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A Review on The Ecological Impacts of Azo Dye and Survey of Bioremediation Potential Strains

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Abstract

A dye is a chemical that gives materials their color. and has become an important component of human life. Azo dyes, which are widely utilized in the textile, leather, pharmaceutical, and cosmetics industries, are the most widely produced synthetic dye-stuffs and pose a hazard to all life forms. As a result, their presence in liquid effluents from textile washing poses a major threat to the quality of receiving habitats if they are not purified. These dyes and pigments are not biodegradable, their presence can have a substantial impact on aquatic wildlife and vegetation. This has a negative impact on the environment's equilibrium by causing serious dangers, including immediate dangers (eutrophication, under-oxygenation, color, turbidity, and odor), long-term dangers (persistence, bioaccumulation of carcinogenic aromatic products, and formation of chlorination by-products), and mutagenicity and carcinogenicity. The dyes are not efficiently removed by the physico-chemical method of industrial wastewater treatment. Microbial degradation of azo dyes has gotten a lot of interest recently because of their eco-friendly and low-cost nature, the dyes could be decolonized by microbes. Therefore, this work presents a review of the hazard caused by azo dye with the pathological basis of the damage and the redemptive influence bacteria.

1. Introduction

Industrial effluents from food, drug, and cosmetic, textile, and dye material manufacturers introduce dyes into the environment [1]. Numerous textile dyes and chemicals are highly structured polymers that are difficult to degrade biologically, and they are divided into several classes based on chemical composition and application. Chromophores (nitrogen, carbon, oxygen, and sulphur with alternate single and double bonds) and Auxophores (amino, carboxyl, hydroxyl, and sulphonic groups) are responsible for the varied color patterns and intensities of textile products.

Globally, processing consumes enormous amounts of water during textile dyeing; the azo textile sector has been identified as one of the largest waste water generating businesses throughout the world. Textile industries release wastewater including a mixture of different colors and chemicals such as dispersants, leveling agents, acids, and alkali into water bodies and the surrounding environment without any efficient treatment, generating major environmental issues [2]. Over the years, it has been stated that over 100,000 distinct synthetic dyes are commercially accessible, with yearly production of over 700,000 metric tons [3]. It was discovered that nearly half of the applied dye was present in the effluent after evaluating the chemical makeup of textile dye effluents. The low absorption characteristics of fibres to dyes in textile manufacturing wastewater have been linked to their high concentration [4]. Synthetic dyes are made up of complex compounds that are usually made up of azo, triphenylmethane, or heterocyclic/polymeric structures, and because of their stable nature, they may stay a long time in the natural ecosystem without being altered or discolored. As a result, effluents from the textile sector may have a negative effect on receiving water bodies or land [5]. The haphazard and excessive use of these dyestuffs has become a growing source of environmental concern [6]. It has been found to be deleterious to biotic components of the ecosystem by its ability to affect the genomes and other cellular organelle. Azo dyes cause mutation in cell which can lead to teratogenesis and development of disease conditions. It is culprit in carcinogenesis as it modulates both genetic and epigenetic factors in the cell. They are thus a considerable threat to natural resources such as soil, water, vegetation, animals, and the human population, and because enormous quantities of dyes are utilized, and thus pollution on a large scale. The pollution occur because of it release into the land (soils) and sections of the environment (lakes, rivers, ponds, streams, etc.). Some bacteria have been found to degrade azo dye to various degree which is natural method of remediation but the efficiency of this system of degradation is quarries or may need biotechnological enhancement [7]. This review is to explore the various damages caused by azo dye, mechanism of pathogenesis and natural remediate by bacteria in the ecosystem.

