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An overview of azo dyes environmental impacts

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Abstract

Intensive industrialization has led to the discharge of increasingly complex wastewater compositions, necessitating advanced treatment methods to safeguard environmental and public health. Among the most persistent and hazardous pollutants are azo dyes, a class of synthetic colorants widely used due to their low cost, vibrant hues, and color fastness. However, their resistance to natural degradation and tendency to bio-accumulate make them significant environmental contaminants. Once released into ecosystems, azo dyes pose allergenic, carcinogenic, mutagenic, and teratogenic risks. Traditional physico-chemical treatment methods, while somewhat effective, are often expensive, generate secondary pollutants, and do not guarantee complete detoxification. In contrast, biotechnological approaches particularly those involving microbial degradation are gaining attention for their ability to achieve complete mineralization of azo dyes in a cost-effective and environmentally friendly manner. In nature, green plants and microorganisms work together to maintain the balance between organic and inorganic matter. While plants fix inorganic compounds through photosynthesis, microbes decompose organic materials, converting them back into inorganic forms. The introduction of synthetic dyes into this cycle disrupts this natural equilibrium. Although natural dyes are safer and more sustainable, they remain economically unfeasible for large-scale use due to their higher costs and more complicated application methods. Thus, azo dyes continue to dominate industrial applications. A significant concern with untreated azo dye effluents is the formation of aromatic amines, highly toxic degradation by-products resulting from the breakdown of azo bonds. These compounds are considered serious carcinogens, posing threats to both human health and ecological systems. This review explores the classification, environmental impact of azo dyes, including their effects on oxygen demand, aquatic ecosystems, and agriculture. It also examines current legislative frameworks and regulatory guidelines aimed at managing the risks associated with azo dyes and their toxic by-products.

Keywords: Azo Dyes; Biotechnology; Climate Change; Environmental Impacts; Toxicological Prospects

1. Introduction

Industries are the most significant contributors to environmental pollution, which in turn causes climate change. The method of dyeing is the main culprit when it comes to water pollution in the textile sector. Visually and ecologically damaging effects can occur when this pollution prevents sunlight from penetrating water, which impacts photosynthetic organisms and the biochemical oxygen demand (BOD) [1].

Natural dyes and dyeing techniques are as old as textiles themselves. Dyes can be derived from plants, animals, or minerals. In Europe, dyeing was already practiced in the Bronze Age. The oldest records of natural dye use were found in China, dating back to around 2600 BC. Despite their non-toxic and environmentally friendly nature, and generally low side effects, natural dyes are scarcely used today compared to synthetic dyes due to limited knowledge about extraction and dyeing efficiency [2-4].

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Plants and animal fats are two examples of the organic ingredients that have historically been used to create natural colors. Other inorganic substances include water, clay, dirt, minerals, metal salts, and semiprecious stones like malachite. Although natural dyes have the potential to be safer and less harmful to the environment than synthetic dyes, they are not practical for use in many commercial applications due to their high cost and difficulty in procurement and application. Specifically, the desirable dyeing components comprise just around 2% of the mass of natural dye sources, meaning that substantial amounts of raw material must be treated. Natural dyes may become inappropriate for mass production due to the high expenses caused by this [5, 6]. The first synthetic dye, Mauveine, was accidentally synthesized in 1856 by William Henry Perkin [7]. Over time, synthetic dyes supplanted natural ones because of their greater color variety, lower production costs, and resistance to fading in water, sunlight, and chemicals [8].

There were 10,000 different synthetic dyes available by the century's close. The current annual dye consumption is 600,000 tons, shared among Taiwan, South Korea, China, Eastern Europe, and India [9]. Discarded synthetic colors make up around 15% of the total. One unintended consequence is that less oxygen is able to reach aquatic species, leading to metabolic stress, flower necrosis, mortality, and stunted animal growth. Another is that sunlight is unable to reach water bodies, which impacts photosynthetic organisms. [10].

