Bacterial Potential in Degradation of Azo Dyes

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Contributor: Lucas Rafael Pinheiro

The use of dyes dates to ancient times and has increased due to population and industrial growth, leading to the rise of synthetic dyes. These pollutants are of great environmental impact and azo dyes deserve special attention due their widespread use and challenging degradation. Among the biological solutions developed to mitigate this issue, bacteria are highlighted for being versatile organisms, which can be applied as single organism cultures, microbial consortia, in bioreactors, acting in the detoxification of azo dyes breakage by-products and have the potential to combine biodegradation with the production of products of economic interest. These characteristics go hand in hand with the ability of various strains to act under various chemical and physical parameters, such as a wide range of pH, salinity, and temperature, with good performance under industry, and environmental, relevant conditions.

sustainability effluent treatment dyes bioremediation bacteria wastewater textile BES bioreactor

1. Introduction

The use of dyes for the aesthetic improvement of objects is an ancient practice, with historical records indicating that dyes of natural origin were already in use 3500 years BC. In the beginning, the coloring agents available (dyes and pigments) were only of natural origin, obtained from mineral sources, vegetables—such as those found in Mediterranean (*Rubia tinctorum*) and Brazilwood (*Paubrasilia echinata*)—which are mostly represented by chemical groups of naphthoquinones, anthraquinones and flavonoids, and those obtained from animals, such as those extracted from some insect species, like the cochineal (*Dactylopiidae coccus*). The coloring obtained with these dyes was applied to utensils, weapons, and dwellings, among others, having aesthetic and cultural importance [1][2].

From ancient times to the present moment of our history, dyeing technology has evolved with the discovery of new matrices and raw materials and the synthesis of new pigments and dyes. In 1856 a major discovery was accidentally made by William Henry Perkins, when he synthesized what came to be the first synthetic dye in history, mauvein [3]. Synthetic dyes have largely replaced natural dyes over the years due to their wide range of colors, cost-effectiveness, and resistance to fading by sunlight, water, perspiration, and different chemicals [4].

It is estimated that around 10,000 different dyes are currently being produced on an industrial scale, with an annual worldwide production volume of around 700,000 tons and about 10 to 15% of those are discarded into nature. This

scenario generates serious consequences for the contaminated environment, such as interference with the entry of sunlight into the water, influencing photosynthetic organisms, causing damage to the oxygen level of the water, metabolic stress, neurosensorial damage, flora necrosis, death, and decreased growth of fauna, among others. Moreover, humans are also potential victims of these compounds, when discharged into nature without treatment, and can be quite toxic, either by oral or respiratory ingestion as well as mere skin contact [2][5][6]. The toxic effects of azo dyes, in particular their ability to promote mutations, are related both to the dyes themselves and to metabolites released upon their breakage or degradation, such as aromatic amines. The possibility of the dye breaking down and releasing these carcinogenic amines on contact with saliva or gastric juice is one of the factors evaluated in classifying the dyes as potentially hazardous to health. However, when ingested, the dye can also be reduced by the action of intestinal bacteria and, possibly, by the enzyme azoreductase present in the liver or intestinal wall, showing how complex the remediation of these toxins can be [7].

Therefore, it is necessary to understand the risks associated with the discarding of these dyes in the environment without prior treatment and how the use of microorganisms in the bioremediation of these contaminants is a viable alternative.

2. Bacteria in the Bioremediation of Azo Dyes

Biotechnology has been widely employed in the search for solutions to the degradation and elimination of dyes, mainly because biological solutions are effective and generate less negative impact on the environment [8]. When dealing with biological processes using bacteria, especially potentially pathogenic genera and species, the concern with a possible biological impact of them, when introduced into the environment for the bioremediation process, may arise. To attend to any unwanted negative effects, some strategies can be used, some of those include: (1) the use of isolated and purified enzymes or other bacterial products that act on the discoloration without needing the bacterial cell itself [9], (2) microbial bacteria/consortia isolated from the contaminated environment itself or similar environments, in order to increase the chance of integration of the bacteria with the environment and the existing microbiota [10], (3) application of genetic engineering techniques that can develop bacterial strains with programmed death, stopping bacterial metabolism in the absence of the target contaminant [11].

Some biological bioremediation systems also have the potential of generating more than one product, in addition of decolorization, following the example of bioelectrochemical systems (BES), which helps in mitigating the costs associated with biological processes [12]. Among these solutions there is bioremediation by heterotrophic bacteria, which have, more broadly, two mechanisms related to the degradation of dyes: biosorption and enzymatic action [13].

