



REVIEW PAPER

## Application of environmental bacteria as potential methods of azo dye degradation systems

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### ABSTRACT

**BACKGROUND AND OBJECTIVES:** The objective of this study is to present a description of the main characteristics of azo dyes and the different treatment methods used to remove them from water. There is a special emphasis given to the benefits associated with biological treatment, predominantly those related to the use of bacteria, which has to do with its competitive advantages over other microorganisms in the dye degradation processes.

**METHODS:** The topic to be addressed was first defined through workshops with the research group. The literature review was carried out following several inclusion/exclusion criteria: the year of publication, as the selection was limited to studies published between 2010 and 2020, the focus of the investigation, which had to be related to the efficiency of different techniques for the remediation of ecosystems contaminated with azo dyes and, lastly, that the studies also discussed the use of environmental bacteria in dye degradation processes.

**FINDINGS:** The efficiency of bacteria to degrade azo dyes ranges from 63-100%, the most efficient being: *Marinobacter* sp, *Sphingobacterium* sp, *Enterococcus faecalis*, *Enterococcus casseliflavus*. The bacteria that, reportedly, have greater efficiency for simultaneously removing the dye-metal complex are *Bacillus circulans* and *Acinetobacter junii*.

**CONCLUSION:** Traditional strategies for the treatment of effluents contaminated with azo dyes are limited to physical and chemical processes that have a high energy and economic cost. For these reasons, current challenges are focused on the use of environmental bacteria capable of transforming dyes into less toxic compounds.

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## INTRODUCTION

Annually more than a million tons of synthetic dyes are produced around the world for use in the leather, textile, pharmaceutical, food, cosmetic, paint, plastic and paper industries (Shamraiz *et al.*, 2016), of which, at least 60% are azo dyes (Shah, 2014; Gürses *et al.*, 2016). In addition to being recalcitrant towards various degradation processes (Singh *et al.*, 2014; Singh *et al.*, 2015), azo dyes produce dangerous chemical substances such as aromatic amines, known for their toxic, allergenic, carcinogenic and mutagenic effect on living organisms (Das *et al.*, 2015). The impact of azo dyes on the environment is proportional to the enormous amounts of hazardous waste associated with industrial processes, which is then released to water bodies, in most cases, without proper treatment. A further aggravating factor is that due to the inability of at least 35% of azo dyes to adhere to substrates, heavy metals have been incorporated during the dyeing process; these act as mordants, favoring the fixation of the dye (Vuthiganond *et al.*, 2018). Colorants associated with metals such as copper, cobalt and especially chromium, are difficult to degrade and represent an important source of environmental contamination considering their increased presence in organic load. They generate adverse and irreversible ecotoxicological effects, bioaccumulation phenomena and biomagnification in flora and aquatic fauna and alteration of biogeochemical cycles (Lončar *et al.*, 2014; Kurade *et al.*, 2016). This powerful metal-dye complex has carcinogenic and mutagenic properties for humans exposed to effluents contaminated with dyes. It can lead to: skin cancer (due to photosensitization), photodynamic damage, allergic contact dermatitis, renal, reproductive, hepatic, cerebral dysfunction, irritation of the respiratory tract and asthma (Mondal *et al.*, 2017; Khan and Malik, 2018). Traditionally, physicochemical methods have been used to treat effluents contaminated with azo dyes, but their high economic and energy cost and the environmental effects associated with their use have pushed technological development towards the use of microorganisms in recent years. These are successful biological alternatives due to their survival properties, adaptability, enzymatic activity and chemical structure. Additionally, hybrid technologies have been developed, which integrate various technologies into one, taking the best of each and