2. Azo dyes

Azo dyes, with over 2,000 structurally different compounds comprising 70% of all dyes, represent the most significant, most important, and most diverse synthetic dyes in wide industrial use [11-14]. Figure 1 shows some commonly used azo dyes' chemical structures, chemical names, and CAS numbers. These dyes are characterized by one or more nitrogen-nitrogen double bonds (–N=N–) between aromatic rings and auxochrome groups such as hydroxyl, sulfonic, or amino groups. Based on the number of azo bonds in their molecular structure, they are classified as monoazo, diazo, triazo, tetraazo, and polyazo dyes [15].

Azo dyes can be grouped according to their solubility and application

- Water-soluble dyes: basic, acidic, substantive (direct), acid mordant, reactive, metal-complex, leuco esters.
- Water-insoluble dyes: vat dyes, disperse dyes, pigments, oil- and fat-soluble dyes, colored lacquers.
- Fiber-developed dyes: such as naphthol AS dyes, oxidation dyes, and dyes for color photography [16].

Since their discovery in 1856, azo dyes have become the most commercially used dyes. They are ubiquitous in the textile industry and used in paper, leather, plastics, and other industries [17, 18]. Most azo dyes are used to color textiles and other fibrous materials via adsorption from aqueous solutions. Those used in non-aqueous media include dyes soluble in mineral oils, fats, alcohols, and organic solvents. Water-insoluble azo dyes are used to color oils, petroleum products, waxes, alcoholic inks, leather, wood, plastics, varnishes, and even food products.

All azo dyes are synthesized to be stable against washing, chemical and microbial actions, and light exposure [19]. Due to these properties, they are highly persistent and difficult to biodegrade [20]. More than 10% of azo dyes used in dyeing processes do not bind to the fibers and enter the environment, posing a serious ecological issue [11]. Many azo dyes are carcinogenic, mutagenic, and allergenic. Their intense coloration also causes visual pollution [21]. Generally, toxicity increases with the number of benzene rings in the structure. Carcinogenicity depends on the molecular structure and degradation mechanisms. Azo dye degradation often results in aromatic amines, which may themselves be carcinogenic. The azo bonds are usually located within benzene or naphthalene rings, and sometimes between aromatic rings and attached aliphatic enolizable groups [22].