2.0. What are Azo Dyes?

Azo dyes are one of the oldest man-made compounds currently in use in the textile printing and food sectors. More than 10% – 15% of dyes are released into the environment during manufacturing and use [8] and more than 700,000 tons are produced yearly worldwide. The textile industry is one of the most important sectors in the world, accounting for the fifth greatest source of foreign cash, but it is also one of the most polluting sources of water and soil [9]. The global production of azo dyes is estimated to be around 1 million tons per year. It comes in a variety of shapes and sizes, and there are currently over 2,000 essentially distinct azo dyes in use. Azo dyes are robust, have good all-around qualities, and are inexpensive, whereas anthraquinone dyes are weak and costly. Acid, mordant, metal complex, direct, basic, and reactive dyes, are classed as soluble dyes, whereas azoic, sulfur, vat, and disperse dyes, are classified as insoluble dyes [7].

Some dyes and their N-substituted aromatic biotransformation products are poisonous or carcinogenic; they are classified as major environmental pollutant. Azo dyes are not destroyed during standard aerobic wastewater treatment, under anaerobic circumstances; however, Azo connections can be easily decreased using digester sludge, anaerobic granular sludge, or sediments [10]. Redox mediators and Azo dyes act as electron acceptors for reduced flavin nucleotides, enhancing reduction. Aromatic amines are formed during the reduction of Azo dyes. With the exception of a few aromatic amines replaced with hydroxyl and carboxyl groups, which were totally destroyed under methanogen conditions, most aromatic amines that accumulate following Azo cleavage are not mineralized anaerobically [7]. Aromatic amines, on the other hand, are easily degraded aerobically. Biodegradation of bacterial Azo dyes usually occurs in two stages; the dyes' Azo links are reductively cleaved in the first stage, resulting in the generation of colorless but potentially dangerous aromatic amines. The aromatic amines are degraded in the second stage. Anaerobic conditions are frequently required for azo dye reduction, but bacterial biodegradation of aromatic amines is almost entirely aerobic. Azo dyes are distinguished by one or more azo bonds (-N=N-) in combination with one or more aromatic structures. They are made to have a lot of photolytic stability and resistance to oxidizing agents. They're used in a wide range of sectors, including textiles, food, paper, and cosmetics [11]. Azo- and nitro-compounds are decreased in sediments and the intestinal environment, resulting in the regeneration of the parent poisonous amines. Every year, 280,000 tons of textile dyes are anticipated to be emitted in such industrial effluents around the world [12]. Under anaerobic conditions, direct discharge of these effluents leads the production of hazardous aromatic amines in receiving media [13] Many synthetic dyes are toxic, mutagenic, and carcinogenic, in addition to their visual effect and COD impact, the search for acceptable treatment methods is a high priority due to the typically large volumetric rate of industrial wastewater discharge, as well as increasingly stringent legislation [14].

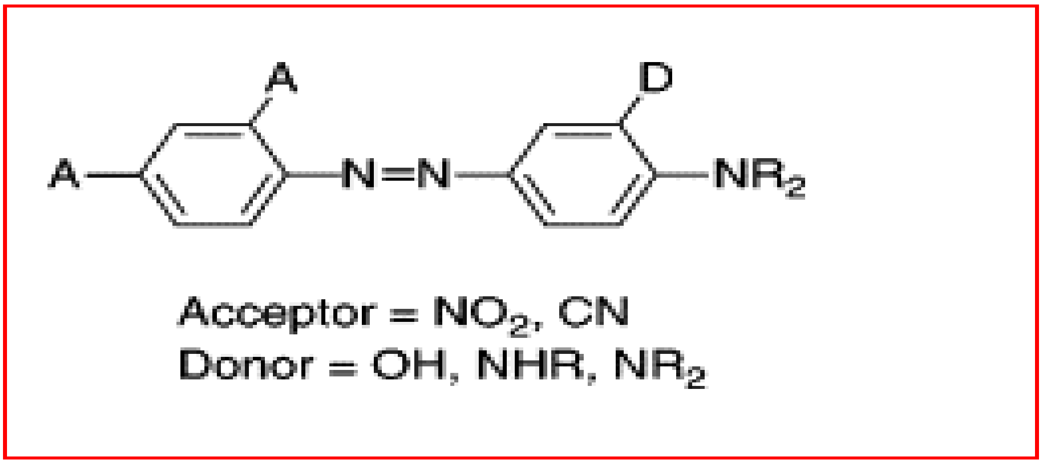

Figure 1: Chemical structure of Azo textile dye [15]