surpassing the limitations of current conventional treatments. (Singh *et al.*, 2015; Ribera, 2019). Recent reports have indicated that molecular techniques such as metagenomics and metaproteomics are being used to explore the molecular degradation mechanism of azo dyes. These technologies can be utilized for screening and identifying crucial genes, proteins and enzymes which will be essential for achieving a deep insight into the intrinsic biodegradation mechanism of dyes. (An *et al.*, 2020; Zhang *et al.*, 2019; Qu *et al.*, 2018). These reports indicated that the application of environmental bacteria capable of degrading azo dyes should mainly focus on bioremediation, clean technologies, genetic engineering, nanotechnology and use of metagenomics and metaproteomics analysis (Fig. 1). The objective of this review is to describe a chemical classification of dyes and their structural characteristics. It presents a description of the main characteristics of azo dyes, the treatments used for their degradation and the potential of bacteria to become an optimal biological alternative in the treatment of effluents contaminated with azo dyes. The main focus of the review are biological treatments using marine bacteria. This is due to their ability to survive in aquatic environments under adverse environmental conditions, as well as their ability to develop multi-resistance mechanisms for antibiotics and heavy metals and the enzyme systems associated with the degradation of dyes. This review also presents the mechanisms of the bacteria-heavy metals interaction and, finally, the bacterial species which are capable of degrading individual and mixed dyes, as well as remove heavy metals and dyes simultaneously and, also, metal-complex dyes. This review is part of a doctoral thesis called: Determination of the capacity of environmental bacteria for the degradation of azo dyes, a study which was carried out at the University of Cartagena, in Cartagena, Colombia during 2019 – 2020.

### Overview of azoic dyes

Dyes are substances of chemical or biological origin with the ability to bind to a substrate and impart color. They can be classified according to their chemical structure, color, application and particle charge in solution. Based on their chemical structure, they are classified as: azo dyes, nitro dyes, phthalein dyes, triphenyl methane dyes, indigoid dyes and anthraquinone dyes (Ngulube *et al.*, 2017; Yagub *et*

*al.*, 2014). Whereas, based on their application, they are classified as: acid dyes, basic dyes, direct dyes, ingrain dyes, disperse dyes, moderate dyes, vat dyes and reactive dyes. In general, dye molecules have a delocalized electron conjugated double bond composed of the auxochrome and the chromophore groups. The chromophores give color to the dye after the excitation of electrons, while the auxochromes intensify the color imparted by the chromophore, conferring the adhesion and solubility properties of the dye (Wardman, 2017). Table 1 describes the classification of dyes according to their chromophore group.

Azo dyes are synthetic compounds widely used due to their brilliant color, ease of handling, usage and economic feasibility in synthesis when compared to other types of dyes. They can be differentiated according to the number of azo linkages ( $-N=N-$ ) present in a molecule of the dye, such as monoazo, diazo, triazo, polyazo and azoic (Pavithra *et al.*, 2019). Azo linkages can bind to benzene rings, naphthalenes, aromatic heterocycles, or essential aliphatic groups, which increases the complexity of the molecule. The binding of azo linkages to these chemical groups gives the molecule special properties

such as photocatalytic stability and resistance to degradation (Shah *et al.*, 2014; Benkhaya *et al.*, 2016). Azo dyes can form complexes with metals called metal complexes, an important feature, exploited for a long time by the textile industry. This is due to the fact that this metal-dye complex increases performance, making them resistant to fading as a result of washing or exposure to sunlight. There are two types of metal complex azo dyes: the first, those in which the azo group is coordinated to the metal (medially metallized) and the second, those in which it is not (terminally metallized). The most important metal complexes are those formed from the reaction of transition metal ions with ligands. In ligands, the ortho positions adjacent to the azo group contain a group which is capable of coordinating with the metal ion. The metals which are used commercially the most in metal complexes are copper(II), cobalt (III) and chromium (III) (Ghosh *et al.*, 2016). The synthesis of these coordination complexes of transition metals with azo ligands is due to the interesting physical, chemical, photophysical, photochemical and catalytic properties. Metal complex dyes play a very important role in the textile industry. Table 2 shows a