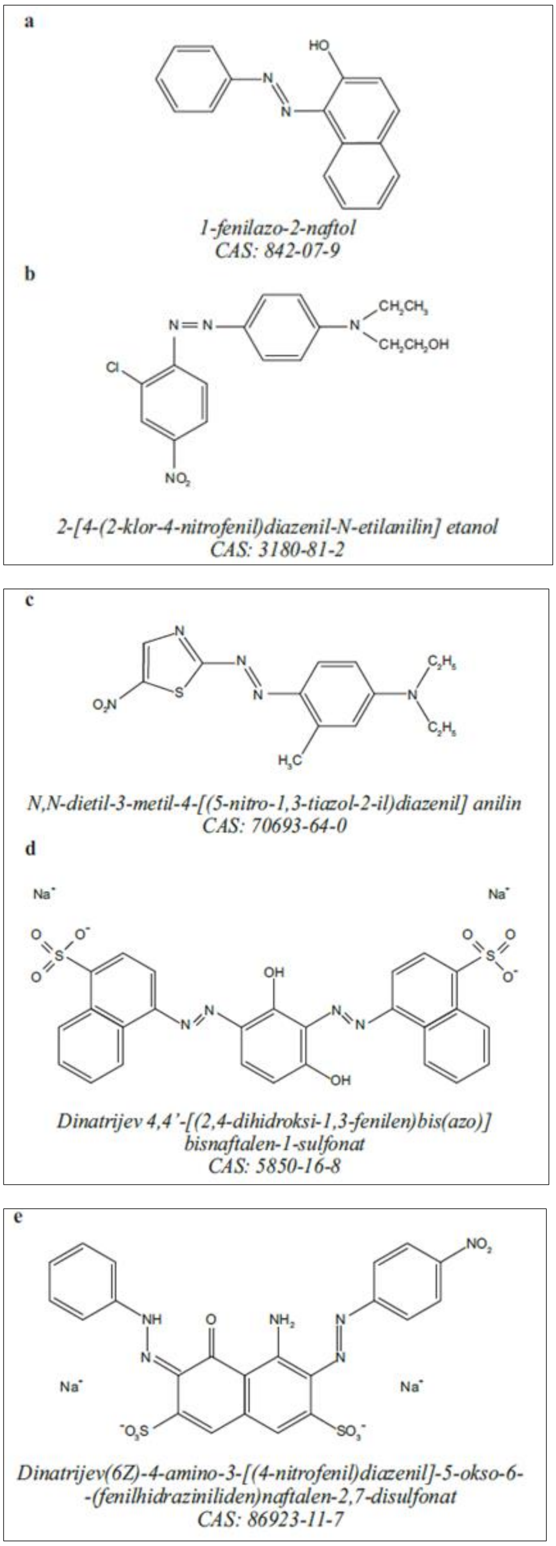

Figure 1 Chemical structures, chemical names and CAS numbers of some azo dyes: a - yellow, b - red, c - blue, d - brown, e – black [15]

3. The Long-Term Effects of Azo Dyes on Ecosystems and Human Well-being

Since azo dyes are xenobiotic by nature and difficult to biodegrade, their removal from wastewater has attracted growing interest from environmental researchers [23]. Due to their synthetic origin, highly complex structure, and stability, they degrade slowly and lose their color. Therefore, they must be removed from water before it is discharged into the environment [20].

Removing color does not necessarily mean removing toxicity. Incomplete degradation can result in the formation of even more toxic by-products. It is therefore essential to verify the success of dye degradation through ecotoxicological testing [24]. Degradation products of azo dyes cause aesthetic problems and contribute to the mutagenicity of soil and surface and groundwater that come into contact with them [14].

Human exposure to dyes occurs primarily in food production, food packaging, pharmaceuticals, and through skin contact. A particular concern regarding the toxicity of azo dyes is that most of them contain known carcinogenic aromatic compounds like benzene and naphthalene [20]. Basic azo and diazo dyes are some of the most toxic [25].

3.1. Key harmful effects of dyes include

- Depending on dye concentration and exposure duration, both acute and toxic effects on organisms may occur.
- Even low concentrations in water can cause intense coloration, attracting public and regulatory attention.
- Dyes present in water interfere with light transmission and thus disrupt organisms at the base of the food chain [26].

3.2. Effects on the growth of plants and crops

There is a lot of worry surrounding azo dyes due to their many dangers to human and environmental health [27]. The amount and distribution of each toxic component in an ecosystem determines its total toxicity, which in turn impacts the biodiversity of that habitat [28]. Potentially genotoxic environmental chemicals are encountered by living things on a cellular and molecular level. The ability to foretell the effects of specific agents on faunas and floras, and by extension, humans, depends on studies of genotoxic potential [29]. When studying the effects of azo dyes and harmful chemicals in contaminated wastewater reservoirs, Wang and Keturi found that root-to-shoot ratios and % seed germination could be helpful, quick, easy, and repeatable methods [30]. In their study, Umesh et al. [31] examined the effects of synthetic azo dyes on five different plant seeds, including their germination and root/shoot elongation.