2.1. Environmental Impact of Azo Dye Effluent

Azo dyes provide bright, vivid hues. Colored cotton, leather, cosmetics, and food are the most common applications [16]. Azo dyes have a long history and are an essential part of our daily life. Natural plant and insect sources were initially used in the dye industry, but synthetic manufacturing technologies were quickly adopted. Unfortunately, certain synthetic dyes, particularly azo dyes have been discovered to be poisonous and mutagenic [13]. However, because of their low cost and other appealing properties, azo dyes are still utilized and manufactured today. Getting azo dyes out of wastewater effluents and treating them is a tough undertaking. In experimental animals, human bladder cancer, splenic sarcomas, hepatocarcinomas, and nuclear anomalies, as well as chromosomal errors in mammalian cells, have all been related to azo dyes [17]. In experimental animals, some azo dyes cause liver nodules, and dye workers exposed to significant amounts of azo dyes have an increased risk of bladder cancer, hepatic and bacterial azoreductases in mammals convert azo compounds to their corresponding amines [18]. Bacterial azoreductases reduce azo dyes more efficiently than hepatic azoreductases, and they can convert some azo dyes to mutagenic and carcinogenic amines [17-18].

2.2. Effect of Azo Dye Effluent on Aquatic Environment:

Water contamination is a result of toxic textile dyeing effluent. Groundwater was discovered to be the most important supply of drinking water, and it has been claimed that the groundwater is still safe to drink [19]. Mills emit tens of thousands of tons of textile dyeing effluent, which is rich in color and organic compounds from dyeing and finishing salts, as hazardous waste. The presence of sulfur and naphthol, as well as vat dyes, heavy metals, and other chemicals, results in toxic textile effluent. Formaldehyde-based color fixing agents, hydrocarbon-based softeners, and non-biodegradable dyeing chemicals are among the other potentially hazardous components in the water. Due to dye process inefficiencies, up to 200000 tons of dyes are wasted to effluents each year during dyeing and finishing activities in the textile sector. Due to the presence of coloring and oily scums, colloidal components increase turbidity and give the water a savage aspect and bad odor [20]. Water pollution happens during scouring, bleaching, and dyeing. The dye plants on the estate require a lot of fresh water for wastewater disposal. These dye effluents permeate and poison the canal, which then flows into a much larger body of water, contaminating the water. Because of the widespread use of salts in dyeing, effluents frequently have an alkaline pH [21].

2.3. Effect of Azo Dye Effluent on Soil

Soil contains mineral and organic materials, and agriculture is founded on it; because soil is a biologically balanced system, changes in the soil microenvironment result in changes in native microbial community profiles [22]. Pollutants like as polycyclic aromatic hydrocarbons (PAHs), petroleum chemicals, and heavy metals, on the other hand, have a significant impact on bacterial diversity. The genetic variation that results in changed metabolic pathways or the selective enrichment of microorganisms that can transform the particular pollution are the main variables that define the abundance of the microbial community in a particular contaminated soil. Textile effluent, on the other hand, pollutes the environment by polluting soil, clogging soil pores, and lowering soil productivity [23]. The soil's consistency has been hardened, preventing roots from penetrating, the dyeing industry's effluents are harmful, posing significant threats to the environment and human health. This is in line with a previous study conducted in India's textile district, which looked at the impact of textile dyeing effluents on soils collected from agricultural fields near the effluents outlet [24]. Several studies have shown that even short-term addition of textile effluent to soil reduces the soil's water-soluble salt and organic matter content when compared to normal water-irrigated soils, such as sodium, calcium, Magnesium, potassium and NH4-N and phosporus content [25]. Many experts predict that at high concentrations, the germination rate of particular effluents, such as textiles, will be reduced [26].