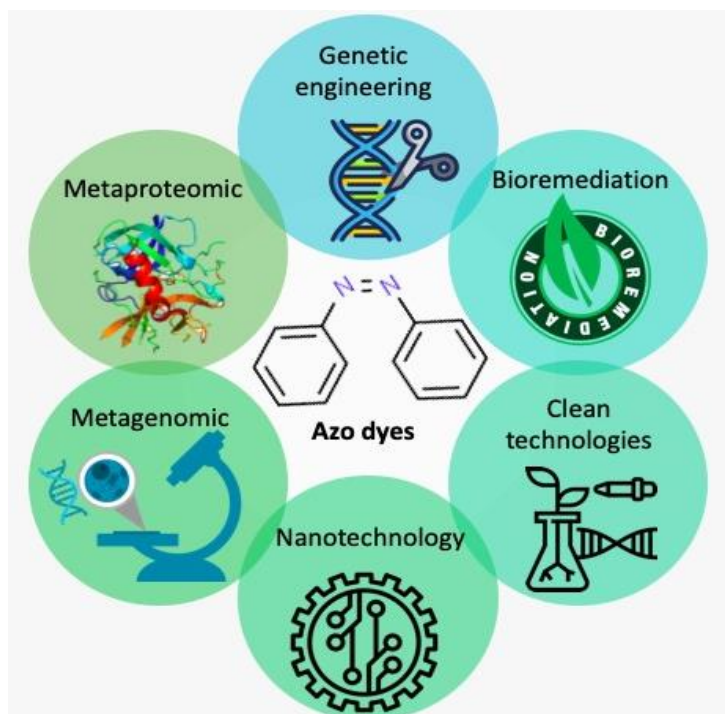


Fig. 1: Technologies for the removal of azo dyes

Potential azoic dye degraders

Table 1: Classification of dyes based on their chemical structure chromophore

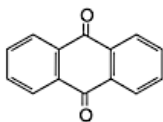
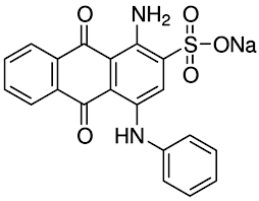
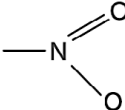
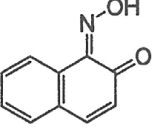
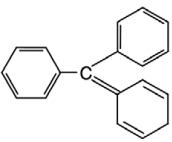
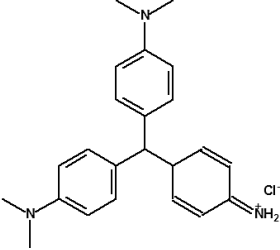
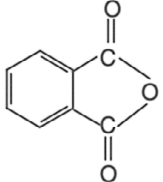
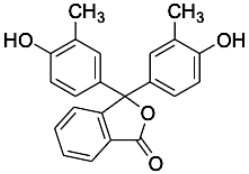
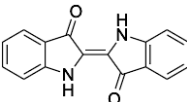
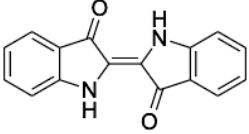
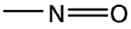
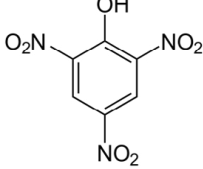
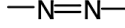
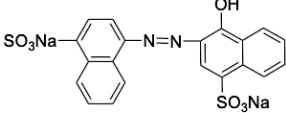
Chemical structure class	Chromophore	Dye	Chemical structure	C.I. name
Anthraquinone		Acid blue 25		62055
Nitro		Mordant green 4		10005
Triphenylmethane		Crystal violet		42555
Phthalein		o-Cresolphthalein		68995
Indigo		Indigo		10215
Nitroso		Picric acid		10305
Azo		Acid red B		14720

Table 2: Classification of azo dyes according to number of azo linkages and metal complex dyes

Chromophore	Azo Dye	Chemical structure	C.I. name
Monoazo	Methyl orange		13025
Diazo	Red ponceau S		103116
Triazo	Direct blue 71		34140
Poliazo	Direct red 80		35780
Naphthol	Naphthol yellow S		10316
Azo lakes	Lithol rubine BK		15850
Benzimidazolone	Benzimidazolone yellow H3G		11781

Continued Table 2: Classification of azo dyes according to number of azo linkages and metal complex dyes

Chromophore	Azo Dye	Chemical structure	C.I. name
	Cu <sup>2+</sup> Reactive blue 13		181575
	Cr <sup>3+</sup> Acid black 172		23976
Metal complex	Co <sup>3+</sup> Acid black 180		13710
	B <sup>3+</sup> Boron-dibenzopyrromethene		131818
	Ni BDN		691182
	Fe <sup>2+</sup> Iron(II)Phthalocyanine		23925

classification of azo dyes according to the number of azo linkages and metal complex dyes.