Practical toxicity assessments for synthetic azo dyes and environmental contamination monitoring are made possible by phytotoxicity bioassays. The mechanism by which plants and crops absorb and store minerals and dyes is dependent on a number of variables, such as the plant type, the soil's metal availability, the dye's water concentration, and the solubility index of the metals [32]. Compared to other plant types, leafy greens absorb and store heavy metals at a higher rate. One possible explanation is that metals accumulate less quickly in fruits because translocation from roots to stems and finally to fruit takes longer in non-leafy vegetables, which have higher transpiration and translocation rates [33]. Percentage growth, dry matter, and heavy metal uptake were measured in potted Amaranthus cruentus L. for various azo dye concentrations by Oguntade et al. [34]. They discovered that when the concentration of the azo dye solution was low, the nutrient components positively affected the growth rate and yield of Amaranthus. However, when the concentration was high, the growth and yield were retarded, and there was an accumulation of heavy metal content ratio and their degraded products in the edible shoots. Eating the shoots instead of the roots increased the concentration of bio-accumulated heavy metals and organic azo-dye molecules. The concentrations of zinc (Zn), manganese (Mn), and iron (Fe) in the edible shoots were significantly higher than the international permissible limits for human consumption, as set by the World Health Organization, the Nigerian Federal Environmental Protection Agency, and the United Nations Food and Agriculture Organization.

Regular consumption of vegetables tainted with dye effluent is associated with health hazards, which is why these limits have been put in place. Furthermore, reactive azo dyes are poisonous and can destroy soil microbes, which in turn reduces agricultural yields, according to research by Savin and Butnaru [35]. Consequently, using wastewater to water crops and vegetables requires industrial effluent pretreatment and detoxification.

3.3. Effects on Water Ecosystems

According to reports, azo dye waste is a major contributor to water contamination and other environmental issues. Studies have demonstrated that aquatic creatures can experience a variety of physiological problems, such as cramps, hypertension, intermittent fever, and kidney impairment, when they drink azo-dye effluent [36]. Toxicants, both organic and inorganic, can build up in waterways depending on how long they stay in the system, how they enter the food chain, and how they affect the body. Like many other aquatic vertebrates, fish and tadpoles are highly vulnerable to aquatic contaminants because their gills, essential for breathing, acid-base balance, and osmoregulation, put them in close contact with the aquatic environment. When air pollution levels are too high, it can disrupt homeostasis in these organs, leading to subpar functioning in daily life [37].

In aquatic environments, azo dyes impede photosynthesis in green biomes and inhibit aquatic biota. Despite the colors' non-degradability, the anaerobic degradation of synthetic azo dyes in sediments produces hazardous amines [37, 38].

Soni et al. [39] conducted a toxicological investigation on the freshwater fish Gambusia affinis, comparing the effects of treated and untreated textile-dye wastewater. Treatment significantly decreased death rates and cytotoxic effects on RBCs, including the frequency and severity of poikilocytosis, an irregular form of the red blood cells. The toxicological and histological effects of synthetic textile-dye effluent on teleost fish (Poecilia reticulata) were investigated by Selvaraj et al. [40] following this work. The aberrant behaviors brought on by the dye effluent included hyper-excitation, irregular swimming, rapid opercular movement, and a thickening of the mucus covering. Furthermore, the study found histopathological alterations, such as an enlarged primary gill bar, detached secondary gill bar, deteriorated intestinal villi, and substantial hemocyte infiltration into the internal lumen.

4. Removal of Azo Dyes

Several treatment strategies, including chemical, physical, biological, and hybrid approaches, have proven efficient in eliminating azo dyes. These procedures are necessary since azo dyes are detrimental to aquatic life and can cause disease in humans (Figure 2). Chemical and physical treatments have one major flaw: they create sludge, which is challenging to manage and necessitates extensive treatment facilities. The enzymes that catalyze the removal and degradation of synthetic dyes are produced most efficiently by white-rot fungi [41]. Variables like pH, nutritional load, treatment time, aeration, carbon (C: N) ratio, biomass shape, inoculum concentration, co-substrates, and salt content affect the effectiveness of azo-dye removal [42].