Textile dyeing businesses use a lot of water and generate a lot of waste water. Because of their imperfect use and washing procedures, azo dyes have been found in significant amounts in textile waste effluents. These dyestuffs are highly structured polymers that are difficult to biologically breakdown. The most noticeable effect of dye-colored effluent discharge is the color's persistence; textile dyeing businesses use a lot of water and generate a lot of waste water. Due to insufficient use and washing procedures, a significant amount of dyes have been detected in textile waste waters [27]. The dye that was discarded can be found in the waste water in dissolved or suspended form. These dyestuffs are complex polymers that are difficult to break down biologically. It is stable and quick, difficult to decompose, and poisonous, making the water unfit for human consumption. Because typical waste water treatment from textile mills and dyestuff facilities is unable to adequately remove most of the vat and other colors, such dyestuff can enter the aquatic environment, primarily dissolved or floating in water [28]. As a result of the usage of excessive amounts of chemicals and dyes, enormous quantities of effluent are generated at various stages of textile dyeing and printing operations. It poses an ecological danger because soils lose their physicochemical features, making them vulnerable to erosion, productivity loss, sustainability, and food chain quality degradation [29].

2.4. Effect of Azo Dye Effluent on Human Beings

In the textile and dyeing industries, the use of dyestuffs and pigments may create health concerns. Chemical compounds used in textiles include optical brighteners, dyes, heavy metals, crease-resistance agents, antibacterial agents, solvents, and flame retardants, to name a few [30]. Organically linked chlorine, a known carcinogen, is found in over 40% of the world's most extensively used colors [9]. Like other environmental toxicants, toxic dyeing effluent impairs cell function, causing changes in animal physiology and biochemical systems, including impairment of vital activities such as respiration, osmoregulation, reproduction, and even death [31].Workers have been found to develop rhinitis, dermatitis, asthma, and nasal difficulties as a result of prolonged exposure to reactive dyes [32].

2.5. Toxicity and Assessment of Azo Dyes Pathogenesis

Extremely electrophilic oxidases produce aromatic oxidative metabolism of azo dye's hydrocarbon, some of which can bind covalently to macromolecules such as DNA, RNA, and proteins, producing mutations. They are active inducers of fish liver enzymes and produce chemicals with cancer-causing potential. Naphthalene can produce liver histopathological changes even at low concentrations, which can lead to severe physio-metabolic dysfunction and mortality. Dye-tainted sediments are also a health risk for humans since they have the potential to infect sea food [18-20]. Aromatic Hydrocarbon breakdown of azo dyes accumulates in fish and shellfish at concentrations several orders of magnitude higher than their aqueous solubility. Acute symptoms of their exposure include skin and lung irritation, as well as cyanosis. In humans and animals, exposure to some dye Polynuclear aromatic Hydrocarbon colors has been linked to carcinogenesis and tumors. Because of the potential dangers to human health, reliable assessments of PAH persistence in the environment are critical in determining the risk posed by PAH pollution [33].

Plant impact of dyeing factory effluent Soil fertility and plant growth are severely harmed when agricultural lands are irrigated with water contaminated with numerous industrial effluents. Chemical and biological condition of soil and water are altered by dissolved compounds in industrial effluents, which can affect plant development and production. Plant susceptibility to diseases is also influenced by effluents. It's impossible to identify all of the biologically active substances in garbage [34].

Bioassay is a more direct method of determining environmental toxicity. To measure plant response to specific contaminants, plant growth indicators such as germination percentage, seedling survival, and seedling height were used as criteria. Some dyes used in the dyeing industry are carcinogenic and mutagenic, and effluents have been shown to impair seed germination and crop plant growth [35]. The elongation of seedling shoots and roots is inhibited by a higher concentration of untreated effluents. High dissolved solids in the effluent diminish the level of dissolved oxygen, limiting seedling growth and development. Inhibition of seed germination may be attributed to high dissolved solids in the effluent disrupting the osmotic relationship of the seedlings.

