

Review

Degradation of Azo Dyes: Bacterial Potential for Bioremediation

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Abstract: 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. This review encompasses studies with promising results related to the use of bacteria in the bioremediation of environments contaminated with azo dyes in the most diverse techniques and parameters, both in environmental and laboratory samples, also addressing their mechanisms and the legislation involving these dyes around the world, showcasing the importance of bacterial bioremediation, specialty in a scenario in an ever-increasing pursuit for sustainable production.

Keywords: sustainability; effluent treatment; dyes; bioremediation; bacteria; wastewater; textile; consortium; BES; bioreactor



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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. The objective of this review is to evaluate the degradation of azo dyes specifically by bacteria, as well as the factors that influence these biological processes and the microbial mechanisms involved. In order to raise awareness about the importance of preventive measures in the discharge of untreated dyes, and some cases in which contamination by these pollutants was found in effluents are also presented, illustrating the importance, and urgency, of bacterial bioremediation for this sector.

2. Azo Dyes

The substances used to add color to many kinds of products are called colorants and include both the class of dyes and pigments. Pigments are mainly inorganic salts and oxides, insoluble in the substrate and commonly dispersed in crystal particles or powder form for application. Dyes, on the other hand, usually refer to organic substances that are soluble in the substrate and dispersed at the molecular level. Dyes promote more vivid colors than conventional pigments, and the characteristics of dyes depend primarily on their chemical structure, while for pigments the physical characteristics of their particles also influences the final color. Some examples of dyes are the azo, coumarin, and perylene groups [8].

Azo dyes are synthetic and organic chemical compounds applied in medicines, food, cosmetics, fabrics, among other products. These chemical classes are widely used in the dyeing industry and are present in 50–65% of commercial formulations, this is because of their stability and chemical versatility, having high fixation and resistance to light and moisture. These characteristics impact directly on their degradation in the environment, which has been a challenge [9,10].

The compounds of the azo group are chemically represented as (R-N = N-R'), with (-N = N-) being the chromophore group referred to as azo. According to the classification carried out by the International Union of Pure and Applied Chemistry (IUPAC), these compounds are derived from diazine HN = NH with substitution of hydrogens by hydrocarbyl, azobenzene, or diphenyldiazene groups, and may contain one to three azo bonds linking phenyl and/or naphthyl rings, which may be substituted by groups such as amino, chloro, nitro, and hydroxyl. They are characterized by their strong coloration and comprise approximately two-thirds of the synthetic dyes produced today, besides being the class of commercial organic dyes with the greatest structural diversity and the widest range of use [11,12].

However, the major problem related to the untreated disposal of these dyes in nature is that they and their byproducts, produced by the breakage of their azo bonds (aromatic amines), have been classified as highly carcinogenic compounds, representing a great risk to humans [13]. Thus, the search for ways to treat industrial effluents, especially those containing dyes of this class, has received much attention worldwide. Among the proposed solutions are chemical, physical, and biological processes. In this scenario bioremediation has received much attention for its lower costs compared to other mechanisms and high effectiveness in decoloring dyes in the affected environment, as well as generating less ecological impact. Among these biotechnological solutions we have the use of microorganisms, including bacteria [14].

Many bacteria have developed systems for decolorizing azo dyes contaminated medium, usually based on enzymatic mechanisms. However, the efficiency of these mechanisms depends on physicochemical parameters that may limit their activity, such as level of agitation, temperature, pH, dye concentration, structure of the dye, oxygen level, and carbon and nitrogen sources [4]. Different combinations of these parameters lead to different results in dye decolorization and degradation by influencing enzyme activity and microbial growth and maintenance in the medium. Understanding these relationships is crucial for improving the bioremediation process for a certain technique or bacterial strain, considering that these parameters in the environment can vary significantly in a few hours and that some dyes, as well as breakdown byproducts, can be toxic for the microbial population. In a laboratory environment it is important to consider that microbial metabolism can alter its degradation activity by nutrient depletion and variation in oxygen levels, for example, which do not necessarily represent real conditions. All of the factors mentioned above can prevent the complete breakdown of dyes and, consequently, the complete decolorization of the medium, since the mineralization efficiency of contaminating compounds relies on the capacity of the microorganism of performing a suitable metabolic response to degrade the contaminant under given environmental conditions, it is also necessary that these organisms are in sufficient concentration to properly handle with the entire amount of dye on the environment [15].

The bacterial degradation of azo dyes typically involves a two-step process, the first being a reductive cleavage of the azo bonds, leading to the formation of aromatic amines, which are potentially toxic and the second step is based on the degradation of these aromatic amines. The bacterial degradation process can occur in the presence or absence of oxygen; however, the biodegradation of these amines happens almost exclusively by aerobic processes. Considering this, the ideal method for the treatment of industrial waste contaminated by azo compounds is a combination, in the same process, of anaerobic and aerobic steps for the safest and most efficient removal of environmental and human risk factors related to these compounds [16].

Many of the azo dyes and their breakdown molecules present toxicity, showing mutagenic and carcinogenic effects, affecting animals, plants, and humans alike, with harmful effects varying with the structure, reactivity, and substitution groups of the dyes. The toxic aromatic amines from dye breakdown are resistant to classical effluent treatment, persisting longer than the dyes and an increase in concentration of these substances in the medium may impair the dye degradation process because of their toxicity to bacterial life itself. Still, there are some microorganisms capable of producing enzymes that degrade these amines in an aerobic environment. For humans, inhalation and oral ingestion of the dyes and their by-products are the primary means of exposure that can lead to acute toxicity [17,18].

The toxicity assessment of the dyes and their by-products can be done using different techniques, which vary in sensitivity, resolution, and cost. Analyses using techniques such as Gas Chromatography–Mass Spectrometry (GC-MS) and Fourier-Transform Infrared Spectroscopy (FTIR) can be performed to confirm the degradation of the dye and/or the presence of aromatic amines, and then the medium containing the by-products of dye degradation is used in tests that evaluate possible negative biological effects being biotoxicity and phytotoxicity assays most often used for this purpose [19]. Another way

to evaluate toxic effects is to expose the living organism to a solution containing different concentrations of dye, also evaluating chronic exposure to these contaminants [20], other toxicity tests that can be performed for these pollutants, including tests performed on human cells [21], with several studies proving the toxic, mutagenic, and carcinogenic effects of dyes and their by-products using plant and animal tests [19–24].

Legislation on the Use and Disposal of Dyes

Over the years the concern regarding the impact of human activities on the environment, especially industrial ones, has grown considerably. In view of this, standards on what is acceptable regarding not only the discarding of potentially toxic substances and waste in nature, but the industrial application of these dyes in some products have been regulated and even prohibited, and control agencies were created for monitoring and enforcing laws of this nature [25].

In the European Union there are some regulations on the use of azo dyes in consumer products, from clothing and toys, to cosmetics, and food, which obviously has stricter guidelines and norms. REACH (Registration, Evaluation, Authorization, and Restriction of Chemicals) lists 24 types of aromatic amines considered hazardous to humans and prohibits the use of azo dyes that produce 30 mg/kg or more of these amines in products that may have direct and prolonged contact with human skin. REACH also lists other colorants that are restricted for use in these same products when in concentrations above 0.1% of its weight. The EN-71 (European Norm 71) deals with the presence of this class of colorants in toys. The regulations (EC) 1223/2009 and (EU) 10/2011 deal, respectively, with the ban in the use of o-Dianisidine and Benzidine in cosmetic products and plastic products that come into contact with foodstuffs, which must keep the release of primary aromatic amines into the food within the limit of 0.01 mg/kg. There are a number of countries which in addition have their own legislation covering limitations related to azo dyes and the release of aromatic amines from them, such as Germany, the Czech Republic, and Switzerland [26].

In the United States there are no laws related specifically to azo dyes or restricting the use of them, however, there are restrictions on aromatic amines from these dyes. Due to the political system in the country, many states have their own laws restricting the presence of certain chemicals in certain products. Some examples that cover aromatic amines derived from certain azo dyes are California's Proposition 65, Washington's Children's Safe Product Act, and Vermont's Act 188 [27].

In Asia, the first example of a country to regulate chemicals, including azo dyes, was India, with a ban in 1997 on the handling of 112 dyes, including representatives of the azo group. In this country the list of banned aromatic amines is the same as in REACH Regulation 1907/2006. Other Asian countries have instituted their own regulations in which they include restrictions on azo dyes and/or aromatic amines, such as China (2005), South Korea (2010), Taiwan (2011), and Egypt (2012). In 2014 Japan joined this list when placed azo dyes as a hazardous substance and restricted the presence of 24 aromatic amines originated from these dyes in all textiles, leather, or fur products in a concentration of 30 mg/kg or more. This same concentration was used for the limitation of 22 aromatic amines in a regulation of Vietnam [28–31].

Other countries like Canada, France, Australia, Brazil, Pakistan, Malaysia, Turkey, and Morocco also have regulations related to industrial effluents that include in their guidelines specifications related to the color of these effluents which, consequently, also influences the concentration of dyes allowed in them [25].

Another relevant insight is that many-if not all-regulations of the cited countries also determine the methods of analysis to which the products will be subjected in order to determine whether they are in order with the regulations of that country. This is important, because different analytical methods have different limitations, ranges, sensibilities, and applications, and these must be considered when carrying out these controls. In addition, other parameters such as chemical and biological oxygen demand are also subject to

regulation, and since the presence of azo dyes has an impact on these factors, the use of these dyes may also be indirectly restricted.

3. 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 [14]. 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 [32], (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 [33], (3) application of genetic engineering techniques that can develop bacterial strains with programmed death, stopping bacterial metabolism in the absence of the target contaminant [34].

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 [35]. Among these solutions we have bioremediation by heterotrophic bacteria, which have, more broadly, two mechanisms related to the degradation of dyes: biosorption and enzymatic action [36].

3.1. Bacterial Mechanisms of Azo Dye Degradation

3.1.1. 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 [36,37].

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 [37,38].

3.1.2. 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 [39,40]. 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 [41–43].