Azo dyes represent a significant and very versatile group of dyes used in numerous industries such as the food industry, printing, leather, pharmaceutical, cosmetic and especially in the textile industry, where they are used to dye fabrics made of protein fibers, cellulose or synthetic fibers such as polyesters and nylon (Tee *et al.*, 2015; Das *et al.*, 2015; Oon *et al.*, 2017). Azo dyes however, are toxic, carcinogenic and mutagenic in nature. They represent a pollution hazard because they include components such as benzidine and aromatic compounds in their structure. Their breakdown products (colorless amines) are also toxic and/or mutagenic to living organisms (Xu *et al.*, 2016). Once released to the environment through colored wastewater, these dyes represent a problem for the receiving waters, due to the reduction of the photosynthetic activity of aquatic plants, the

decrease in the concentration of dissolved oxygen and the increase in oxygen biochemical demand (Liu *et al.*, 2015; Orts *et al.*, 2018; Sarkar *et al.*, 2020). The bioaccumulation of these dyes in sediments and soil can generate modifications in the microbial communities and enzymatic activities, can inhibit the nitrification process, alter the activity of the urease enzyme, the ammonification rate of arginine and reduce oxidative bacteria. In plants, these dyes decrease the germination rate and produce chlorosis and anatomical changes in leaves that ultimately lead to plant death (Imran *et al.*, 2015; Rehman *et al.*, 2018).

#### *Traditional methods for the treatment of contaminated effluents*

Taking into account the negative environmental impact generated by the discharge of untreated or partially treated colored effluents to receiving

Table 3: Physical and chemical methods most used for the treatment of colored effluents

Method	Rationale	Limitations	References
Adsorption	Uses absorbents to remove colorants, such as activated carbon and other materials such as cobs, sawdust, vegetable and fruit peels, among others.	High cost for the preparation of activated carbon. Low cost absorbents have low dye removal efficiency. After treatment the absorbents are polluting.	Mincea <i>et al.</i> , 2013 Zhao <i>et al.</i> , 2014
Coagulation	Adding coagulants to water to form flocs. With the proper weight, size, and strength, sedimentation of macro-flocs occurs.	It requires the use of chemical products, and generates a large quantity of sludge that must be treated later.	Ayanda, 2018
Membrane Filtration	Uses special pore-size membranes to filter contaminants through techniques such as reverse osmosis, ultra, micro, and nanofiltration.	More efficient as a pretreatment for separation processes. It is a method of high initial and limited cost for the elimination of dyes due to their solubility in water.	Ahmad <i>et al.</i> , 2012
Advanced oxidation	It involves techniques such as the oxidation of Fenton's reagent, ultraviolet photolysis, sonolysis, use of ozone and hydrogen peroxide to degrade organic pollutants at room temperature and pressure.	Inefficient for the removal of insoluble dyes and limited life. The use of the Fenton's reagent generates a large quantity of sludge	Ayanda, 2018 Gupta <i>et al.</i> , 2015
Electrochemical oxidation	They use the anode of the electrolytic cell to electrochemically oxidize wastewater contaminated with dyes.	Its efficiency depends on the operating conditions and variables such as the support electrolyte, pH of the medium, temperature, concentration of the organic compound, and the type of anode material.	Ayanda, 2014 Gupta <i>et al.</i> , 2015

water bodies, various treatment methods have been used. The most widely used physicochemical methods include advanced oxidation processes, adsorption, ozonation, membrane filtration, photocatalytic degradation, coagulation and flocculation, electrocoagulation, photo-electrocatalysis, and electrochemical oxidation (Gupta *et al.*, 2015; Fajardo *et al.*, 2015; Solano *et al.*, 2015). Unfortunately, although these methods are considered effective for removing dyes from wastewater, there are many drawbacks to these strategies: complex infrastructures, high cost, inefficient color removal, production of secondary pollutants or generation of large amounts of contaminated sludge and toxic by-products (Balapure *et al.*, 2014; Liu *et al.*, 2015). To minimize these drawbacks, a combination of two or more physicochemical techniques are frequently used for the treatment of waste water contaminated with dyes; however, the results remain unpromising given the recalcitrant nature of the dyes and their resistance to degradation processes (Ayanda, 2018; Kertes *et al.*, 2014). Table 3 presents the rationale and limitations of the most widely used physicochemical methods for treating colored effluents. Currently, wastewater treatment systems are not only focused on guaranteeing the ecosystem quality of the receiving water bodies and minimizing the impact to human health. Based on the principles of the circular economy, they are also focused on the need to develop treatments that allow the reuse of wastewater in response to the issue of the growing demand for water and the depletion of natural sources (Ribera *et al.*, 2019).