2.1. Bacterial Mechanisms of Azo Dye Degradation

2.1.1. Biosorption

The biosorption is related to both the adsorption and absorption processes, and bacteria capable of performing the removal of dyes by adsorption have already been described in the literature. Biosorption is directly correlated with the composition of lipids and heteropolysaccharides of the cell wall, in which different charged groups generate attractions between it and the azo dyes, therefore, dead and living cells, in this latter case called bioaccumulation, have the ability to perform biosorption. Taking into account the range of charged groups existing in the cell walls of microorganisms and the variety of structures of the dyes, a microorganism X that adsorbs/absorbs dye A may not adsorb/absorb dye B, which is processed by a microorganism Y. Pretreatment can promote changes in the biosorption capacity of cells, optimizing the process and achieving a better fit to a certain need, among the substances capable of performing these changes are acids, formaldehyde, bases, among others [13][14].

To be used as biosorbents, dead cells are more advantageous than living cells because they do not require nutrients, can be stored for a longer time, and can be regenerated by the application of organic solvents or surfactants. However, biosorption is not the most suitable mechanism for dye treatment, since the treatment of large volumes of contaminated material would lead to the generation of large amounts of biomass containing dyes and possibly other toxic products that should have a proper disposal, i.e., it does not completely solve the problem, since it often does not destroy the dye, only seizes it in a matrix: the biomass [14][15].

2.1.2. Enzymatic Degradation

The initial step for the decolorization of solutions with azo dyes, being it waste, industrial effluents, or environmental samples, is the reduction of the azo bond (-N = N-) in the chromophore group, this step can occur intra- or extracellularly and involves the transfer of four electrons in two steps, where in each step two electrons are transferred from the dye to its final electron acceptor, leading to its decolorization. Some groups of enzymes already identified as capable of performing this reduction are azoreductases and laccases. These two are the most addressed groups in the literature regarding these decolorization reactions [16][17]. The **Figure 1** presents the general action mechanisms of these two enzymatic groups plus the peroxidase group which also acts on the azo chromophore group [18][19][20].

