMI 3WL, the American Dye Manufacturers Institute's tri-stimulus filter technique. The UV-Vis spectra of Navy blue 2GL, a textile azo dye, following treatment with *Bacillus* sp. VUS indicated decolorization and a reduction in dye concentration with time, with no change in max until full decolorization of the medium [83].
- 2. Fourier Transform Infrared Spectroscopy:** Fourier transform infrared (FTIR) spectroscopy includes the absorption of infrared light by a molecule at wavelengths spanning from 700 to 25,000 nm, resulting in a shift to higher degrees of vibration. When a molecule absorbs incident light of a specific wavelength, the ensuing infrared spectrum is produced, and the molecule then vanishes from the transmitted light. An absorption band will be apparent in the measured spectra. Because atom bonds are flexible springs,

bond vibrations may be thought of as either stretching or bending (deformation) activities. Because the wavenumber related with a single functional group varies somewhat because of the effect of the molecular environment, a detailed analysis of a molecule's structure is feasible. It is possible, for example, to identify the C-H vibrations (-CH<sub>3</sub>) of methyl and methylene (-CH-) groups. FTIR is increasingly being used to examine peptides and proteins [81]. As a result, it is a significant analytical tool since the FTIR spectrum makes it simpler to evaluate the kind and intensity of interactions between the various functional groups of the azo dye molecule after exposure to bacteria. The FTIR spectra of an azo dye and its biodegradable byproducts are examined, suggesting dye breakdown by bacterial species. The FTIR spectrum of dye intermediates revealed the removal of particular peaks in the region of 1575.0-1630.0 cm<sup>-1</sup> of azo compounds, indicating the dissolution of the dye's azo links [84]. The other peaks assist to dye degradation by indicating distinct dye groups' twisting and/or vibrations of stretching [83].

- 3. High-Performance Liquid Chromatography:** A chromatogram is a graphical depiction of the detector's output in terms of retention time or elution volume, consisting of a succession of peaks or bands with preferably uniform shapes illustrating the removal of separate analytes. This duration of time serves as a distinguishing feature for the analyte and assists in identification [81]. By injecting new peaks in the chromatogram with different retention periods than the original dye, the high-performance liquid chromatography (HPLC) method may be used to examine dye degradation. These metabolites formed during dye biodegradation are shown by the emergence of extra peaks on the spectrum.
- 4. Mass Spectrometry:** Mass spectrometry (MS) is used to get the mass spectra of a chemical combination, making it an incredibly effective qualitative instrument for studying dye degradation. When a liquid is used as the mobile phase, the method is known as liquid chromatography-mass spectrometry (LC-MS), but when a gas is used as the mobile phase, the procedure is known as gas chromatography-mass spectrometry. Both approaches have been proven to be useful for analysing the molecular weights and structural properties of dye breakdown metabolites formed by the bacterial operation, and they can assist in the identification of azo dye degradation microbiological processes. The metabolites produced by *Providencia* sp. SRS83 during the process of biodegradation of triazo dye Acid Black 210 were evaluated using GCMS; retention duration and m/z values aided in predicting the intermediates produced and the likely method subsequently to the organism for dye breakdown [83].

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