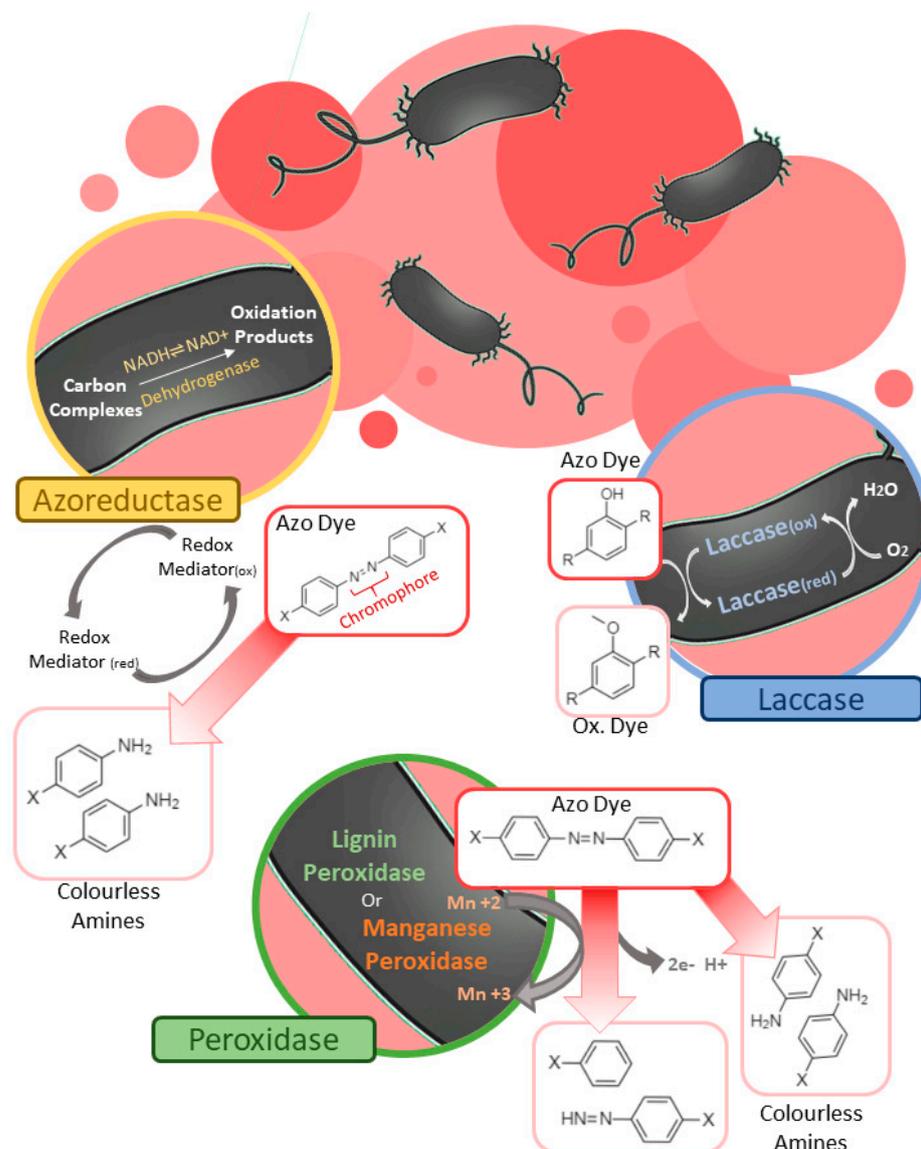


Figure 1. Schematic representation of three general bacterial enzymatic degradation mechanisms of azo chromophore group. Firstly, showing the enzymatic degradation by the action of azoreductases—yellow—in this example using NADH as an essential reducing agent for the cleavage of azo bonds, generating aromatic amines and thus discoloring the medium. Then—clockwise—we have 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.

3.1.3. 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 [40,44].

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 [45].

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 [37,40,44].

3.1.4. 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 [36,44,46]. 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 [35]. 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 [46].

3.1.5. 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 [47]. They possess the ability to degrade a wide range of dyes, as cited by Paszczynski and co-workers [48], 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 [44]. Another class, versatile peroxidase (1.11.1.16), was pointed out by Đurđić and co-workers [49] as having the ability to perform structure breakdown of azo dyes.

3.2. 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 physicochemical 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 [50].

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

Species	Dye	Optimum Values of Physicochemical Parameters for Bacterial Decolorization	Degradation Mechanism	Local of Bacterial Isolation	Maximum Degradation	Reference
<i>Shewanella marisflavi</i>	Xylydine Ponceau 2R	20–30% of salinity	Flocculation and Enzymatic	China	≈100% (30% of salinity, anaerobic conditions and 22h incubation)	[51]
<i>Pseudomonas extremorientalis</i>	Congo Red	50 mg/L of dye concentration, 2.5–5% of salinity and 0.6 U/mL enzyme concentration	Enzymatic-Laccase	Tunisia	79.8 ± 2.1% (50 mg/L of dye concentration, 2.5–5% of salinity, 24h incubation and 0.6 U/mL enzyme concentration)	[52]
<i>Aliiglaciecola lipolytica</i>	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)	[53]
<i>Enterococcus faecalis</i> , <i>Shewanella indica</i> , <i>Oceanimonas smirnovii</i> and <i>Clostridium bufermentans</i>	8 different dyes	Varied depending of bacteria strain and dye	Enzymatic-Azoreductase and phenol oxidases	China	96.5% (<i>E. faecalis</i> strain and <i>C. bufermentans</i> with Dye Acid Orange 7 when pH ranged from 5 to 8, respectively)	[54]
<i>Bacillus</i> 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)	[55]
<i>Aeromonas hydrophila</i>	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)	[56]
<i>Comamonas</i> 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)	[57]
<i>Halomonas</i> sp.	Remazol Black B	Varied depending of bacteria strain.	-	Iran	≈100% (40 °C)	[58]
<i>Aeromonas</i> sp.	Reactive Black	Microaerophilic conditions	-	India	≈100% (Microaerophilic conditions)	[59]
<i>Oerskovia paurometabola</i>	Acid Red 14	Anaerobic conditions	Enzymatic	Portugal	91% (anaerobic conditions)	[60]
<i>Aeromonas hydrophila</i> , <i>Lysinibacillus sphaericus</i>	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)	[61]
<i>Bacillus</i> sp.	4 different dyes	-	Enzymatic-Azoreductase	-	-	[62]
<i>Bacillus</i> sp.	5 different dyes	-	Enzymatic-Azoreductase	-	-	[63]
<i>Aeromonas hydrophila</i> , <i>Lysinibacillus sphaericus</i>	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)	[64]
<i>Lysinibacillus fusiformis</i>	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)	[65]
<i>Pseudomonas stutzeri</i>	Acid Blue 113	-	Enzymatic-Azoreductase and Laccase	India	86.2% (static conditions, 37 °C and 300 ppm of dye)	[66]

Table 1. Cont.

Species	Dye	Optimum Values of Physicochemical Parameters for Bacterial Decolorization	Degradation Mechanism	Local of Bacterial Isolation	Maximum Degradation	Reference
<i>Aeromonas</i> 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)	[67]
<i>Proteus mirabilis</i>	Reactive Blue 13	pH 7, 35 °C and anoxic conditions.	Enzymatic-Laccase, azoreductase and veratryl alcohol oxidase	Nigeria	≈90% (pH 7)	[68]
<i>Pseudomonas putida</i> , <i>Bacillus subtilis</i>	18 different dyes	-	Enzymatic-Azoreductase and Laccase	-	≈100%	[69]
<i>Bacillus</i> sp.	Red HE7B	-	Enzymatic-Azoreductase and Laccase	-	89% (30 °C, 50 mg/L of dye concentration, 24h incubation and under agitation conditions)	[70]

3.2.1. Pure Bacterial Colonies

Degradation in the Presence of Salts

As mentioned, bacteria are able to degrade dyes in different salt concentrations, a desirable attribute since inorganic salts are used in dyeing processes as mordants, helping in color fixation process, especially sodium chloride (NaCl) and sodium sulfate (Na₂SO₄). The bacterial degradation process has proven effective for dyeing at salt concentrations ranging from 0.25% to 10%. Many countries have no regulations on the amount of salt that can be discharged into the environment, or when they do, many allow the presence of these salts in high concentrations [25]. Taking this into account, the ability of bacteria to degrade dyes in saline environments becomes an important differential for the selection of microorganisms with real biotechnological potential of application in dyes bioremediation processes.

The work of Xu and co-workers [51] used a strain of *Shewanella marisflavi*, an exoelectrogenic bacterium-bacteria that has extracellular electron transfer pathways-isolated from marine sediments in China. Tests performed with the dye Xylidine Ponceau 2R showed that this strain is able to decolorize the medium at concentrations ranging from 0% to 20% NaCl. Higher salt concentrations also influence the solubility of the dye by increasing the ionic strength of the solution, leading to floc formation, and flocculation was also observed at lower NaCl concentrations when in the presence of *Shewanella marisflavi*. The researchers concluded that the tested bacteria had two decolorization mechanisms, performing only dye degradation up to 6% NaCl, then degrading and flocculating from 8% to 10% NaCl in the medium, and above this percentage, only underwent flocculation process.

Laccases are widely studied in fungi and plants whose ability to degrade phenolic compounds is well known. These enzymes have also been found in bacteria, showing ability to act in environments with high salt concentration and alkaline pH, presenting an advantage over fungi and plant laccases [52]. Neifar and co-workers [52] worked with a laccase-producing *Pseudomonas extremorientalis* strain isolated from oil-contaminated sediments in Tunisia, evaluating the degradation of Congo red dye. The enzyme produced by this strain showed great resistance to alkalinity, maintaining its activity in pH 7 to 10 and also resisted to salinity, maintaining almost 90% of its activity in the presence of sodium chloride at 17.5%.

Wang and co-workers [53] demonstrated the ability of a strain of the marine bacterium *Aliiglaciicola lipolytica* to decolorize medium containing Congo red dye at salt concentrations as high as 4%. However, the strain did not tolerate pH increase and has glucose as the best carbon source for its dye degradation activity, increasing the costs of the process. The study also pointed out that the bacteria use a degradation mechanism that involves the adsorption of the dye in their cells by means of extracellular polymeric substances (EPS), where part of it is degraded by a process involving the co-metabolism with glucose and

the mediation of the enzymes azoreductase and intracellular laccase, in this process the non-degraded dye is encapsulated in the bacterial cell.

In a study by Zhuang and co-workers [54] the degradation of azo dyes used four bacteria isolated from coastal region in China, *Enterococcus faecalis*, *Shewanella indica*, *Oceanimonas smirnovii*, and *Clostridium bufermentans*. The isolated strains were able to degrade eight different dyes, achieving removal percentages above 70% for most of them. *E. faecalis* and *C. bufermentans* maintained dye decolorization rates above 80% at salt concentrations up to 7%. The four also demonstrated to have great resistance of their decolorization activity in the presence of ions, being little affected by the great majority of those tested; however, Cadmium (Cd^{2+}) and Copper (Cu^{2+}) were the ions that most interfered in the decolorization results, which is a problem to be considered, since they are metals frequently found in industrial effluents.

