

Comprehensive Review on Microbial Degradation of Dyes

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Abstract: The extensive use of synthetic dyes in industries such as textiles, paper, and leather has led to severe environmental pollution due to the release of toxic and non-biodegradable dye effluents. Conventional physico-chemical treatment methods, such as adsorption, coagulation, and chemical oxidation, are often inefficient, costly, and environmentally unsustainable. Microbial degradation has emerged as an eco-friendly and cost-effective alternative for dye removal, utilization of bacteria, fungi, and algae to break down the complex dye molecules into less toxic or non-toxic metabolites. Various microbial species, including *Pseudomonas*, *Bacillus*, *Phanerochaete chrysosporium*, and *Aspergillus*, produce enzymes such as laccases, peroxidases, and azoreductases that facilitate the degradation of different dye classes, including azo, anthraquinone, and triphenylmethane dyes. Factors such as pH, temperature, oxygen availability, and microbial adaptability influence the efficiency of dye biodegradation. Recent advancements in genetic engineering and microbial consortia optimization have further enhanced degradation rates and efficiency. Additionally, immobilization techniques and bioreactor-based systems offer promising solutions for large-scale applications. Despite its potential, challenges such as the toxicity of dye intermediates, microbial resistance, and the need for process optimization remain. Future research should focus on metabolic pathway elucidation, strain improvement, and the integration of microbial degradation with other treatment technologies to achieve complete mineralization of dyes. Overall, microbial degradation provides a sustainable and efficient approach to mitigating dye pollution, promoting environmental safety, and supporting circular bioeconomy initiatives.

Keywords: environmental pollution, physico-chemical treatment, Bacteria & fungi, biodegradable, sustainable approach

I. INTRODUCTION

Colors play an important role to different industries and in consumer choices. It also contributes to the aesthetic quality of a product, which drives consumers to purchase and therefore contributes to economic growth. The different colors and hues that we see are often derived from the dyes that were used. Natural dyes are the safer and more eco-friendly option compared to synthetic dyes. They also pose other advantages such as having antimicrobial properties and offering protection from UV light [1].

Although natural dyes are the safer and the more environmentally friendly option to use, they are quite costly, more tedious to apply, and more difficult to procure. Tyrian purple, for example, is a natural dye from the mucus of *Murex* sp. snails that retails for about €2000 per gram. Therefore, the use of natural dyes has been deemed to be impractical for many commercial applications. Moreover, natural sources of dyes usually contain only about 2% of actual coloring material, which means that uneconomic amounts of raw material might be needed to obtain the desired shades and hues. This drives up its cost and is thus undesirable for mass production use. It is nearly impossible to reproduce the same shade from batch to batch and fastness properties are rather poor, which make it difficult to apply on textiles.

As an alternative, synthetic dyes such as azo dyes have become the primary coloring agent in industries such as the food, cosmetics, and textile industries as trace amounts of dyeing material already produce an intense color. Azo dyes take account for most of the synthetic dyes used as they are easy to synthesize, thus cost-efficient to produce, and generate a variety of colors as there are about 10,000 different azo dyes available [2]. Since azo dyes are synthesized chemically, careful downstream treatments are needed to ensure safety in its usage and disposal. It is important to