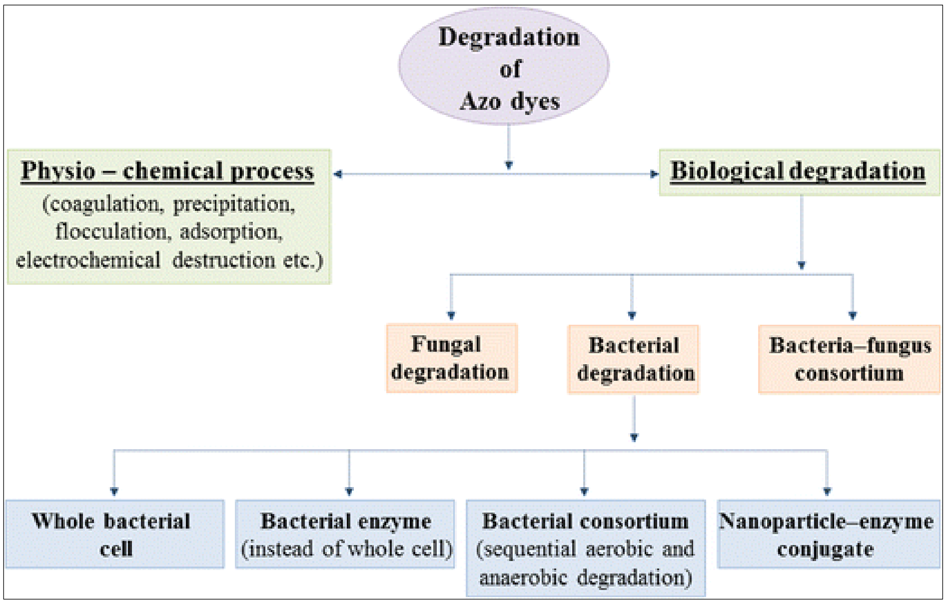

Figure 2 Methods of Azo dye removal

4.1. Physical-Chemical Removal Methods

Physical methods remove substances from wastewater without chemical reactions. The most common approaches include filtration, adsorption, and precipitation, which are based on physical factors like filterability, adsorption affinity, and the ability of substances to precipitate. When added chemical agents intensify the removal of pollutants, whether suspended particles or dissolved organic matter, this becomes a physico-chemical treatment [43]. Physico-chemical methods specialized in dye removal either concentrate the dyes into a sludge or aim to destroy their molecular structure [44] completely. These processes include:

- Adsorption
- Flocculation
- Coagulation

- Precipitation
- Membrane filtration
- Ion exchange
- Radiation
- Fenton process
- Chemical oxidation [20, 45]

The ideal goal is full mineralization of dyes into CO_2, H_2O, NO_3^-, and SO_4^{2-}. However, many dyes are resistant to light and water-soluble, which makes it difficult for these methods to reduce dye concentration to acceptable levels (e.g., 0.003 mg/L), significantly lower than the limits for other organic pollutants in treated water [44].

Although these methods can be effective, they have technical and economic limitations, as shown in Table 1 [21].

Table 1 Methods of Physical-chemical Removal of dyes

Physico-chemical Method	Advantages	Disadvantages
Oxidation	Fast processing time	High energy demand and formation of degradation by-products
Adsorption	Effective in removing a wide range of dyes	Adsorbent requires regeneration or disposal
Membrane Technology	Capable of removing all dyes	Formation of concentrated dye waste
Coagulation / Flocculation	Economically viable	Generates large amounts of sludge

Advanced Oxidation Processes (AOPs) are among the most promising recent developments, which have shown the best results in physical-chemical treatment [46]. However, due to the drawbacks of these methods, there is increasing interest in alternative technologies, especially biological processes, which are more environmentally friendly [47].

4.2. Biological Removing Methods

Biological treatment is more acceptable than physical-chemical methods due to its cost-effectiveness, lower sludge production, and environmental friendliness. Using microorganisms to remove dyes from wastewater offers advantages such as low maintenance costs and the potential for complete mineralization of pollutants into non-toxic end-products [12, 48].

Since azo dyes are designed to resist degradation, they are also highly resistant to biodegradation using conventional activated sludge technology. The chemical structure of dye molecules plays a key role in the efficiency of biodegradation. However, a clear relationship between structure, dye concentration, and microbial selectivity has not yet been fully established [49].

De-colorization of wastewater containing dyes occurs through the breakdown of C=C and N=N bonds or heterocyclic aromatic rings, shifting the light absorption out of the visible spectrum [20].