PH Range: Degradation in Alkaline Medium

Due to the use of sodium hydroxide and other basic components in textile dyeing processes and with the textile industry being one of the three sectors with the highest release of dye contaminated effluents into nature, it is important for this scenario that we use bacteria resistant to alkaline environments for the treatment of these effluents which can have pH up to 11.5. In this sense, the work of Guadie and co-workers [55] tested the decolorization capacity of a *Bacillus* sp. strain isolated from lakes with an alkaline characteristic. This strain proved to be able to decolorize medium with pH from 9 to 11 with efficiency above 90% and was also effective against the seven different dyes tested with complete decolorization been achieved in anoxic and anaerobic environments, and in the presence of oxygen, there was almost no decolorization of the medium.

Several studies have isolated and identified bacterial strains capable of decolorizing dyes in alkaline solutions. Chen et al. [56] worked with a strain of *Aeromonas hydrophila* capable of degrading the dyes Reactive Red 198 and Reactive Black 5 over a pH range of 5.5 to 10. The research of Jadhav and co-workers [57] isolated a strain of *Comamonas* sp. from contaminated soil of industrial environment able to degrade the dye Direct Red 5B in a pH range of 6–12, but having its best activity in neutral pH range, different from that was found by of Asad group [58] who worked with three different strains of bacteria belonging to genus *Halomonas* sp. isolated from textile effluents and observed that all of them performed the best decolorization in alkaline pH, with the highest activity achieved at the highest pH tested: 11. These three studies also showed, again, little or no decolorization activity in an aerobic environment.

Bioremediation in Aerobic and Anaerobic Environments

One of the major problems faced in the degradation of azo dyes in anaerobic environments is the formation of toxic products, notably mutagenic and/or carcinogenic aromatic amines (e.g., benzidine and 4-biphenylamine) that are only degraded in aerobic environments [7,59]. The work of Shah [59] evaluated the degradation of the dye Reactive Black using a strain of *Aeromonas* sp. in a microaerophilic environment until the color was no longer noticeable in the medium and then promoted the aeration of it to stimulate the oxidation of the aromatic amines-formed by the breakdown of the dye into non-toxic products, obtaining a discolored medium with a low degree of toxicity. This work proves that a dye bioremediation process performed outside anaerobic conditions can also be successful.

Franca and co-workers [60] used a different approach, where they performed the decolorization tests in an anaerobic environment and, after complete removal of the color, they used an aerobic environment to evaluate the ability of a *Oerskovia paurometabola* strain to metabolize the toxic products generated by the previous step. The result obtained was positive, with decolorization above 90% and removal of toxic products above 63%.

The research by Srinivasan and Sadasivam [61] also worked with the aerobic-microaerophilic approach for the degradation of azo dye, in this case Reactive Red 195 using strains of *Aeromonas hydrophila* and *Lysinibacillus sphaericus*. This work also used

the molecular docking tool, in addition to decolorization tests, in order to understand the interaction between the amino acid residues of the enzymes—laccase and azoreductase—and the dye. The study pointed out the high efficiency of these strains in degrading the dye used and a positive correlation between the score of the docking studies and the percentage results of dye degradation. In silico approaches for azo dye degradation studies have been increasingly employed, also as a strategy to better use resources and research efforts [62–64].

Although the mechanism of azo dye decolorization commonly occurs under anaerobic conditions, the work of Sari and Simarani [65] indicates that there are bacteria capable of performing azo dye degradation in an aerobic environment. The study identified a strain of *Lysinibacillus fusiformis* capable of achieving a 96% decolorization rate of Methyl Red dye in 2 hours under neutral pH and temperature of 30 ± 2 °C under aerobic conditions. The study further pointed out that the bacteria's oxidoreduction mechanism involved the enzymes laccase, azoreductase and lignin peroxidase. As seen, azoreductase and laccase are highlighted in several studies related to the bioremediation of azo dyes by bacteria, such as the action of *Pseudomonas stutzeri* in the degradation of the dye Acid Blue 113 [66]. This strain obtained good decolorization results and a high resistance to elevated concentrations of the dye. Genomic studies indicated the presence of both enzymes in the decolorization system of the microorganism. Several other works pointed out the presence of these two enzymes in the bioremediation activity of azo dyes [58,67–70].

3.2.2. Microbial Consortia

The application of microbial consortia instead of isolated organisms for the treatment of effluents contaminated with dyes presents advantages (Table 2), especially in the fact that each strain can act on different targets of the organic molecule and/or consume different products generated by the breakdown of the dyes, thus generating a synergistic effects action where the enzymatic activity of a bacterial strain is positively influenced by the presence of other microorganisms, thus, increasing the degradation rate [71].

An example of this is recorded in the work of Masarbo and co-workers [72] in which the researchers evaluated the decolorization activity of pure bacterial strains and in consortia. The result shows that two of the consortia tested were able to achieve higher percentages of decolorization and in shorter time, when compared to the results of the strains tested individually. Furthermore, at the highest dye concentration tested, the percentage of degradation was at least 10% higher than the best result achieved by a single bacterial species.

Physicochemical Parameters in Microbial Consortia: Salinity, PH and Oxygenation

Other studies have identified consortia capable of decolorizing environments with a combination of parameters, such as high salinity and pH, closer to those found in real remediation scenarios. Guo and co-workers [73] assembled a consortium consisting mostly of genus *Halomonas*, *Marinobacter*, and *Clostridiisalibacter*, which was able to decolorize over 90% of the dye at 40 °C, withstand 10% salinity, and to achieve a decolorization rate above 70% at pH 8–12. However, the different strain's ability to endure increasing concentrations of the dye was low. Guo's group [74] also studied a consortium consisting mostly of the genus *Bacillus* and *Piscibacillus*. This consortium showed best activity at 50 °C, pH 10, and between 1% and 10% salinity. However, it poorly withstood higher pH values as well as the increase in dye concentration. Another factor worth mentioning is that, in both studies, the supplementation of the medium with yeast extract was essential for higher decolorization results.

Table 2. Dye degradation by bacterial consortium under various medium conditions.

Main Consortium Species	Dye	Best Physicochemical Parameters for Bacterial Decolorization	Degradation Mechanism	Local of Bacterial Isolation	Maximum Degradation	Reference
<i>Pseudoarthrobacter</i> sp. and <i>Gordonia</i> sp., (consortium PsGo); <i>Stenotrophomonas</i> sp., and <i>Sphingomonas</i> sp. (consortium StSp)	Reactive Black 5	pH 11 (for PsGo) and Glucose as carbon source (for StSp)	-	Iran	85% (for PsGo) 75% (for StSp)	[71]
<i>Bacillus</i> sp., <i>Lysinibacillus</i> sp. and <i>Kerstersia</i> sp. at different combinations	Ponceau 4R	200 mg/L of dye	Enzymatic-Azoreductase	India	100% (with 200 mg/L of dye)	[72]
<i>Halomonas</i> sp., <i>Marinobacter</i> sp. and <i>Clostridiisalibacter</i> sp.	Methanil Yellow G	pH 8, salinity 5–10%, 40 °C, 100 mg/l of dye concentration and presence of yeast extract	Enzymatic	China	93.3% (with yeast extract and 100 mg/L of dye)	[73]
<i>Piscibacillus</i> sp. and <i>Bacillus</i> sp.	Methanil Yellow G	pH 10, 50 °C, yeast extract, 1% salinity and 100mg/L of dye concentration	Enzymatic-Laccase, Lignin peroxidase, Nicotinamide adenine dinucleotide-dichlorophenol indophenol reductase and Azoreductase	China	94.26% (with yeast extract)	[74]
<i>Zobellella</i> sp., <i>Rheinheimera</i> sp. and <i>Marinobacterium</i> sp.	Methanil Yellow	Microaerophilic conditions, pH 6, 400 mg/L of dye concentration and 3% salinity	Enzymatic-Azoreductase, Laccase and Lignin Peroxidase	-	≈100% (with 400 mg/L of dye concentration)	[75]
<i>Staphylococcus</i> sp. and <i>Bacillus</i> sp.	Remazole Brilliant Violet 5R	-	Enzymatic-Azoreductase, Laccase and Lignin Peroxidase	India	100% (with pH 6.5 and 37 °C)	[76]
<i>Pseudomonas</i> sp., <i>Bacillus</i> sp., <i>Bacillus</i> sp. and <i>Ochrobactrum</i> sp.	15 different dyes	pH 8, 40 °C, 100 mg/L of dye concentration and no salt presence	Enzymatic	India	100% (with pH 8)	[77]
<i>Bacillus cereus</i> , <i>Pseudomonas fluorescenc</i> , <i>Staphylococcus aureus</i> , <i>Escherichia coli</i> and <i>Lactobacillus</i> sp.	Remazol Yellow	-	-	India	97.84%	[78]
<i>Enterococcus faecalis</i> and <i>Klebsiella variicola</i>	Reactive Red 198	10–25 mg/L of dye concentration, pH 8 and 37 °C	Enzymatic	Iran	99.26% (with pH 8)	[79]
-	5 different dyes	pH 7–8, 35 ± 2 °C, static conditions, 0.5–1% salinity and 200 mg/L of dye concentration	-	India	98.8%	[80]

As we have seen before, another factor that affects the decolorization process is the presence of oxygen. In the work of Guo and co-workers [75], a consortium consisting mainly of *Zobellella*, *Rheinheimera*, and *Marinobacterium* was used for the degradation of the dye Methanil Yellow in a saline and microaerophilic environment. In a short time, assay, five hours, the consortium achieved above 80% decolorization at up to 5% salt concentration in the medium and optimum pH of 6. However, it showed great resistance to increasing dye concentration, decolorizing almost 100% at 400 mg/L concentration within those five hours. Laccase, azoreductase and lignin peroxidase were, once more, the enzymes identified in the degradation mechanism of this consortium.

The group of Shah [76] studied the behavior of a bacterial consortium consisting of five bacterial strains in microaerophilic and aerobic environments based on the reduction of the azo dye Remazole Brilliant Violet 5R. A difference was observed in the efficiency of the consortium regarding the degradation of the dye between these two environments, where, in a microaerophilic environment, the degradation reached 100% in less than 24 hours, this result was not seen in an aerobic environment. Another factor pointed out in the study as influencing the efficiency of degradation was the structure of the dye, where 29 different dyes were tested, and the efficiency of the consortium varied from less than 20% of degradation to more than 80% in some cases.

Khan and co-workers [77] also compared the efficiency of a bacterial consortium in microaerophilic and aerobic environments and with respect to several structurally different

dyes. In the microaerophilic environment the decolorization was 100% and in the aerobic environment it was less than 10%. The consortium was found to withstand the presence of salt and temperature up to 45 °C, as well as a slightly alkaline pH. For the different dyes tested, the decolorization ranged from values above 92% to less than 25%.