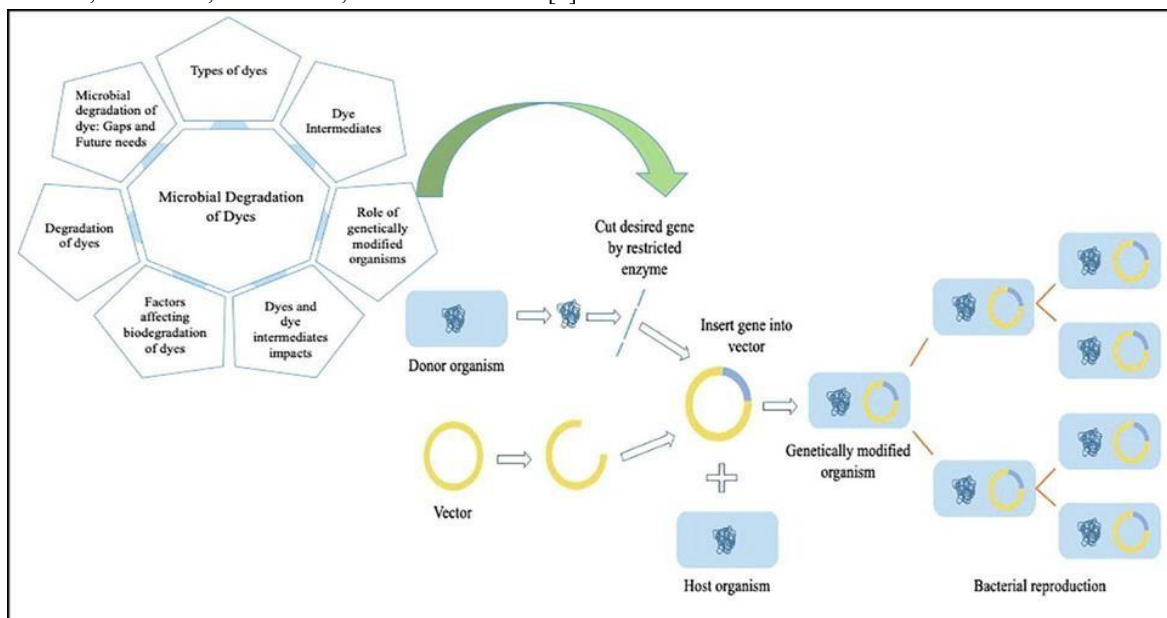


consider that only 10% of the dye is transferred into the material permanently, and the majority goes into wastewater as effluents. At the same time, trace amounts of dye can lead to severe environmental and health hazards as some azo dyes are toxic, carcinogenic, and mutagenic. The presence of these synthetic dyes can also hamper various biological activities. Therefore, it is important to implement tight regulations for their treatment and disposal.

Several physical and chemical procedures are available for the downstream treatment and waste handling of azo dye containing effluents[3]. Quite often, these approaches are met with skepticism, as these have major downsides such as the ecologically questionable waste disposal for filters and charcoals, generation of toxic intermediates, production of sludge, and the high costs of equipment. Thus, finding a more environmentally friendly approach is essential. Here, biological methods can be a more promising solution. This review tackles the treatment methods available for azo dyes especially on the biological context (primarily bacteria) with emphasis on the approach of whole-cell biocatalysis and/or enzymatic degradation. Physical parameters that affect dye decolorization and degradation, such as pH and temperature, are not within the scope of this review.

Impacts of Azo Dyes on Human Health and the Environment

Azo dyes are characterized by the presence of an azo bond ($-N=N-$) between two or more aromatic rings. The versatility of azo dyes renders them very appealing for various industries such as food and textiles. However, the xenobiotic nature of these dyes calls for proper evaluation of their harmful effects. In the food industry, the use of azo dyes should be critically assessed as they are often used as colorants for sweets and desserts with children as target consumers. Although there are regulations on which dyes may be used or not, these are different by country, which adds to the difficulty of standardizing protocols for the usage of dyes. There are thousands of azo dyes used in various industries. Some of the better-known azo dyes used in the food industry are Brilliant Black BN, Sunset Yellow FCF, Amaranth, Azorubine, Ponceau 4R, and Allura Red AC[4].



The toxicity of some of these azo dyes can be attributed to the reduction of the azo bond, which produces aromatic amines. When ingested orally, the dye reaches the gastrointestinal tract, and the intestinal microflora or mammalian azoreductases cleave the azo bond. The aromatic amines, which are often the final product of azo dye reduction, are subsequently hydroxylated or acetylated, and this adds to the mutagenicity and carcinogenicity of these compounds. The intake of azo dyes can also increase the risk of human bladder cancer, splenic sarcomas, hepatocarcinomas, and nuclear anomalies. They can also cause allergies, dermatitis, and even DNA damage that results in the formation of malignant tumors. Methyl Yellow, which is now banned in different countries, was formerly used for dyeing butter, and



it was found to cause liver cancer in rats after two to three months of exposure. Another example is the dye Amaranth, which was shown to induce DNA damage in the colon epithelium of mice, while Brilliant Black BN has shown genotoxicity in human lymphocytes based on in vitro experiments. Sunset Yellow FCF can cause DNA damage and has shown to have toxic effects on the reproductive and neurobehavioral system of tested rodents and chick embryos. Tartrazine was shown to bind to albumin, induce neurotoxicity, impair mental functions, and promote various reactions such as angioedema, nasal congestion, itchy skin, and hives. In the cosmetic and textile industries, 4-aminobenzene or Aniline Yellow, a dye used for printers and as a precursor of other dyes, has been shown to cause high hepatocarcinogenicity and induce tumors to rats. Sudan III, used for coloring non-polar substances such as acrylic emulsions, was also reported as a carcinogen [5].

Impact of Azo Dye Metabolites

As mentioned earlier, not all azo dyes are harmful. However, dyes that were not found to be harmful still pose a threat, since oxidative and reductive metabolism could lead to the formation of toxic aromatic amines. Different studies have identified compounds derived from azo dye metabolism such as benzidine, p-phenylenediamine, aniline, and toluene, which show carcinogenic and mutagenic properties.