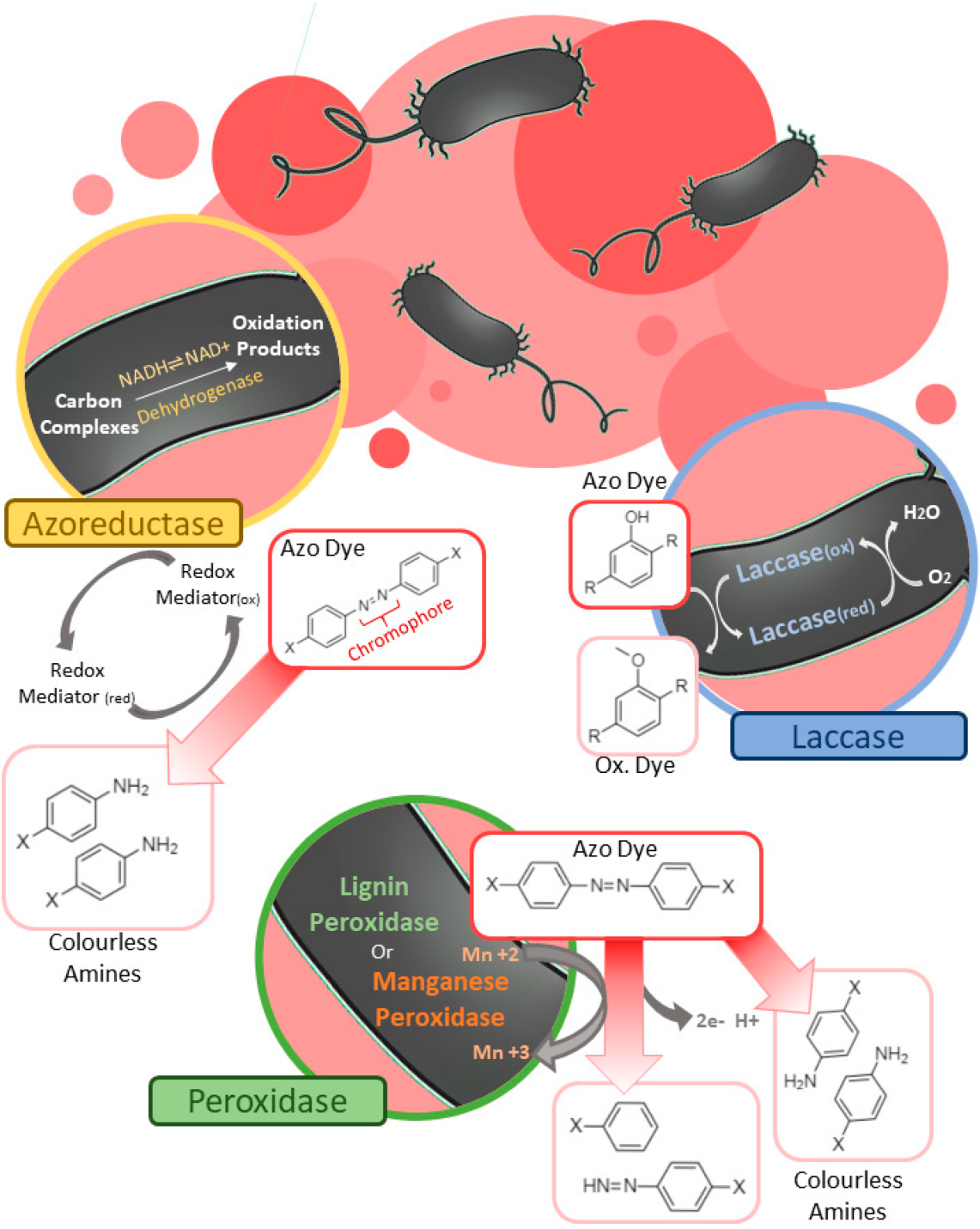

Figure 1. Schematic representation of three general bacterial enzymatic degradation mechanisms of azo chromophore group. Firstly, showing the enzymatic degradation by the action of azoreductases—yellow—in this example using NADH as an essential reducing agent for the cleavage of azo bonds, generating aromatic amines and thus discoloring the medium. Then—clockwise—there is the catalytic reaction cycle mediated by laccase—blue—with generation of oxidized substrate instead of potentially toxic amines, in addition to not requiring cofactors. Finally, peroxidase enzymes—green—such as lignin peroxidase and manganese peroxidase, two enzymes most commonly used for dye degradation, illustrating some possible products according to the cleavage of their bonds, which can be symmetric or asymmetric.

2.1.3. Enzymatic Degradation by Azoreductases

The azoreductases (e.g., EC 1.7.1.6 and EC 1.7.1.17) are the largest group of enzymes active in the biodegradation of azo dyes. They have the specific activity of reductive cleavage of azo bonds, resulting in aromatic amines, but to promote this reaction, azoreductases require reducing agents, such as $FADH_2$, NADPH, and NADH. They are more related to the anaerobic degradation of dyes because the presence of oxygen impairs this azo bond reduction step by competing for the reducing agents needed as electron acceptors for the azo bonds, which are also used by aerobic respiration. These enzymes are classified based on function into flavin-dependent and flavin-independent. The former class is subdivided into those enzymes that use as coenzymes: (1) NADH only; (2) NADPH only; and (3) both NADH and NADPH [17][21].

This group is quite varied and, depending on the source in which it is found, i.e., which organism, and even species, it is obtained from, it is possible to observe differences, such as in its catalytic activity, cofactor requirement and biophysical characteristics. Because of it, there is specificity between substrate and the types of azoreductases described so far, which varies in the requirement for cofactors and reducing agents and in the ability to resist oxygen [22].

Most azo dyes have a high molecular mass and are unlikely to cross the plasma membrane of cells. Therefore, microorganisms have a reduction mechanism related to the electron transport of azo dyes in the extracellular medium, so that there is a need for connection between the intracellular electron transport system and the dye molecules for degradation to occur. However, the action of azoreductases in the intracellular medium has also been identified and enzymes of this group have been found in bacteria, including in halophilic and halotolerant microorganisms [14][17][21].

2.1.4. Enzymatic Degradation by Laccases

Laccases (EC 1.10.3.2) are oxidases that have multiple structurally attached copper ions and are of great industrial interest due to their ability to utilize different substrates. They are able to non-specifically catalyze the degradation of azo dyes by acting on the phenolic group of the dye using a free radical mechanism that forms phenolic compounds generating fewer toxic aromatic amines. Moreover, these enzymes do not need other cofactors for their activation [13][21][23]. Although laccases do not need other cofactors to carry out their activity, they benefit from their presence in the medium. The presence of redox mediators can extend the range of dyes that this enzyme can degrade and significantly improve the degradation of dyes already covered in its range of action [12]. Bacterial laccases have great potential as biocatalysts due to their properties of high thermal stability, activity over a wide pH range, and resistance to denaturation by detergents, being already used to remove textile dyes and treat industrial effluents [23].