Microbial Bioprospecting in Textile Effluents

The search for bacteria capable of degrading dyes can take advantage of the natural, or not so natural, selection of these organisms through bioprospecting in contaminated environments, selecting bacteria that underwent great environmental pressure, thanks to human action. Kannan and co-workers [78] evaluated the results of microbial consortia assembled with different combinations of five strains isolated from textile effluent. The best result obtained was from a consortium composed of *Bacillus cereus* and *Pseudomonas fluorescens* that achieved nearly 100% decolorization of Remazol Yellow dye, with nine of the ten combinations tested achieving decolorization above 78%. Another work that used microorganisms isolated from textile effluents was that of Eslami's group [79], which evaluated the action of a consortium of *Enterococcus faecalis* and *Klebsiella variicola* which obtained results of almost 100% removal of the dye Reactive Red 198.

However, it is worth noting that these two studies achieved good results with their consortia at mild parameters of temperature, pH, and salinity, which is often not the case with effluents contaminated by dyes, in a real case scenario. In addition, the consortia evaluated in both studies required more than two days for almost complete decolorization to occur.

Eskandari and co-workers [71] tested in their study two consortia with bacterial strains isolated from textile effluent and soil from a typically cold region, the Zagros Mountains in Iran. Their ability to degrade the dye Reactive Black 5 was evaluated, achieving the best results at mild temperatures, but in an alkaline range with pH between 9 and 11, which already represents an advantage over previous works, since it is common for industrial effluents containing dyes to have alkaline pH due to the use of substances of basic character in industrial processes.

The azo dyes have some representatives called pre-metallized dyes, which have metals in their structure previous to their application in the staining process [81]. Eleven consortia formed by bacteria isolated from areas contaminated with pre-metallized dyes in India were tested in the degradation of eighteen of these dyes. There was a wide variation in the percentage of degradation between consortia and dyes, with values ranging from 7.4% to 98.8% [80].

3.2.3. Bioreactors and Their Potential in the Bioremediation of Azo Dyes by Bacteria

Bioelectrochemical systems (BES) are represented by microbial fuel cells, microbial desalination cells, and microbial electrolysis cells. In these systems microorganisms perform the oxidation of compounds and the electrons generated in this process can be used in the production of energy and other compounds of interest [82]. BES have proven to be better compared to conventional anaerobic and electric or electrochemical processes by performing well in a shorter time, in a more cost-effective manner, and causing less negative environmental impact [83].

Microbial fuel cells have already been explored to generate energy allied to the treatment of effluents containing dyes. This type of treatment presents advantages such as: potential for energy production instead of its consumption; low sludge formation; operation at mild temperatures and atmospheric pressure and offers the possibility of performing oxidation (anode) and reduction (cathode) of the dyes [84]. In line with this, several works explore this possibility in the process of dye decolorization (Table 3), even adding steps for the detoxification of other harmful compounds.

Table 3. Bacterial dye degradation in bioreactor systems.

Main Bacteria	Dye	Reaction System	Parallel Study	Best Parallel Study Results	Maximum Degradation	Reference
<i>Enterobacter</i> , <i>Desulfovibrio</i> and <i>Enterococcus</i>	Alizarin Yellow R	Single chamber up-flow bioelectrochemical system (UBES)	-	-	87.74 ± 3.52%	[83]
<i>Shewanella oneidensis</i>	Acid Orange 7	Microbial Fuel Cell	COD * Reduction and Electricity production	Power density of 50 ± 4 mW m ⁻² and COD reduction 80.4 ± 1.2%	80%	[84]
<i>Proteobacteria phyla</i>	Congo red	Single chamber air-breathing cathode Microbial Fuel Cell	Sulfide removal and Electricity production	Maximum power density of 23.50 mW m ⁻² and 98% of Sulfide removal	81.5%	[85]
-	Acid Orange 7	Bioelectrochemical system (BES) combined with a membrane biofilm reactor	COD Reduction and Degradation of Sulfanilic acid	52.6 ± 3.2% of COD reduction and 64.7 ± 2.7% of Sulfanilic acid reduction	96.5 ± 0.6%	[86]
<i>Unclassified genus</i>	Reactive Brilliant Red X-3B	Biofilm electrode reactors (BERs)	COD Reduction	75.65% of COD removal and 21.13 mA m ⁻² of current density	75.27%	[87]
-	Reactive Brilliant Red X-3B	Microbial fuel cell (MFC)-biofilm electrode reactor (BER) coupled system	COD Reduction and Electricity production	Power density of 0.257 W m ⁻³ and 88.62% of COD removal	97.77%	[88]
<i>Geobacter. sulfurreducens</i> and <i>Beta proteobacteria</i>	Reactive Brilliant Red X-3B	Microbial fuel cell coupled constructed wetlands (CW-MFCs)	Electricity production	0.256 W m ⁻³	92.7%	[89]
<i>Enterobacter</i> and <i>Enterococcus</i>		Up-flow anaerobic sludge blanket (UASB) reactor	VFA production and COD removal efficiency	≈100% of COD removal efficiency	95.84 ± 2.60	[90]
-	Direct Black 22	Sequencing batch reactors	COD Reduction and Ecotoxicity	81.4% of COD removal	81.4%	[91]
-	C.I. Basic Red 46	Anaerobic-aerobic sequencing batch reactor (SBR)	COD Reduction	>90% of COD removal	98%	[92]
<i>Citrobacter sp.</i> , <i>Enterococcus sp.</i> and <i>Enterobacter i</i>	Remazol Black B	Upflow packed-bed reactor for continuous sequential microaerophilic-aerobic batch operations	-	-	95.87%	[93]
<i>Serratia marcescens</i> and <i>Klebsiella oxytoca</i>	Nylosan Yellow E2RL SGR	Sequencing batch reactor system, followed by ultrafiltration	COD Reduction	94% of COD removal	97%	[94]
<i>Paludibacter</i> , <i>Trichococcus</i> and <i>Methanosarcina</i>	Reactive Red 2	Anaerobic sequencing batch reactor	Ammonium removal and The effect of Fe3O4 on anaerobic treatment of azo dye	≈100% of Ammonium removal	≈100%	[95]
-	Yellow Dye	Aerobic Ganular Sludge (AGS)	Ammonium removal	≈100% of Ammonium removal	≈100%	[96]

* COD = Chemical Oxygen Demand.

Sulfide is considered to be toxic, corrosive and a threat to human health. It is commonly present in textile effluents containing dyes, formed due to the addition of sodium sulfide for reduction processes of azo compounds or conversion of other sulfur-containing substances. With this in mind, a single-chamber air-cathode microbial fuel cell was used by Dai and co-workers [85] for the simultaneous degradation of Congo red dye, bioelectricity generation, and sulfide oxidation. The results showed 98% sulfide removal and 88% decolorization, accompanied by the formation of maximum power of 23.50 mW m⁻². It was also evaluated that the sulfide concentration affects the sulfide oxidation rate as well as the dye degradation [85].

Mani and co-workers [84] studied the difference between the decolorization efficiency, electricity production and the decrease in chemical oxygen demand (COD) between feeding the fuel cell via the anode chamber containing the electrochemically active bacterium *Shewanella oneidensis* or via the cathode chamber containing the enzyme laccase. The conclusion of this work was that degradation with the laccase at the cathode is a more

advantageous process, as it generates more stable and chemically simpler products, as well as lower COD (80.4% versus 69% of the anode) and higher power generation efficiency (50 mW m^{-2} versus 42.5 mW m^{-2} of the anode).

Other BES have already been studied in relation to their potential use for industrial effluent treatment, for example, the evaluation of their use in the removal and recovery of nutrients, especially with regard to nitrogen present in industrial effluents [82], as well as in the decrease of (COD) allied with the degradation of azo dyes, and the effects of their coupling with a continuous stirred reactor system and the increase of modules in a stacked BES has also been studied. In these with the following advantages were pointed out: (1) the use of a three-module system improved decolorization by 15% and 33% compared to systems with only two or one modules, respectively, achieving up to 80% removal [86]; (2) coupling with a continuous stirring reactor achieved 97% of color removal from the medium in just seven hours, being superior to the results obtained with these techniques alone, where approximately 54.9% and 91.4% decolorization were achieved [83]; (3) there was 75.6% reduction in (COD) and voltage has shown to affect the decolorization efficiency [87].

Other studies have performed couplings of systems aiming to improve the decolorization process and for the evaluation of energy generation. A microbial fuel cell system was coupled to a biofilm electrode reactor and the results indicated an increase of almost 30% in color removal efficiency compared to the process performed with these mechanisms decoupled [88]. In the system assembled with microbial fuel cells in combination with a constructed wetland it was observed that the higher the substrate biomass, the higher the decolorization and the lower the power generation, with these varying among the groups tested from 76.3% to 92.7% and 0.117 W m^{-3} to 0.256 W m^{-3} , respectively [89].

The use of bioreactors can also be achieved with various combinations and parameters variations such as concentrations of salts, presence of oxygen, and feed rate. Regarding the presence of oxygen, several possibilities have already been studied, namely tests in an anaerobic environment [90]; with continuous micro aeration; intermittent and without aeration [91]; anaerobic starting followed by aerobic [92]; and microaerophilic environments followed by aerobic [93].

In tests performed in a reactor with a fully anaerobic environment, the decolorization rate ranged from 62.98% to 95.84%, depending on the loading rate of dye in the reactor, which decreased the decolorization with its increase [90]. In the study by Menezes and co-workers [91] the reactor without aeration had the highest decolorization, followed by the intermittent aeration reactor and lastly the continuous aeration reactor, the rates being 81.4%, 76.8%, and 74.5%, respectively. However, the reactor without aeration produced waste with toxic substances, which was not observed in other conditions. Assadi and co-workers [92] pointed out better dye removal in an anaerobic environment, reaching almost 98%, and indicate that increasing the concentration of the dye negatively affects the decrease in COD. This same work also showed that the decolorization is also negatively affected by increasing the concentration of salt and nitrate ions in the medium. The assays without an anaerobic environment obtained maximum decolorization of 95.87% in the micro-aeration stage and removal of 23 mg/L of aromatic amines in the aerobic stage compared to the previous environment with micro-aeration [93].

Bioremediation can also be employed in other treatment processes; an example is the study by Korenak and his group [94] who combined the treatment of contaminated effluent performed in bioreactors with bacteria to the subsequent process of ultrafiltration. This combination of methods improved both the dye removal rate, 85% before ultrafiltration and 97% after, and the decrease in COD, 91% before and 94% after.

Studies also indicate the possibility of improving the anaerobic treatment with the use of Fe_3O_4 , which generated a decrease in the lag phase of microbial growth, improved the decolorization rate, increased microbial resistance to increasing dye concentration, among other factors [95]. Moreover, ammonium removal has already been achieved with 92% to

100% rates, coupled with 89% to 100% dye removal in microbial treatment in bioreactors using aerobic granular sludge [96].