2.6. Microbial Degeneration of Azo Textile Dyes

The bacterial reduction of the azo dye is usually nonspecific and bacterial decolorization is normally faster. A wide range of aerobic and anaerobic bacteria such as *Bacilus subtilis, Pseudomonas* sp, *Escherichia coli, Rhabdobacter* sp, *Enterococcus* sp, *Staphylococcus* sp, *Xenophilus* sp, *Corneybaterium* sp, *Clostridium* sp.,*Micrococcus dermacoccus* sp, *Acinetobacter* sp, *Geobacillus*, *Lactobacillus*, *Rhizobium*, *Proteus* sp, *Morganella* sp, *Aeromonas* sp. and *Klebsiella* sp have been extensively reported as degraders of azo dyes [37] . Some aerobic strains rely only on azo dyes for carbon and nitrogen, whereas others need on particular oxygen-tolerant azo reductases to reduce the azo group. The biological treatment of textile wastewater ranges from bacterial culture to fungal culture to yeast culture [38]. Because enzymes have catalytic activity, they can be used in very little amounts to speed up reactions. As a result, microorganisms that generate enzymes could be a viable solution for reducing water contamination. In both anaerobic and aerobic settings, bacteria are capable of degrading azo dye to a large extent, anaerobes and facultative anaerobes both produce good degradation results [39]. The reduction of the azo dye is usually non-specific and decolorization is faster during the bacterial degradation process. A wide range of aerobic and anaerobic bacteria such as *Pseudomonas* sp., *Bacillus subtilis, Geobacillus* sp., *Eschericheria coli*, *Rhabdobacter* sp., *Enterococcus* sp., *Staphylococcus* sp., *Coryneybacterium* sp., *Lactobacillus* sp., *Xenophilus* sp., *Clostridium* sp., *Acinetobacter* sp., *Micrococcus* sp., *Dermacoccus* sp., *Rhizobium* sp., *Proteus* sp., *Morganella* sp., *Aeromonas* sp., *Alcaligenes* sp., *Klebsiella* sp., *Shewanella* sp. and *Alishewanella* sp. have been extensively reported for resulting good biodegradation of Azo dyes. *Pseudomonas* spp. [40] had been used in decolorization and degradation studies of commercial azo dye from textile waste water [41]. Some aerobic bacterial strains use Azo dyes as their primary carbon and nitrogen source in their metabolic pathway, while others merely use as oxygen-tolerant enzyme called azoreductase to decrease the azo group [40-41].