Studies show that dyes are not readily biodegradable because microorganisms cannot use them as substrates [20]. Most microbial communities are applied without detailed knowledge of their composition [50]. In addition to classic methods of isolating and selecting microorganisms, genetic modifications and mutations are also used to improve the bioremediation of hard-to-degrade compounds like dyes. Due to synergistic interactions among microbial species, mixed microbial cultures are more effective for decolorization than pure cultures. It has been found that transferring pure culture-based processes from the lab to real systems is more difficult than with mixed cultures. In general, bacteria degrade dyes faster than yeasts [51]. Another benefit of microbial diversity is deleting a wider range of pollutants. The process efficiency can be further improved by adding a co-substrate (e.g., glucose, lactose, yeast extract), which serves as an additional nutrient source and provides electrons necessary for breaking azo bonds, accelerating degradation by up to 36% [51].

Microorganisms can, under certain conditions, develop enzymatic mechanisms to degrade azo dyes. Although limited, research has shown that yeasts like Cunninghamella elegans ATCC 3612 can metabolize up to 85% of the dye malachite green in 24 hours, although the mechanism is still unclear [49].

Bacteria can degrade azo dyes aerobically and anaerobically [52], but aromatic amines produced during anaerobic degradation may be toxic or more toxic than the original dye [49]. Azo dyes are particularly resistant to aerobic microbial degradation, especially in activated sludge systems. Their resistance is due to electron-deficient azo (-N=N-) and sulfonic (-SO_3^-) groups, which make them less susceptible to bacterial oxidation [53].

In aerobic conditions, decolorization often occurs through adsorption onto bacterial cells rather than oxidation. In contrast, anaerobic systems offer advantages:

- No need for aeration
- Less sludge production
- Methane generation as a byproduct [51].

However, the resulting aromatic amines require further aerobic treatment for mineralization. Therefore, combining anaerobic and aerobic conditions is considered optimal for complete degradation. Over the last two decades, several technological methods for removing color from industrial wastewater have been developed, with some already used in industry [54]. Yet, there remains a need for more cost-effective biological processes capable of treating large volumes of colored wastewater [20]. Due to their genetic diversity and metabolic potential, microorganisms can be powerful agents for bioremediation. Some capable of azo dye reduction include: Obligate anaerobes: *Bacteroides, Eubacterium, Clostridium,* Facultative anaerobes: *Sphingomonas, Pseudomonas luteola, Proteus vulgaris, Streptococcus faecalis* [55].

Azo reduction usually occurs under anaerobic conditions, but bacteria can also aerobically mineralize some aromatic amines [54, 55]. Certain fungi *Irpex lacteus, Pleurotus ostreatus, Trametes modesta, Phlebia tremellosa* also participate in decolorization, though the biochemical pathways are not fully understood [56]. Yeasts can use dye components (e.g., aniline) as sole sources of carbon and nitrogen. Co-substrates again help reduce process duration and cost [49, 51].

5. Conclusion

The color industry has been radically changed by azo dyes, which are utilized in numerous industrial sectors thanks to their color fastness, low cost, and great intensity. But azo dyes are harmful to the environment and a significant factor in climate change, even at low doses. The removal of the majority of pollutants from wastewater does not necessarily mean that toxicity has been eliminated. On the contrary, incomplete degradation and the formation of unwanted by-products during degradation can even increase toxicity. By applying multi-stage biotechnological processes, efficient, cost-effective, and ecologically acceptable biodegradation of the complex components of azo dyes can be accomplished. These processes use mixed microbial cultures of microorganisms with specific enzymatic potential and metabolic efficiency. To reduce environmental problems caused by the persistence of azo dyes and other widely used xenobiotic compounds, it would be generally beneficial to develop and manufacture substances whose structure is compatible with the naturally existing catabolic potential of microorganisms. This would require intensive cooperation and dialogue between chemists, microbiologists, biotechnologists, and toxicologists.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare no conflict of interest.

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