As for the inoculation of bacteria into the reactors, it can occur in multiple ways, depending on the operation system proposed. For example, sludge obtained from a wastewater treatment location can be added into the bioreactor to serve as a inoculum source, being composed, in theory, of a myriad of organisms adapted to the contaminated environment and in balanced association [85], or a specific pure culture bacteria can be grown in order to be inoculated directly in a anode of a microbial fuel cell [84]. A packed-bed column reactor can be inoculated by the circulation of the bacteria culture in the packed-bed column [93]. As demonstrated, there is more than one form of inoculating a bioreactor for bioremediation use, and the initial bacteria acclimatation/growth also varies with the bacteria strain and bioreactor operation. Because of the various possibilities when it comes to bacterial inoculum, this step is susceptible to improvement, being an interesting hub for microbial prospection and cultivation optimizations, including here the pre-treatment of the sludge for better results [97–99].

3.3. Degradation of Environmental and Industrial Samples

The use of bacteria to treat contamination caused by azo dyes can aim at both the treatment of effluents before their release into nature and the bioremediation of already contaminated natural environments. This topic deals with research conducted on the treatment of samples taken from contaminated environments and industrial effluents (Table 4) to show how efficient bacteria can be applied in remediating real samples in real cases of contamination.

Table 4. Bacterial degradation of azo dyes contaminated industrial effluents.

Main Bacteria	Wastewater Source	Degradation Mechanism	Country	Maximum Degradation and Experiment Conditions	Reference
<i>Micrococcus luteus</i>	Dyehouse	Adsorption and Enzymatic	Japan	Laboratory	[99]
<i>Pseudomonas aeruginosa</i>	-	Enzymatic-Azoreductase	India	62%-Laboratory	[100]
<i>Pseudomonas</i> sp.	Textile Industries	Enzymatic-Laccase	India	90%-Laboratory	[101]
<i>Pseudomonas</i> sp. and <i>Bacillus</i> sp.	Mill effluent outlet	-	India	<i>Pseudomonas</i> 95% <i>Bacillus</i> 97%-Laboratory	[102]
<i>Pseudomonas aeruginosa</i> , <i>Pseudomonas putida</i> and <i>Bacillus cereus</i>	Textile Factory	-	Egypt	92%-Laboratory	[103]
-	Dye Wastewater Plant	-	Korea	75%-Real production facility	[104]

3.3.1. Industrial Effluents

To illustrate how diverse the sources of bacteria capable of degrading azo dyes can be, the study of Ito and co-workers [99] isolated bacteria from the microbiota of human hands and classified them into two groups: azo dye decolorizers and anthraquinone dye decolorizers. The two strains chosen for further work, one from each group, were able to perform decolorization of the industrial effluent sample collected from a dyeing plant. Other works used *Pseudomonas* sp. strains in the decolorization of industrial effluent samples. The decolorization rates were different, of 62% when supplemented with nutrients and in the time of 7 days [100], up to 90% in sixty hours [101] and ranging between 87–95% in 48 hours [102]. The latter work further tested a *Bacillus* sp. strain and obtained decolorization of the textile effluent samples in the range of 92–97% in 48 hours. The work of Bayoumi and co-workers [103] also focused on bacteria from the genera *Bacillus* sp. and *Pseudomonas* sp. in the decolorization of textile effluent samples from an industrial city in Egypt, obtaining results between 84% and 92% of decolorization in a period of 48 hours.

Kalathil and co-workers [104] worked with microbial fuel cells to treat wastewater containing dyes in Daegu, South Korea, the tests were conducted with a retention time of forty-eight hours and the system was operated in open loop and in closed loop. The closed system had the highest color removal-almost 80%-while the open system showed only 62% decolorization. In addition, the closed loop also presented higher toxicity removal and a decrease in COD.

3.3.2. Environmental Samples

Still in the efforts to find bacterial strains capable of degrading azo dyes under conditions relevant to real-world application, different studies have evaluated the ability of these organisms to degrade dyes in environmental samples (Table 5). The work of Tara and co-workers [105] used a pilot scale floating wetland system coupled with dye degrading bacteria to treat wastewater from a textile industry in Pakistan. These macrocosms were installed using separate or combined plants and bacteria, and the symbiose between them improved the removal of organic and inorganic pollutants by decreasing chemical and biochemical oxygen demand by 92% and 91%, respectively, staining was reduced by 86%, and trace metals by 87%. Furthermore, the combination of these organisms also resulted in improved detoxification of the effluent, where no fish kills were observed after exposure to the treated textile effluent. When treated without the combination of bacteria and plants, the effluent still caused the death of some fish, highlighting the benefits of this synergy for bioremediation efforts.

Table 5. Bacterial degradation of azo dyes contaminated environmental samples.

Remediation site	Parallel Study	Degradation System	Country	Maximum Degradation	Reference
On-site Textile Industrial Wastewater	COD *, BOD ** and Trace metals removal	Floating Wetlands	Pakistan	86%	[105]
On-site Textile Industrial Wastewater	COD, BOD, heavy metals, nitrogen, phosphorous and total dissolved solids decrease.	Vertical flow constructed wetlands (VFCW) with bacterial endophytes	Pakistan	74%	[106]
On-site Textile Industrial Wastewater	-	Aerated wetland	Italy	82%	[107]
Dye Contaminated Soil	-	Continuous flow reactor	India	98%	[108]
Dye ontaminated soil	COD, BOD, TOC ***, heavy metals, nitrogen reduction and nitrogen, phosphorous and potassium increase.	Microbial consortia	India	98.87%(after 30 days) 99.25% (after 60 days)	[109]
Dye spiked soil	Optimum pH and temperature decolorization and effect of carbon and nitrogen sources addition	Bacterial consortia	India	97%	[110]
Yabagawa river sediments	Aromatic amines persistence	Natural river microbiota	Japan	-	[111]

* COD = Chemical Oxygen Demand. ** BOD = Biological Oxygen Demand. *** TOC = Total Organic Carbon.

Hussain and co-workers [106] also focused on a wetlands system to treat wastewater from a Pakistani industry but using pilot-scale vertical flow constructed wetlands (VFCW) system augmented with bacterial endophytes, which were selected based on their capabilities to improve plant growth and degrade dyes. The combination proposed was able to decrease chemical oxygen demand (81%), biochemical oxygen demand (72%), total dissolved solids (32%), color (74%), nitrogen (84%), phosphorous (79%), and heavy metals (Cr (97%), Fe (89%), Ni (88%), Cd (72%)). In addition, the treated wastewater was found to cause no harm based on a fish toxicity assay. Another study based on wetlands system (but with aeration) was carried out by Masi and co-workers [107]. The group assessed color removal based on three different wavelengths and different influent concentrations, achieving results that varied from a negative color removal (−58%) to a positive decolorization of 82%.

In India a continuous flow reactor was tested for the bioremediation of contaminated soil using a consortium of bacteria, achieving 85% color removal on the first day of operation, 90% on the second, and a steady 98% removal rate from the 13th day of

operation [108]. Other groups have also worked with soil bioremediation. Vipul and co-workers [109] treated soil samples, collected from industrial area, with a bacterial community previously isolated from sludge samples of six sites contaminated with different organic compounds containing bacteria and fungi organisms. The microbial community was able to achieve a decolorization of 98.87%, 82.88% of COD removal and 89.82% of BOD removal after 30 days. As for the Tandon and co-workers' group [110], they treated dye contaminated soil with a bacterial consortium, achieving 97% and 96.25% of decolorization for two different dyes.

The Yabagawa River in Japan was studied over three years to assess the natural degradation of dyes and their breakdown products, the aromatic amines, by bacteria. This river had been suffering from the dumping of dyes and industrial effluents by a dye factory for more than 50 years, and with the closure of the factory in 2012, Ito and co-workers [111] were able to evaluate the natural recovery of this environment.

This work pointed out the persistence of dyes and their aromatic amines in the river sediments even years after the end of the discharge of industrial effluent in that environment and even without the presence of visible coloration in the water. It was also observed that the degradation rate of the dye varies with its concentration in the medium, i.e., the less dye the lower the rate of degradation, and the opposite occurred with the degradation of aromatic amines, which increased over time, reaching its highest rate one year after the end of the effluent discharge on the river. The variety of bacteria itself changed over time, going from an abundant variety of genera related to the degradation of azo dyes to a decrease in these and an increase in groups related to aniline degradation [111].

4. 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, among other factors more specific to the numerous systems under study.

Despite the challenges in this sector, which this review tried to address under a critical approach, the good results obtained in laboratory, pilot scale and in some specific cases applied to real situations, together with the urgency for new sustainable solutions in large-scale industries make the search for biotechnological solutions a possible path in collective efforts in the search for cleaner and more responsible forms of production.