As a consequence of the degradation processes of azo dyes, chemical compounds known as aromatic amines are generated, whose molecular structure is characterized by having one or more aromatic rings with amino substituents. The toxicity of amines depends on the metabolic activation of the amino group, which generates metabolic intermediates capable of binding to DNA molecules, producing genotoxicity and mutagenicity (Brüschweiler *et al.*, 2017). Although azo dyes are considered recalcitrant, recent studies have reported that aromatic amines can be biodegraded taking into account important factors such as the type of microbial population, their conditions to adapt and the availability of oxygen (Pietruk *et al.*, 2019).

#### *Methods for removing azo dyes in different industries*

The release of dyes into the environment is a consequence of industrial processes. The textile industry releases 54% of the existing dyes in the world, the dyeing industry itself releases 21%, the pulp and paper industry releases 10%, the leather tanning industry releases 8%, and 7% is released by the dye manufacturing industry. Dyes used in the textile industry are xenobiotic and recalcitrant compounds (Kurade *et al.*, 2016; Ajaz *et al.*, 2019). Physico-chemical alternatives for treating waste water resulting from the textile industry include: advanced oxidation processes AOP (Mondal *et al.*, 2017), coagulation/flocculation (Butani *et al.*, 2017), electrochemical treatments (Chellam and Sari, 2016) and physical methods (Khan *et al.*, 2018). Biological alternatives are: bacterial cultures- either pure or in consortia (Kurade *et al.*, 2016; Kuppusamy *et al.*, 2017), algal biomass (Elgarahy *et al.*, 2019), fungi cultures (Dayi *et al.*, 2019) and enzymatic methods (Katheresan *et al.*, 2018). The use of emerging technologies to treat waste water resulting from the dyeing industry has been reported recently. These technologies include: the combination of TiO<sub>2</sub> microreactor and electroflotation (Talaiekhosravi *et al.*, 2020), itaconic acid hydrogels (Bharathiraja *et al.*, 2019) ion exchange resins (Wu *et al.*, 2020), catalytic ozonation (Ghuge and Saroha, 2018) advanced photocatalytic processes (Kim and Jo, 2019; Bahadur and Bhargava, 2019) and pulsed light (Martínez-López *et al.*, 2019). The pulp and paper industry treats effluents contaminated with azo dyes using advanced oxidation processes (Cesaro *et al.*, 2019), ion exchange (Jaria *et al.*, 2017), ozonation and biological treatment (Kumar *et al.*, 2019), coagulation using polymeric ferric chloride, (Yang *et al.*, 2019), aerobic granulation (Morais *et al.*, 2016), bio-adsorbents (Kakkar *et al.*, 2018), the microbial fuel cell, enzymatic methods with laccases, peroxidases and xylanases (Sharma *et al.*, 2020; Singh *et al.*, 2019) and microorganisms such as *Clostridium* (An *et al.*, 2020), *Diaphorobacter nitroreducens* (Zhong *et al.*, 2020) and *Saccharomyces cerevisiae* (Lin *et al.*, 2012; Lin *et al.*, 2017). Advanced oxidation processes such as electrochemical oxidation, electro-fenton and photoelectro-fenton are the most commonly used in the treatment of effluents contaminated with azo dyes in the leather tanning industry. The use of



biosorption using solid waste from tanneries has also been reported (Gomes *et al.*, 2016), as well as the use of biomass from microalgae (Da Fontoura *et al.*, 2017) and fungi such as *Trametes versicolor*, *Ganoderma lucidum* and *Irpex* (Baccar *et al.*, 2011). For the treatment of effluents contaminated with dyes in the dye manufacturing industry other methods are used: adsorption with magnesium oxide nanopores (Pourrahim *et al.*, 2020), activated carbon (Ilnicka *et al.*, 2020) and capsules with hierarchical Mg(OH)<sub>2</sub> nanostructures (Akbari, 2017).