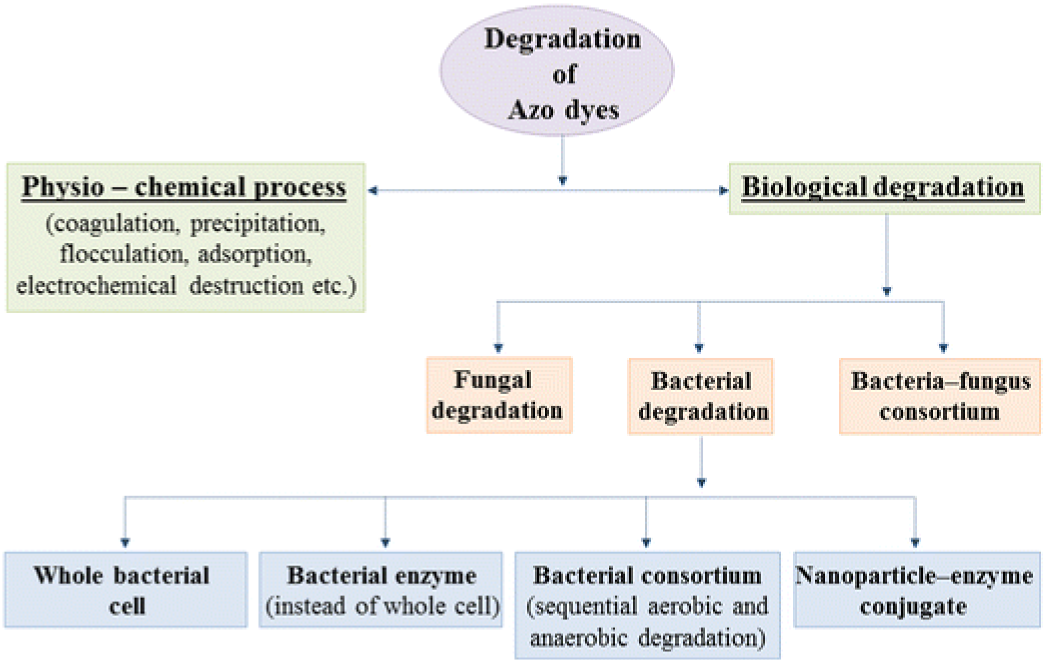

Figure 2: Treatment methods for the removal of dyes from wastewater effluent [41]

Precipitation, coagulation, adsorption, and flocculation, as well as electrochemical destruction, mineralization, and decolorization processes [42] have certain drawbacks in terms of cost, time, and residue release. All of these approaches are aimed at reducing toxicity rather than neutralizing it [43]. Microorganisms decrease azo dyes by secreting enzymes such as laccase, azoreductase, peroxidase, and hydrogenase, they can be utilized to totally break them down, the reduced azo dyes are then mineralized into simple compounds [44].

2.6. Degradation of Azo Dyes by Enzymes

The reduction of the azo bond (-N = N-) in the chromophore group, which can occur intracellularly or extracellularly, it is the first step in the decolorization of azo dye solutions. Whether waste industrial effluents or environmental samples. This phase entails the transfer of four electrons in two steps, with two electrons being transferred from the dye to the dye's ultimate electron acceptor, resulting in decolorization [45]. A set of enzymes known as azoreductases and laccases has already been discovered as capable of this reduction. These two groups are the most discussed in the literature when it comes to decolorization reactions [46]. Figure following depicts the general action methods of these two enzyme groups, as well as the peroxidase group, which also works on the azo chromophore group. Figure 3 depicts three general azo chromophore enzymatic breakdown mechanisms in bacteria. To begin, illustrating azoreductases' (yellow) enzymatic degradation, in this case using NADH as a key reducing agent for the breakdown of azo bonds, resulting in aromatic amines and therefore discoloring the medium. Then there's the laccase-mediated catalytic reaction cycle (blue), which creates oxidized substrate instead of potentially toxic amines and doesn't require cofactors. Finally, green peroxidase enzymes, such as lignin peroxidase and manganese peroxidase, two of the most widely employed enzymes for dye degradation, show some of the possible products based on the cleavage of their bonds, which can be symmetric or asymmetric [46].