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References

- De Araújo, M.E.M. Corantes naturais para têxteis—Da antiguidade aos tempos modernos. *Conserv. Patrim.* **2006**, *3–4*, 39–51.
- Kant, R. Textile Dyeing Industry an Environmental Hazard. *Nat. Sci.* **2012**, *4*, 22–26. [[CrossRef](#)]
- Abel, A. The history of dyes and pigments: From natural dyes to high performance pigments. In *Colour Design: Theories and Applications*; Woodhead Publishing: Sawston, UK, 2012; pp. 557–587. [[CrossRef](#)]
- Ajaz, M.; Shakeel, S.; Rehman, A. Microbial Use for Azo Dye Degradation—A Strategy for Dye Bioremediation. *Int. Microbiol.* **2020**, *23*, 149–159. [[CrossRef](#)] [[PubMed](#)]
- Guaratini, C.C.L.; Zanoni, M.V.B. Corantes Têxteis. *Química Nova* **2000**, *23*, 71–78. [[CrossRef](#)]
- Saxena, A.; Gupta, S. Bioefficacies of Microbes for Mitigation of Azo Dyes in Textile Industry Effluent: A Review. *BioResources* **2020**, *15*, 9858–9881. [[CrossRef](#)]
- Chequer, F.M.D.; Lizier, T.M.; de Felício, R.; Zanoni, M.V.B.; Debonsi, H.M.; Lopes, N.P.; Marcos, R.; de Oliveira, D.P. Analyses of the Genotoxic and Mutagenic Potential of the Products Formed after the Biotransformation of the Azo Dye Disperse Red 1. *Toxicol. In Vitro.* **2011**, *25*, 2054–2063. [[CrossRef](#)] [[PubMed](#)]
- Gürses, A.; Açıkıldız, M.; Güneş, K.; Gürses, M.S. Dyes and Pigments: Their Structure and Properties. In *Dyes and Pigments*; Springer: Cham, Switzerland, 2016; pp. 13–29. ISBN 9783319338903.
- Oliveira, D.P.D. Corantes como Importante Classe de Contaminantes Ambientais—Um Estudo de Caso. Ph.D. Thesis, Universidade de São Paulo, São Paulo, Brasil, 1 March 2005.
- Gomes, L.M. Estudo da otimização do processo Fenton para o descolorimento de corantes azo. Master's Thesis, Universidade de São Paulo, São Paulo, Brasil, 2009.
- Bell, J.; Plumb, J.J.; Buckley, C.A.; Stuckey, D.C. Treatment and Decolorization of Dyes in an Anaerobic Baffled Reactor. *J. Environ. Eng.* **2000**, *126*, 1026–1032. [[CrossRef](#)]
- Chung, K.-T. Azo Dyes and Human Health: A Review. *J. Environ. Sci. Health Part C* **2016**, *34*, 233–261. [[CrossRef](#)] [[PubMed](#)]
- De Amorim, C.C.; Leão, M.M.D.; Moreira, R.d.F.P.M. Comparação Entre Diferentes Processos Oxidativos Avançados Para Degradação de Corante Azo. *Eng. Sanit. Ambient.* **2009**, *14*, 543–550. [[CrossRef](#)]
- Singh, A.L.; Chaudhary, S.; Kayastha, A.M.; Yadav, A. Decolorization and degradation of textile effluent with the help of *Enterobacter asburiae*. *Indian J. Biotechnol.* **2015**, *14*, 101–106.
- Kumar, A.; Kumar, A.; Singh, R.; Singh, R.; Pandey, S.; Rai, A.; Singh, V.K.; Rahul, B. Genetically Engineered Bacteria for the Degradation of Dye and Other Organic Compounds. In *Abatement of Environmental Pollutants*; Elsevier: Amsterdam, The Netherlands, 2020; pp. 331–350. ISBN 9780128180952.
- van der Zee, F.P.; Villaverde, S. Combined Anaerobic–Aerobic Treatment of Azo Dyes—A Short Review of Bioreactor Studies. *Water Res.* **2005**, *39*, 1425–1440. [[CrossRef](#)] [[PubMed](#)]
- Mahmood, S.; Khalid, A.; Arshad, M.; Mahmood, T.; Crowley, D.E. Detoxification of Azo Dyes by Bacterial Oxidoreductase Enzymes. *Crit. Rev. Biotechnol.* **2016**, *36*, 639–651. [[CrossRef](#)]
- Lellis, B.; Fávaro-Polonio, C.Z.; Pamphile, J.A.; Polonio, J.C. Effects of Textile Dyes on Health and the Environment and Bioremediation Potential of Living Organisms. *Biotechnol. Res. Innov.* **2019**, *3*, 275–290. [[CrossRef](#)]

19. Rawat, D.; Sharma, R.S.; Karmakar, S.; Arora, L.S.; Mishra, V. Ecotoxic Potential of a Presumably Non-Toxic Azo Dye. *Ecotoxicol. Environ. Saf.* **2018**, *148*, 528–537. [CrossRef]
20. Parrott, J.L.; Bartlett, A.J.; Balakrishnan, V.K. Chronic Toxicity of Azo and Anthracenedione Dyes to Embryo-Larval Fathead Minnow. *Environ. Pollut.* **2016**, *210*, 40–47. [CrossRef] [PubMed]
21. Barathi, S.; Karthik, C.; Nadanasabapathi, S.; Padikasan, I.A. Biodegradation of Textile Dye Reactive Blue 160 by *Bacillus firmus* (*Bacillaceae*: Bacillales) and Non-Target Toxicity Screening of Their Degraded Products. *Toxicol. Rep.* **2020**, *7*, 16–22. [CrossRef]
22. Croce, R.; Cinà, F.; Lombardo, A.; Crispeyn, G.; Cappelli, C.I.; Vian, M.; Maiorana, S.; Benfenati, E.; Baderna, D. Aquatic Toxicity of Several Textile Dye Formulations: Acute and Chronic Assays with *Daphnia magna* and *Raphidocelis subcapitata*. *Ecotoxicol. Environ. Saf.* **2017**, *144*, 79–87. [CrossRef]
23. Duarte Baumer, J.; Valério, A.; de Souza, S.M.A.G.U.; Erzinger, G.S.; Furigo, A.; de Souza, A.A.U. Toxicity of Enzymatically Decolored Textile Dyes Solution by Horseradish Peroxidase. *J. Hazard. Mater.* **2018**, *360*, 82–88. [CrossRef]
24. North, A. Impact of Chronic Exposure of Selected Azo Dyes on the Model Organism *Caenorhabditis elegans*. Ph.D. Thesis, Texas Southern University, Houston, TX, USA, 2021.
25. Hessel, C.; Allegre, C.; Maisseu, M.; Charbit, F.; Moulin, P. Guidelines and Legislation for Dye House Effluents. *J. Environ. Manag.* **2007**, *83*, 171–180. [CrossRef]
26. Compliance Gate. Are Azo Dyes Banned in the European Union? Available online: https://www.compliancegate.com/azo-dye-regulations-european-union/#Are_azo_dyes_banned_in_the_European_Union (accessed on 6 April 2021).
27. Compliance Gate. Azo Dye Regulations in the United States: An Overview. Available online: <https://www.compliancegate.com/azo-dye-regulations-united-states/> (accessed on 6 April 2021).
28. SGS. Textile and Clothing Chemical Safety Criteria in Asian Countries—When East Meets West. Available online: <https://www.sgs.com/en/news/2012/04/textile-and-clothing-chemical-safety-criteria-in-asian-countries-when-east-meets-west> (accessed on 6 April 2021).
29. Qima. Japan Regulation Restricting Azo Dyes in Household Products Comes into Force. Available online: <https://www.qima.com/regulation/03-16/japan-azo> (accessed on 6 April 2021).
30. Hktdc Hong Kong Means Business. India—Azo Requirement on Imported Apparel and Textile Products. Available online: <https://hkmb.hktdc.com/en/1X09YJMT/hktdc-research/India-Azo-Requirement-on-Imported-Apparel-and-Textile-Products> (accessed on 6 April 2021).
31. Tuvsud. Vietnam: New Regulation on Azo Dyes and Formaldehyde in Textile Products Published. Available online: <https://www.tuvsud.com/en/e-ssentials-newsletter/consumer-products-and-retail-essentials/e-ssentials-1-2018/new-regulation-on-azo-dyes-and-formaldehyde-in-textile-products-published> (accessed on 6 April 2021).
32. Buthelezi, S.; Olaniran, A.; Pillay, B. Textile Dye Removal from Wastewater Effluents Using Biofloculants Produced by Indigenous Bacterial Isolates. *Molecules* **2012**, *17*, 14260–14274. [CrossRef]
33. Sriram, N.; Reetha, D.; Saranraj, P. Biological Degradation of Reactive Dyes by Using Bacteria Isolated from Dye Effluent Contaminated Soil. *Middle-East J. Sci. Res.* **2013**, *12*, 1695–1700. [CrossRef]
34. Li, Q.; Wu, Y.-J. A Fluorescent, Genetically Engineered Microorganism That Degrades Organophosphates and Commits Suicide When Required. *Appl. Microbiol. Biotechnol.* **2009**, *82*, 749–756. [CrossRef] [PubMed]
35. Pan, Y.; Zhu, T.; He, Z. Enhanced Removal of Azo Dye by a Bioelectrochemical System Integrated with a Membrane Biofilm Reactor. *Ind. Eng. Chem. Res.* **2018**, *57*, 16433–16441. [CrossRef]
36. Kuhad, R.C.; Sood, N.; Tripathi, K.K.; Singh, A.; Ward, O.P. Developments in Microbial Methods for the Treatment of Dye Effluents. In *Advances in Applied Microbiology*; Elsevier: Amsterdam, The Netherlands, 2004; Volume 56, pp. 185–213. ISBN 9780120026586.
37. Solís, M.; Solís, A.; Pérez, H.I.; Manjarrez, N.; Flores, M. Microbial Decolouration of Azo Dyes: A Review. *Process Biochem.* **2012**, *47*, 1723–1748. [CrossRef]
38. Khan, R.; Bhawana, P.; Fulekar, M.H. Microbial Decolorization and Degradation of Synthetic Dyes: A Review. *Rev. Environ. Sci. Biotechnol.* **2013**, *12*, 75–97. [CrossRef]
39. Saratale, R.G.; Saratale, G.D.; Chang, J.S.; Govindwar, S.P. Bacterial Decolorization and Degradation of Azo Dyes: A Review. *J. Taiwan Inst. Chem. Eng.* **2011**, *42*, 138–157. [CrossRef]
40. Singh, R.L.; Singh, P.K.; Singh, R.P. Enzymatic Decolorization and Degradation of Azo Dyes—A Review. *Int. Biodeterior. Biodegrad.* **2015**, *104*, 21–31. [CrossRef]
41. Keck, A.; Klein, J.; Kudlich, M.; Stolz, A.; Knackmuss, H.J.; Mattes, R. Reduction of Azo Dyes by Redox Mediators Originating in the Naphthalenesulfonic Acid Degradation Pathway of *Sphingomonas* Sp. Strain BN6. *Appl. Environ. Microbiol.* **1997**, *63*, 3684–3690. [CrossRef]
42. Chivukula, M.; Renganathan, V. Phenolic Azo Dye Oxidation by Laccase from *Pyricularia Oryzae*. *Appl. Environ. Microbiol.* **1995**, *61*, 4374–4377. [CrossRef]
43. Garcia, F.D.S. Enzimas Oxidorredutases Produzidas por Fungos Filamentosos. Master’s Thesis, Universidade de São Paulo, São Paulo, Brasil, 13 June 2018.
44. Chacko, J.T.; Subramaniam, K. Enzymatic degradation of azo dyes—a review. *Int. J. Environ. Sci.* **2011**, *1*, 1250, 2011.
45. Misal, S.A.; Gawai, K.R. Azoreductase: A Key Player of Xenobiotic Metabolism. *Bioresour. Bioprocess.* **2018**, *5*, 17. [CrossRef]
46. Sharma, V.; Upadhyay, L.S.B.; Vasanth, D. Extracellular Thermostable Laccase-Like Enzymes from *Bacillus licheniformis* Strains: Production, Purification and Characterization. *Appl. Biochem. Microbiol.* **2020**, *56*, 420–432. [CrossRef]