2.1.5. Enzymatic Degradation by Peroxidase

Peroxidases (EC 1.11.1) are hemoproteins that catalyze reactions in the presence of hydrogen peroxide and are mostly present in fungi, but also occur in some bacteria [24]. They possess the ability to degrade a wide range of

dyes, as cited by Paszczynski and co-workers [25], where lignin peroxidase (EC 1.11.1.14) and manganese peroxidase (1.11.1.13) were indicated as directly involved in the degradation of dyes and xenobiotic compounds [21]. Another class, versatile peroxidase (1.11.1.16), was pointed out by Đurđić and co-workers [26] as having the ability to perform structure breakdown of azo dyes.

2.2. Bacterial Degradation of Commercial Colorants

The occurrence of bacteria in different environments and physicochemical conditions makes them an interesting focus of prospection (**Table 1**). In the case of dyes degradation, a wide range of variables has already been explored and it was identified that this group of microorganisms can degrade azo dyes under aerobic, microaerophilic, and anaerobic conditions, as isolated cultures or as microbial consortia, in the presence of various sources of carbon and nitrogen and in wide ranges of pH, temperature, salinity and other physical-chemical parameters. In addition, bioreactors have been used in several works in an attempt to increase the efficiency of the degradation process, especially by immobilization of microorganism or redox mediators [27].

Table 1. Dye degradation by bacterial strains—pure cultures—under various medium conditions.

Species	Dye	Optimum Values of Phisicochemical Parameters for Bacterial Decolorization	Degradation Mechanism	Local of Bacterial Isolation	Maximum Degradation	Reference
Shewanella marisflavi	Xylidine Ponceau 2R	20–30% of salinity	Flocculation and Enzymatic	China	≈100% (30% of salinity, anaerobic conditions and 22h incubation)	[28]
Pseudomonas extremorientalis	Congo Red	50 mg/L of dye concentration, 2.5–5% of salinity and 0.6 U/mL enzyme concentration	Enzymatic-Laccase	Tunísia	79.8 ± 2.1% (50 mg/L of dye concentration, 2.5–5% of salinity, 24h incubation and 0.6 U/mL enzyme concentration)	[29]
Aliiglaciecola lipolytica	Congo Red	35 °C, <100 mg/L of dye concentration, 0–1% of salinity, pH 6–7, >4 g/L of glucose.	Adsorption and Enzymatic-Laccase and Azoreductase	-	>90% (35 °C, 25 mg/L of dye concentration, 1% of salinity, pH 6 and 4 g/L of glucose)	[30]
Enterococcus faecalis, Shewanella indica, Oceanimonas smirnovii and Clostridium bufermentans	8 different dyes	Varied depending of bacteria strain and dye	Enzymatic-Azoreductase and phenol oxidases	China	96.5% (*E. faecalis* strain and *C. bufermentans* with Dye Acid Orange 7 when pH ranged from 5 to 8, respectively)	[31]

Species	Dye	Optimum Values of Phisicochemical Parameters for Bacterial Decolorization	Degradation Mechanism	Local of Bacterial Isolation	Maximum Degradation	Reference
Bacillus sp.	7 different dyes	50–100 mg/L of dye concentration, pH 10, 30 °C, with glucose and yeast extract supplementation.	Enzymatic	Ethiopia	100% (pH 10, 30 °C, anoxic and anaerobic conditions)	[32]
Aeromonas hydrophila	Reactive Red 198 e Reactive Black 5	pH 5.5–10.0, temperature were and 20–35 °C under anoxic culture	Adsorption and Enzymatic	Taiwan	>90% (pH 5.5–10.0, temperature were and 20–35 °C under anoxic culture)	[33]
Comamonas sp.	Direct Red 5B	pH 6.5, 40 °C, static incubation conditions and 300–1100 mg/L of dye concentration.	Enzymatic-Laccase and Lignin Peroxidase	India	100% (pH 6.5, 40 °C and static incubation conditions)	[34]
Halomnas sp.	Remazol Black B	Varied depending of bacteria strain.	-	Iran	≈100% (40 °C)	[35]
Aeromonas sp.	Reactive Black	Microaerophilic conditions	-	India	≈100% (Microaerophilic conditions)	[36]
Oerskovia paurometabola	Acid Red 14	Anaerobic conditions	Enzymatic	Portugal	91% (anaerobic conditions)	[37]
Aeromonas hydrophila, Lysinibacillus sphaericus	Reactive Red 195	-	Enzymatic-Laccase and Azoreductase	India	91.96% (pH 8, 37 °C, 100 mg/L of dye concentration and sequential aerobic-microaerophilic conditions)	[38]
Bacillus sp.	4 different dyes	-	Enzymatic-Azoreductase	-	-	[39]
Bacillus sp.	5 different dyes	-	Enzymatic-Azoreductase	-	-	[40]