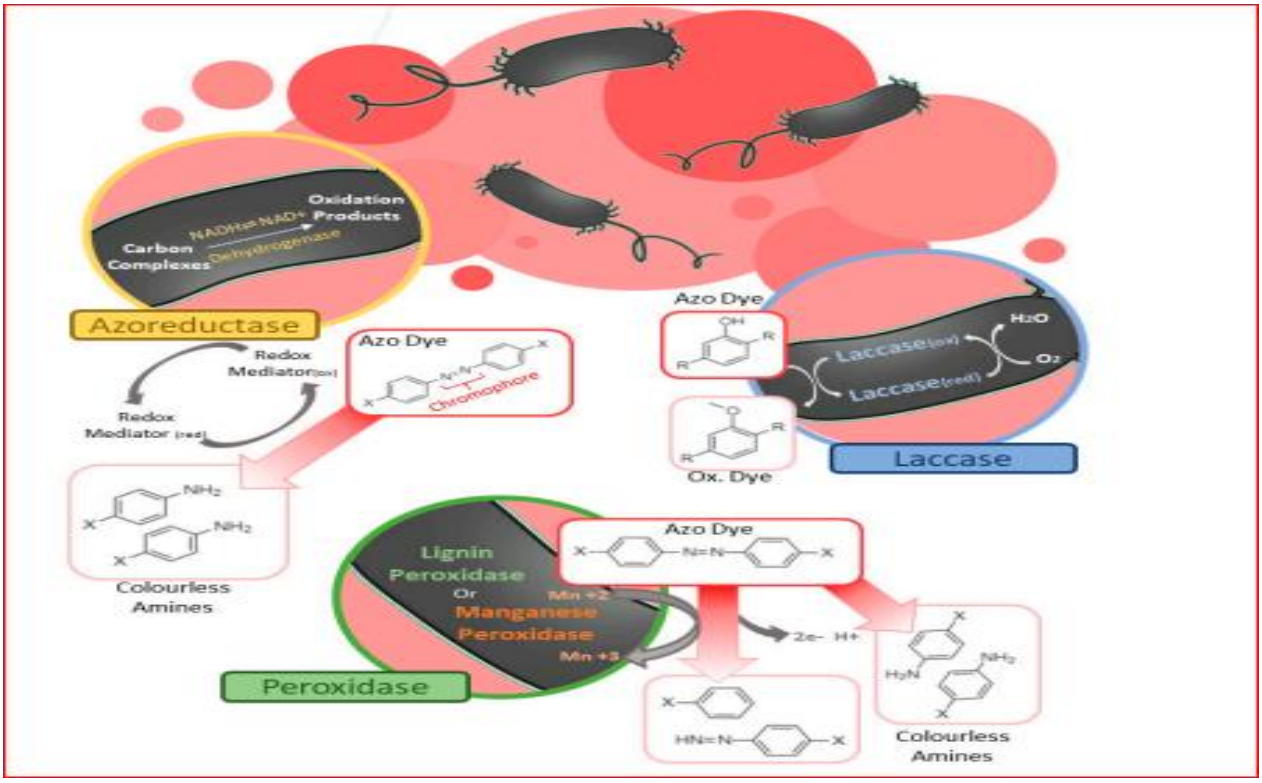

Figure 3: Schematic display of bacterial enzymatic degradation processes of the azo chromophore group

2.7. Significance of Azo Reduction

Humans can be exposed to azo dyes through ingestion, inhalation, or skin contact. Once within the body, azo dyes are biotransformed into aromatic amines [47] The most important Intestinal microflora azo reductases reductively hydrolyze biotransformation into aromatic amines. Various dietary factors influenced azoreductase activity in a range of intestinal preparations, including cellulose, proteins, fiber, antibiotics, and supplementation containing live lactobacilli cultures [48] also showed that azo dyes such as Methyl Red and Orange II were cleaved by human skin microbiota including many species in the genera of *Staphylococcus, Corynebacterium, Micrococcus and Dermacoccus*. The human skin is home to hundreds of species, many of which have azo reductase activity. Under aerobic conditions, only a few aerobic bacteria have been found to reduce azo dyes. There are three types of azo reductases that have been identified. Flavin dependent NADH preferred azo reductase, flavin dependent NADPH preferred azo reductase, and flavin free NADPH preferred azo reductase are the three flavin dependent azo reductases [49]. Each enzyme was purified from separate bacteria and studied in *Bacillus subtilis, Escherichia coli, Enteroccus fecalis,* and *Pseudomonas aeruginosa* for azo reductase characterization and crystal structure. *Shewanella* strain azo reduction is linked to electron donor oxidation, electron transport, and energy conservation in cell membranes, according [50], Anaerobic azo compound reduction by bacteria has been shown to be capable of simultaneously transforming hazardous chemical molecules and reducing azo compounds. It would be perfect for bioremediation of azo dyes and other harmful organic chemicals-contaminated environments [51].