47. Kandelbauer, A.; Guebitz, G.M. Bioremediation for the Decolorization of Textile Dyes—A Review. In *Environmental Chemistry*; Lichtfouse, E., Schwarzbauer, J., Robert, D., Eds.; Springer: Berlin/Heidelberg, Germany, 2005; pp. 269–288. ISBN 9783540228608.
48. Paszczynski, A.; Crawford, R.L. Degradation of Azo Compounds by Ligninase from *Phanerochaete Chrysosporium*: Involvement of Veratryl Alcohol. *Biochem. Biophys. Res. Commun.* **1991**, *178*, 1056–1063. [[CrossRef](#)]
49. Ilić Đurđić, K.; Ostafe, R.; Đurđević Đelmaš, A.; Popović, N.; Schillberg, S.; Fischer, R.; Prodanović, R. Saturation Mutagenesis to Improve the Degradation of Azo Dyes by Versatile Peroxidase and Application in Form of VP-Coated Yeast Cell Walls. *Enzym. Microb. Technol.* **2020**, *136*, 109509. [[CrossRef](#)] [[PubMed](#)]
50. Singh, P.; Iyengar, L.; Pandey, A. Bacterial Decolorization and Degradation of Azo Dyes. In *Microbial Degradation of Xenobiotics*; Singh, S.N., Ed.; Springer: Berlin/Heidelberg, Germany, 2012; pp. 101–133. ISBN 9783642237881.
51. Xu, F.; Mou, Z.; Geng, J.; Zhang, X.; Li, C. Azo Dye Decolorization by a Halotolerant Exoelectrogenic Decolorizer Isolated from Marine Sediment. *Chemosphere* **2016**, *158*, 30–36. [[CrossRef](#)] [[PubMed](#)]
52. Neifar, M.; Chouchane, H.; Mahjoubi, M.; Jaouani, A.; Cherif, A. *Pseudomonas extremorientalis* BU118: A New Salt-Tolerant Laccase-Secreting Bacterium with Biotechnological Potential in Textile Azo Dye Decolourization. *3 Biotech* **2016**, *6*, 107. [[CrossRef](#)] [[PubMed](#)]
53. Wang, Y.; Jiang, L.; Shang, H.; Li, Q.; Zhou, W. Treatment of Azo Dye Wastewater by the Self-Flocculating Marine Bacterium *Aliiglaciicola Lipolytica*. *Environ. Technol. Innov.* **2020**, *19*, 100810. [[CrossRef](#)]
54. Zhuang, M.; Sanganyado, E.; Zhang, X.; Xu, L.; Zhu, J.; Liu, W.; Song, H. Azo Dye Degrading Bacteria Tolerant to Extreme Conditions Inhabit Nearshore Ecosystems: Optimization and Degradation Pathways. *J. Environ. Manag.* **2020**, *261*, 110222. [[CrossRef](#)] [[PubMed](#)]
55. Guadie, A.; Tizazu, S.; Melese, M.; Guo, W.; Ngo, H.H.; Xia, S. Biodecolorization of Textile Azo Dye Using *Bacillus* sp. Strain CH12 Isolated from Alkaline Lake. *Biotechnol. Rep.* **2017**, *15*, 92–100. [[CrossRef](#)]
56. Chen, K.-C.; Wu, J.-Y.; Liou, D.-J.; Hwang, S.-C.J. Decolorization of the Textile Dyes by Newly Isolated Bacterial Strains. *J. Biotechnol.* **2003**, *101*, 57–68. [[CrossRef](#)]
57. Jadhav, U.U.; Dawkar, V.V.; Ghodake, G.S.; Govindwar, S.P. Biodegradation of Direct Red 5B, a Textile Dye by Newly Isolated *Comamonas* Sp. UVS. *J. Hazard. Mater.* **2008**, *158*, 507–516. [[CrossRef](#)]
58. Asad, S.; Amoozegar, M.A.; Pourbabaee, A.A.; Sarbolouki, M.N.; Dastgheib, S.M.M. Decolorization of Textile Azo Dyes by Newly Isolated Halophilic and Halotolerant Bacteria. *Bioresour. Technol.* **2007**, *98*, 2082–2088. [[CrossRef](#)] [[PubMed](#)]
59. Shah, M. Evaluation of *Aeromonas* Spp. In Microbial Degradation and Decolorization of Reactive Black in Microaerophilic—Aerobic Condition. *J. Bioremed. Biodeg.* **2014**, *5*, 246. [[CrossRef](#)]
60. Franca, R.D.G.; Vieira, A.; Carvalho, G.; Oehmen, A.; Pinheiro, H.M.; Barreto Crespo, M.T.; Lourenço, N.D. *Oerskovia Paurometabola* Can Efficiently Decolorize Azo Dye Acid Red 14 and Remove Its Recalcitrant Metabolite. *Ecotoxicol. Environ. Saf.* **2020**, *191*, 110007. [[CrossRef](#)] [[PubMed](#)]
61. Srinivasan, S.; Sadasivam, S.K. Exploring Docking and Aerobic-Microaerophilic Biodegradation of Textile Azo Dye by Bacterial Systems. *J. Water Process Eng.* **2018**, *22*, 180–191. [[CrossRef](#)]
62. Dehghanian, F.; Kay, M.; Kahrizi, D. A Novel Recombinant AzrC Protein Proposed by Molecular Docking and in Silico Analyses to Improve Azo Dye's Binding Affinity. *Gene* **2015**, *569*, 233–238. [[CrossRef](#)] [[PubMed](#)]
63. Haghshenas, H.; Kay, M.; Dehghanian, F.; Tavakol, H. Molecular Dynamics Study of Biodegradation of Azo Dyes via Their Interactions with AzrC Azoreductase. *J. Biomol. Struct. Dyn.* **2016**, *34*, 453–462. [[CrossRef](#)]
64. Srinivasan, S.; Shanmugam, G.; Surwase, S.V.; Jadhav, J.P.; Sadasivam, S.K. In Silico Analysis of Bacterial Systems for Textile Azo Dye Decolorization and Affirmation with Wetlab Studies: General. *Clean Soil Air Water* **2017**, *45*, 1600734. [[CrossRef](#)]
65. Sari, I.P.; Simarani, K. Decolorization of Selected Azo Dye by *Lysinibacillus fusiformis* W1B6: Biodegradation Optimization, Isotherm, and Kinetic Study Biosorption Mechanism. *Adsorpt. Sci. Technol.* **2019**, *37*, 492–508. [[CrossRef](#)]
66. Joshi, A.U.; Hinsu, A.T.; Kotadiya, R.J.; Rank, J.K.; Andharia, K.N.; Kothari, R.K. Decolorization and Biodegradation of Textile Di-Azo Dye Acid Blue 113 by *Pseudomonas stutzeri* AK6. *3 Biotech* **2020**, *10*, 214. [[CrossRef](#)]
67. Du, L.-N.; Li, G.; Zhao, Y.-H.; Xu, H.-K.; Wang, Y.; Zhou, Y.; Wang, L. Efficient Metabolism of the Azo Dye Methyl Orange by *Aeromonas* Sp. Strain DH-6: Characteristics and Partial Mechanism. *Int. Biodeterior. Biodegrad.* **2015**, *105*, 66–72. [[CrossRef](#)]
68. Olukanni, O.D.; Osuntoki, A.A.; Kalyani, D.C.; Gbenle, G.O.; Govindwar, S.P. Decolorization and Biodegradation of Reactive Blue 13 by *Proteus Mirabilis* LAG. *J. Hazard. Mater.* **2010**, *184*, 290–298. [[CrossRef](#)]
69. Mendes, S.; Farinha, A.; Ramos, C.G.; Leitão, J.H.; Viegas, C.A.; Martins, L.O. Synergistic Action of Azoreductase and Laccase Leads to Maximal Decolourization and Detoxification of Model Dye-Containing Wastewaters. *Bioresour. Technol.* **2011**, *102*, 9852–9859. [[CrossRef](#)]
70. Thakur, J.K.; Paul, S.; Dureja, P.; Annapurna, K.; Padaria, J.C.; Gopal, M. Degradation of Sulphonated Azo Dye Red HE7B by *Bacillus* sp. and Elucidation of Degradative Pathways. *Curr. Microbiol.* **2014**, *69*, 183–191. [[CrossRef](#)]
71. Eskandari, F.; Shahnavaz, B.; Mashreghi, M. Optimization of Complete RB-5 Azo Dye Decolorization Using Novel Cold-Adapted and Mesophilic Bacterial Consortia. *J. Environ. Manag.* **2019**, *241*, 91–98. [[CrossRef](#)]
72. Masarbo, R.S.; Niranjana, S.R.; Monisha, T.R.; Nayak, A.S.; Karegoudar, T.B. Efficient Decolorization and Detoxification of Sulphonated Azo Dye Ponceau 4R by Using Single and Mixed Bacterial Consortia. *Biocatal. Biotransform.* **2019**, *37*, 367–376. [[CrossRef](#)]