Benzidine is a building block of azo dyes such as Congo Red, Direct Black 38, and Direct Red 39. Earlier studies have shown that it can induce tumor cells on different body parts such as the gastrointestinal tract, pancreas, liver, and gallbladder. Benzidine and its congeners, such as 3',3'-dimethylbenzidine and 3',3'-dichlorobenzidine, were shown to induce carcinoma and tumors. 3',3',5,5'-Tetramethylbenzidine was the only congener of benzidine that was not found to be carcinogenic.

Meanwhile, p-phenylenediamine is another compound that is used as a henna substitute, for the manufacturing of certain polymers in different industries, as a developing agent for films, and as a major component for hair dyes. Initial investigations on p-phenylenediamine showed contrasting results. On the one hand, it was shown to be carcinogenic via an Ames test. On the other hand, it was shown that it did not pose any carcinogenic potential to F344 rats regardless of the sex and the exposure time [6,7]. It was also shown to not affect pregnant rats, and a multigenerational reproduction study also showed no negative effects. However, it was discovered that p-phenylenediamine only becomes mutagenic after oxidation. This was corroborated when p-phenylenediamine, in the presence of microsomal fraction and after H₂O₂ oxidation, became mutagenic to a Salmonella typhimurium tester strain TA1538. This poses a problem as most permanent hair dyes need to be oxidized upon usage to exert their dyeing properties. It was shown that upon absorption, p-phenylenediamine can lead to the formation of tumors in the liver, kidney, urinary bladder, and thyroid gland of rats. It was also found that it can increase the expression of p53 proteins, thereby suggesting an increase in apoptosis and affecting cell viability. A correlation was also observed on the usage of hair dyes and Non-Hodgkin lymphoma and cancer.

Other monocyclic aromatic amines such as p-nitroaniline, o-toluidine (2-methylaniline), 2-nitro-p-phenylenediamine, and o-phenylenediamine were also shown to have carcinogenic and mutagenic properties. These compounds are all highly relevant toxins, as occupational exposure to aromatic amines can explain 25% of bladder cancer cases [8].

Physical and Chemical Treatment of Dyes

As azo dyes pose different risks and hazards to health and the environment, it is necessary to find ways to treat these dyes. Various physical and chemical methods have been explored. Physical methods include techniques such as adsorption and filtration. Adsorption uses materials such as activated carbon that can accumulate compounds to be removed from wastewater at their surface. Although activated carbon is effective and has been the primary option for this kind of treatment, it is not often used due to its high cost. Alternatives have been explored, such as peat, banana peels, clay, corn cob, maize, and wheat straw. However, there are some drawbacks due to the problematic waste disposal of these cheaper alternatives. Another frequently used physical method is filtration. It often involves the use of membranes to remove suspended solids and other unwanted materials from water. Although effective, it likewise has



some drawbacks such as similarly high costs for investment and of materials, deterioration of the membrane or membrane fouling, production of potentially toxic sludge, and again problematic waste disposal.

Chemical treatment involves the use of different chemicals or techniques such as coagulation– flocculation, Fenton’s reagent, ozone, and electrochemical methods. Coagulation–flocculation followed by sedimentation are processes used in conventional wastewater treatment facilities [9]. Coagulation involves the use of coagulants to neutralize suspended solids (often with an opposite charge to the coagulant). Once neutralized, the suspended solids can collide and form microflocs. During flocculation, these microflocs can form macroflocs and sediment, upon which they can be removed from water using gravity. Furthermore, the Fenton reaction allows the generation of hydroxyl radicals using Fenton reagent (H₂O₂ and Fe²⁺ ions), which can destroy toxic pollutants in wastewater. Although the overall process is cheap, it can lead to a high sludge production as secondary waste. Ozonation uses reactive ozone (O₃) to oxidize and disassemble preferentially the chromophores of dyes, but its unstable nature makes it undesirable for wastewater treatment.

These physical and chemical treatments have been used traditionally for wastewater treatment. However, as mentioned earlier, these approaches can pose several drawbacks. Furthermore, dyes may be resistant or recalcitrant to these available treatments [10].

II. CONCLUSION

Azo dyes have become important in different industries, especially because color plays a huge role in consumer choices. However, with the increased usage of azo dyes, several health and environmental problems have emerged, which are caused by some of these azo dyes and its metabolites. As modern societies are striving toward greener solutions, bioremediation should be taken advantage of. Microorganisms have shown versatile performance not only in the biomedical field but also in the realms of environmental application. Microorganisms have tremendous potential still to be explored, and there are numerous enzymes from various microorganisms that should be further studied. The ability of these microorganisms to accept a broad spectrum of xenobiotics must also be also looked upon. Modern technology has displayed tremendous progress over the past decades.

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