Species	Dye	Optimum Values of Phisicochemical Parameters for Bacterial Decolorization	Degradation Mechanism	Local of Bacterial Isolation	Maximum Degradation	Reference
Aeromonas hydrophila, Lysinibacillus sphaericus	5 different dyes	-	Enzymatic-Azoreductase and Laccase	India	90.4% (pH 8, 37 °C, 100 mg/L of dye concentration and sequential aerobic-microaerophilic conditions)	[41]
Lysinibacillus fusiformis	Methyl Red	pH 7.5–8, 30 °C, 100 mg/L of dye concentration and 10–20% (*v*/*v*) of inoculum size	Enzymatic-Laccase, Azoreductase and Lignin Peroxidase	-	96% (aerobic condition, pH 7.5, 30 ± 2 °C, dye concentration of 100 mg/L and 10% (*v*/*v*) inoculum size)	[42]
Pseudomonas stutzeri	Acid Blue 113	-	Enzymatic-Azoreductase and Laccase	India	86.2% (static conditions, 37 °C and 300 ppm of dye)	[43]
Aeromonas sp.	Methyl Orange	pH 6, 5–45 °C, 100–200 mg/L of dye concentration	Enzymatic-laccase, NADH-DCIP reductase, and azo reductase	China	≈100% (100–200 mg/L of dye concentration; with carbon and nitrogen supplementation; pH 6; 5–45 °C)	[44]

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Main Bacteria	Wastewater Source	Degradation Mechanism	Country	Maximum Degradation and Experiment Conditions	Reference
Micrococcus luteus	Dyehouse	Adsorption and Enzymatic	Japan	Laboratory	[48]
Pseudomonas aeruginosa	-	Enzymatic-Azoreductase	India	62%-Laboratory	[49]
Pseudomonas sp.	Textile Industries	Enzymatic-Laccase	India	90%-Laboratory	[50]
Pseudomonas sp. and *Bacillus* sp.	Mill effluent outlet	-	India	*Pseudomonas* 95% *Bacillus* 97%-Laboratory	[51]
Pseudomonas aeruginosa, Pseudomonas putida and *Bacillus cereus*	Textile Factory	-	Egypt	92%-Laboratory	[52]
-	Dye Wastewater Plant	-	Korea	75%-Real production facility	[53]

3. Conclusions

Azo dyes can be harmful to the environment and human health when disposed of without prior treatment, and the search for sustainable and less harmful production processes requires the development of new alternatives for effluent treatment that are efficient, cost-effective and of low environmental impact. Thus, bacterial bioremediation is a good alternative, given the versatility of this phylum that offers a range of possibilities, either with pure cultures or in consortia, tolerating different physicochemical parameters, in order to better adapt this process to various industrial wastes.

The application of these organisms in BES also brings the possibility of generating more than one salable product or service, making this process more attractive in terms of cost, an important bottleneck to be overcome in the implementation of biological systems. The application of bacteria to environmental samples also attests to this viability, being able to degrade dyes and their toxic by-products in environmentally relevant concentrations. Through the critical reading of the literature presented, scientific advances in this area can be evaluated, as well as the efforts to remedy the still deficient points, showing bacterial bioremediation to be an increasingly feasible process.

For the widespread application of bacterial bioremediation, several factors have to be considered, depending on the technique used, the characteristics of the environment to be remediated and of the bacteria strain, in this sense, the following points are relevant bottlenecks for large-scale application: (1) Bioreactor implementation and maintenance costs, (2) physicochemical parameters—which may vary over time, (3) space available for use of, e.g., wetlands or bioreactors, (4) availability of nutrients in the environment or in the textile effluent to be decontaminated, (5) presence/generation of suitable redox mediators for the enzymatic action of azo bond breaking, (6) engineering optimization in the transition from laboratory/pilot to industrial scale, (7) stricter local legislation forcing companies to treat their effluents properly, (8) co-relation between dye and bacteria/bacterial consortia or the presence of mixed dyes that can affect the bleaching given the bacterial suitability to each dye, (9) the use of industrial chemicals not considered in the laboratory tests, (10) changes in industrial dyeing techniques that modify the characteristics of its effluent and require adaptation of the bioremediation technique used, and (11) generation of toxic by-products that bacteria are not able to degrade

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