73. Guo, G.; Hao, J.; Tian, F.; Liu, C.; Ding, K.; Xu, J.; Zhou, W.; Guan, Z. Decolorization and Detoxification of Azo Dye by Halo-Alkaliphilic Bacterial Consortium: Systematic Investigations of Performance, Pathway and Metagenome. *Ecotoxicol. Environ. Saf.* **2020**, *204*, 111073. [[CrossRef](#)]
74. Guo, G.; Hao, J.; Tian, F.; Liu, C.; Ding, K.; Zhang, C.; Yang, F.; Xu, J. Decolorization of Metanil Yellow G by a Halophilic Alkalithermophilic Bacterial Consortium. *Bioresour. Technol.* **2020**, *316*, 123923. [[CrossRef](#)] [[PubMed](#)]
75. Guo, G.; Li, X.; Tian, F.; Liu, T.; Yang, F.; Ding, K.; Liu, C.; Chen, J.; Wang, C. Azo Dye Decolorization by a Halotolerant Consortium under Microaerophilic Conditions. *Chemosphere* **2020**, *244*, 125510. [[CrossRef](#)] [[PubMed](#)]
76. Shah, B.; Jain, K.; Jiyani, H.; Mohan, V.; Madamwar, D. Microaerophilic Symmetric Reductive Cleavage of Reactive Azo Dye—Remazole Brilliant Violet 5R by Consortium VIE6: Community Synergism. *Appl. Biochem. Biotechnol.* **2016**, *180*, 1029–1042. [[CrossRef](#)] [[PubMed](#)]
77. Khan, Z.; Jain, K.; Soni, A.; Madamwar, D. Microaerophilic Degradation of Sulphonated Azo Dye—Reactive Red 195 by Bacterial Consortium AR1 through Co-Metabolism. *Int. Biodeterior. Biodegrad.* **2014**, *94*, 167–175. [[CrossRef](#)]
78. Kannan, D.; Rajan, S.; Murugesan, A.G. Decolourization of azo dye by native microbial consortium. *J. Adv. Sci. Res.* **2019**, *10*, 95–99.
79. Eslami, H.; Shariatifar, A.; Rafiee, E.; Shiranian, M.; Salehi, F.; Hosseini, S.S.; Eslami, G.; Ghanbari, R.; Ebrahimi, A.A. Decolorization and Biodegradation of Reactive Red 198 Azo Dye by a New Enterococcus Faecalis–Klebsiella Variicola Bacterial Consortium Isolated from Textile Wastewater Sludge. *World J. Microbiol. Biotechnol.* **2019**, *35*, 38. [[CrossRef](#)] [[PubMed](#)]
80. Patel, D.K.; Tiple, D.R.; Dave, S.R. Selection and Development of Efficient Consortia for Decolorization of Metal Complex Dyes. *Toxicol. Environ. Chem.* **2017**, *99*, 252–264. [[CrossRef](#)]
81. Chakraborty, J.N. Metal-complex dyes. In *Handbook of Textile and Industrial Dyeing*; Elsevier: Amsterdam, The Netherlands, 2011; pp. 446–465. ISBN 9781845696955.
82. Kelly, P.T.; He, Z. Nutrients Removal and Recovery in Bioelectrochemical Systems: A Review. *Bioresour. Technol.* **2014**, *153*, 351–360. [[CrossRef](#)] [[PubMed](#)]
83. Cui, M.-H.; Cui, D.; Gao, L.; Cheng, H.-Y.; Wang, A.-J. Analysis of Electrode Microbial Communities in an Up-Flow Bioelectrochemical System Treating Azo Dye Wastewater. *Electrochim. Acta* **2016**, *220*, 252–257. [[CrossRef](#)]
84. Mani, P.; Fidal, V.T.; Bowman, K.; Breheny, M.; Chandra, T.S.; Keshavarz, T.; Kyazze, G. Degradation of Azo Dye (Acid Orange 7) in a Microbial Fuel Cell: Comparison Between Anodic Microbial-Mediated Reduction and Cathodic Laccase-Mediated Oxidation. *Front. Energy Res.* **2019**, *7*, 101. [[CrossRef](#)]
85. Dai, Q.; Zhang, S.; Liu, H.; Huang, J.; Li, L. Sulfide-Mediated Azo Dye Degradation and Microbial Community Analysis in a Single-Chamber Air Cathode Microbial Fuel Cell. *Bioelectrochemistry* **2020**, *131*, 107349. [[CrossRef](#)]
86. Kong, F.; Ren, H.-Y.; Pavlostathis, S.G.; Wang, A.; Nan, J.; Ren, N.-Q. Enhanced Azo Dye Decolorization and Microbial Community Analysis in a Stacked Bioelectrochemical System. *Chem. Eng. J.* **2018**, *354*, 351–362. [[CrossRef](#)]
87. Cao, X.; Wang, H.; Zhang, S.; Nishimura, O.; Li, X. Azo Dye Degradation Pathway and Bacterial Community Structure in Biofilm Electrode Reactors. *Chemosphere* **2018**, *208*, 219–225. [[CrossRef](#)]
88. Cao, X.; Wang, H.; Li, X.; Fang, Z.; Li, X. Enhanced Degradation of Azo Dye by a Stacked Microbial Fuel Cell-Biofilm Electrode Reactor Coupled System. *Bioresour. Technol.* **2017**, *227*, 273–278. [[CrossRef](#)]
89. Fang, Z.; Cheng, S.; Wang, H.; Cao, X.; Li, X. Feasibility Study of Simultaneous Azo Dye Decolorization and Bioelectricity Generation by Microbial Fuel Cell-Coupled Constructed Wetland: Substrate Effects. *RSC Adv.* **2017**, *7*, 16542–16552. [[CrossRef](#)]
90. Cui, M.-H.; Cui, D.; Liang, B.; Sangeetha, T.; Wang, A.-J.; Cheng, H.-Y. Decolorization Enhancement by Optimizing Azo Dye Loading Rate in an Anaerobic Reactor. *RSC Adv.* **2016**, *6*, 49995–50001. [[CrossRef](#)]
91. Menezes, O.; Brito, R.; Hallwass, F.; Florêncio, L.; Kato, M.T.; Gavazza, S. Coupling Intermittent Micro-Aeration to Anaerobic Digestion Improves Tetra-Azo Dye Direct Black 22 Treatment in Sequencing Batch Reactors. *Chem. Eng. Res. Des.* **2019**, *146*, 369–378. [[CrossRef](#)]
92. Assadi, A.; Naderi, M.; Mehrasbi, M.R. Anaerobic–Aerobic Sequencing Batch Reactor Treating Azo Dye Containing Wastewater: Effect of High Nitrate Ions and Salt. *J. Water Reuse Desalination* **2018**, *8*, 251–261. [[CrossRef](#)]
93. Kardi, S.N.; Rashid, N.A.A.; Ibrahim, N.; Ahmad, A. Biodegradation of Remazol Black B in Sequential Microaerophilic–Aerobic Operations by NAR-2 Bacterial Consortium. *Environ. Earth Sci.* **2016**, *75*, 1172. [[CrossRef](#)]
94. Korenak, J.; Ploder, J.; Trček, J.; Hélix-Nielsen, C.; Petrinic, I. Decolourisations and Biodegradations of Model Azo Dye Solutions Using a Sequence Batch Reactor, Followed by Ultrafiltration. *Int. J. Environ. Sci. Technol.* **2018**, *15*, 483–492. [[CrossRef](#)]
95. Wang, Z.; Yin, Q.; Gu, M.; He, K.; Wu, G. Enhanced Azo Dye Reactive Red 2 Degradation in Anaerobic Reactors by Dosing Conductive Material of Ferroferric Oxide. *J. Hazard. Mater.* **2018**, *357*, 226–234. [[CrossRef](#)] [[PubMed](#)]
96. Sarvajith, M.; Reddy, G.K.K.; Nancharaiyah, Y.V. Textile Dye Biodecolourization and Ammonium Removal over Nitrite in Aerobic Granular Sludge Sequencing Batch Reactors. *J. Hazard. Mater.* **2018**, *342*, 536–543. [[CrossRef](#)]
97. Al-Hoqani, M.; Zafar, M.; Al Musharafi, S.K.; Mahanty, B.; Behera, S.K. COD Fractionation and Solubility Assessment of Sonicated Waste-activated Sludge. *Environ. Qual. Manag.* **2021**, *1–8*, tqem.21789. [[CrossRef](#)]
98. Haddad, M.; Abid, S.; Hamdi, M.; Bouallagui, H. Reduction of Adsorbed Dyes Content in the Discharged Sludge Coming from an Industrial Textile Wastewater Treatment Plant Using Aerobic Activated Sludge Process. *J. Environ. Manag.* **2018**, *223*, 936–946. [[CrossRef](#)]

99. Ito, T.; Shimada, Y.; Suto, T. Potential Use of Bacteria Collected from Human Hands for Textile Dye Decolorization. *Water Resour. Ind.* **2018**, *20*, 46–53. [[CrossRef](#)]
100. Sarayu, K.; Sandhya, S. Aerobic Biodegradation Pathway for Remazol Orange by *Pseudomonas aeruginosa*. *Appl. Biochem. Biotechnol.* **2010**, *160*, 1241–1253. [[CrossRef](#)]
101. Telke, A.A.; Joshi, S.M.; Jadhav, S.U.; Tamboli, D.P.; Govindwar, S.P. Decolorization and Detoxification of Congo Red and Textile Industry Effluent by an Isolated Bacterium *Pseudomonas* sp. SU-EBT. *Biodegradation* **2010**, *21*, 283–296. [[CrossRef](#)]
102. Srinivasan, V.; Bhavan, P.S.; Krishnakumar, J. Bioremediation of textile dye effluent by *Bacillus* and *Pseudomonas* spp. *Int. J. Environ. Sci. Technol.* **2014**, *3*, 2215–2224.
103. Bayoumi, M.N.; Al-Wasify, R.S.; Hamed, S.R. Bioremediation of textile wastewater dyes using local bacterial isolates. *Int. J. Curr. Microbiol. App. Sci.* **2014**, *3*, 962–970.
104. Kalathil, S.; Lee, J.; Cho, M.H. Efficient Decolorization of Real Dye Wastewater and Bioelectricity Generation Using a Novel Single Chamber Biocathode-Microbial Fuel Cell. *Bioresour. Technol.* **2012**, *119*, 22–27. [[CrossRef](#)]
105. Tara, N.; Arslan, M.; Hussain, Z.; Iqbal, M.; Khan, Q.M.; Afzal, M. On-Site Performance of Floating Treatment Wetland Macrocosms Augmented with Dye-Degrading Bacteria for the Remediation of Textile Industry Wastewater. *J. Clean. Prod.* **2019**, *217*, 541–548. [[CrossRef](#)]
106. Hussain, Z.; Arslan, M.; Malik, M.H.; Mohsin, M.; Iqbal, S.; Afzal, M. Treatment of the Textile Industry Effluent in a Pilot-Scale Vertical Flow Constructed Wetland System Augmented with Bacterial Endophytes. *Sci. Total Environ.* **2018**, *645*, 966–973. [[CrossRef](#)] [[PubMed](#)]
107. Masi, F.; Rizzo, A.; Bresciani, R.; Martinuzzi, N.; Wallace, S.D.; Van Oirschot, D.; Macor, F.; Rossini, T.; Fornaroli, R.; Mezzanotte, V. Lessons Learnt from a Pilot Study on Residual Dye Removal by an Aerated Treatment Wetland. *Sci. Total Environ.* **2019**, *648*, 144–152. [[CrossRef](#)]
108. Daniel, D.; Jegathambal, P.; Bevers, B. In Situ Bioremediation of Textile Dye Effluent-Contaminated Soils Using Mixed Microbial Culture. *Int. J. Civ. Eng.* **2019**, *17*, 1527–1536. [[CrossRef](#)]
109. Patel, V.R.; Khan, R.; Bhatt, N. Cost-Effective in-Situ Remediation Technologies for Complete Mineralization of Dyes Contaminated Soils. *Chemosphere* **2020**, *243*, 125253. [[CrossRef](#)] [[PubMed](#)]
110. Tandon, S.A.; Shaikh, S.; Kumar, R. Development and use of soil bacterial consortia for bioremediation of dye polluted soil and municipal waste water. *GJ Biosci. Biotech.* **2014**, *3*, 284–291.
111. Ito, T.; Adachi, Y.; Yamanashi, Y.; Shimada, Y. Long-Term Natural Remediation Process in Textile Dye-Polluted River Sediment Driven by Bacterial Community Changes. *Water Res.* **2016**, *100*, 458–465. [[CrossRef](#)] [[PubMed](#)]