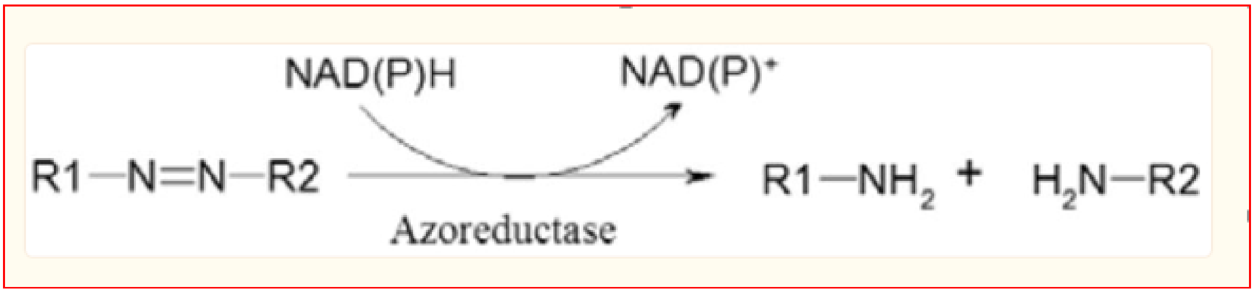

Figure 4: The reduction of Azo dyes by NAD(P)H catalyzed by azoreductase [50]

2.8. Bacterial isolates involved in azo dye degradation

Bacillus, Acinetobacter, Staphylococcus, Legionella, and *Pseudomonas* bacterial isolates had been tested for their ability to decolorize textile effluent [51]. *Staphylococcus aureus, Bacterioides fragilis, Bacillus subtilis, Bacillus cereus, Clostridium perifringens* and *Escherichia coli* were tested for their ability to decrease and regulate textile waste water comprising primarily Indigo Blue [52]. Spectroscopic analysis was used to investigate bacterial dye decolorization. The bacterial inoculums were inoculated into flasks containing azo dyes, yeast extract, glucose, and sucrose, and incubated for four days. The degree of decolorization was measured in percentages. The best decolorizer of Blue RR was *Psuedomonas putida* (95%). The best Black B decolorizer was *Pseduomonas aeruginosa* (93%). *Bacillus subtilis* was the greatest decolorizer for Red RR (91 percent) [53]. Yello RR decolorizes well with *Bacillus subtillis* (65%). The best decolorizer of navy blue was *Pseudomonas aeruginosa* (70.58 %). *Clostridium biofermentans* isolated from a contaminated site was used to investigate the decolorization of textile reactive azo dyes in aerobic conditions [53-54]. After 36 hours post-inoculation, *Clostridium biofermentans* decolorized the dyes Reactive red 3B-A, Reactive black 5, and Reactive yellow 3B-A by over 90%. Spectrophotometric analyses of the reactive dyes revealed no distinct peak indicating aromatic amines. *Clostridium biofermentans* was found to be a suitable bacterium for biological digestion of dye-contaminated waste water [54]. For both aerobic and facultative anaerobic bacteria, decolorization of several azo dyes occurs in anaerobic circumstances by reducing the azo bond [55].

3.0. Conclusion

Azo dyes and the aromatic amines that are produced are dangerous pollutants for our environment as well as carcinogenic and mutagenic when disposed off without prior treatment to the environment and human health. The indiscriminate discharge of industrial dye effluents into the environment is a huge threat to human life. Detoxification of sludge is best accomplished through microbiological treatment. Numerous bacterial species have created biological processes, especially enzymatic ones for removing azo dye contamination from medium but physiochemical parameters have an upper limit on the effectiveness of these systems. To enhance the bioremediation process, it is essential to comprehend the interaction between these variables. Cancer and other pertinent human diseases can be significantly decreased if we can successfully limit the use of azo dyes and stop the contamination of our environment with azo dyes and their hazardous aromatic amines.

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