#### *Mechanism of microorganisms on degradation of azo dyes*

Microorganisms can degrade azo dyes by means of biosorption mechanisms and/or enzymatic degradation. The biosorption capacity of a microorganism is associated with the attraction generated between the azo dye and the components of the bacterial cell wall. This mechanism depends on pH, temperature, ionic strength, contact time, adsorbent and dye concentration, dye structure and type of microorganism. Enzymatic degradation is an anaerobic mechanism favored by the deficiency of electrons in the dye. The reduction of azo binding of the dye is mediated by azoreductase enzymes and oxidative degradation is catalyzed by peroxidases and phenoloxidases such as: manganese peroxidase, lignin peroxidase, laccase, tyrosinase, N-demethylase (Wu *et al.*, 2012; Ambrosio *et al.*, 2012; Solis *et al.*, 2012). Fungi have ligninolytic enzymes such as manganese peroxidase enzyme, laccase and lignin peroxidase with excellent catalytic power, capable of degrading dyes using biosorption mechanisms, biotransformation or complete removal by mineralization. These mechanisms are favored by the addition of carbon and nitrogen sources, aeration, humidity and use of mixed crops (Asgher *et al.*, 2014; Akdogan *et al.*, 2014). For the degradation of azo dyes, bacteria have an efficient enzymatic system that allows them to carry out a series of catabolic activities, with azoreductase and laccase enzymes being responsible for the transfer of electrons to the azo bond of the dye and the production of aromatic amines. (González *et al.*, 2018). The mechanism of degradation by azoreductase enzymes consists of two phases; the first, called the reducing phase, begins with the cleavage of the azo bond (-N=N-) by catalyzed reduction of the enzyme under anaerobic/anoxic or

microaerophilic conditions, where NADH molecules, derived from carbohydrate metabolism are used as electron donors (Elfarash *et al.*, 2017; González *et al.*, 2018). In the second phase, as a result of this division, relatively simple intermediate aromatic amines are generated, which are deaminated or dehydrogenated by bacteria through aerobic processes (Garg *et al.*, 2012) under aerobic conditions, which leads to complete degradation of azo dyes (Saratale *et al.*, 2011; Garg *et al.*, 2012; Al-Amrani *et al.*, 2014). Laccases, on the other hand, are copper oxidases that degrade dyes in the presence of oxygen through mechanisms that involve direct or indirect oxidation using redox mediators to accelerate the reaction, which involves the removal of a hydrogen atom from the hydroxyl and amino groups, replacing it with phenolic substrates and aromatic amines (Tišma *et al.*, 2020). Bacterial peroxidases are also involved in the degradation of dyes. These enzymes need H<sub>2</sub>O<sub>2</sub> as a terminal electron acceptor rather than oxygen. Their mechanism of action is similar to that of laccases and leads to degradation of the dye without production of toxic aromatic amines (Imran *et al.*, 2014). For the degradation of azo dyes, algae may involve enzymatic degradation processes, adsorption, or both. They degrade azo dyes through azoreductase enzymes or oxidative enzymes. Adsorption efficiency is influenced by dye structure, algal species and pH. Microalgae that are immobilized in alginate may remove a higher percentage of color than algae in suspension (Priya *et al.*, 2011). Similar to microalgae, yeast discoloration mechanisms involve adsorption, enzymatic degradation or a combination of both. Adsorption by yeast biomass is more efficient at a pH between 2 and 4, while, degradation is associated with the presence of oxidase and reductases enzymes and the addition of carbon or glucose as an energy source. Genetically modified organisms can also degrade azo dyes through mechanisms involving genetic modification or gene transfer, that encode enzymes with different characteristics or biochemical pathway variants in a microorganism (Martorell *et al.*, 2012; Solis *et al.*, 2012).

#### *The potential of bacteria for the degradation of azo dyes*

The limitations associated with the use of physicochemical methods for the treatment of effluents contaminated with azo dyes have promoted

the development of new treatment alternatives that are attractive, efficient, profitable, environmentally friendly and produce less sludge (Balapure et al., 2014; Liu et al., 2015; Sabaruddin et al., 2018; Zhuang et al., 2020). Table 4 compares efficiency, environmental impact and costs between biological methods and physico-chemical methods.

The effectiveness of microorganisms for the degradation of compounds depends on various factors such as survival, adaptability, the activity of the microorganism and the chemical structure (Amoozegar et al., 2011; Agrawal et al., 2014). Among the biological alternatives for dye removal is phytoremediation, which uses plants such as *Aster amellus*, which removes azo dyes mainly through their roots (Khandare et al., 2011). On the other hand, algae such as *Chara* sp and *Comarium* sp, are resistant to the conditions found in textile effluents and are capable of removing malachite green through degradation and sorption mechanisms. However, the long amount of time necessary to carry out these processes constitutes a disadvantage (Khandare et al., 2011; González et al., 2018). The ability of fungi to adapt their metabolism to the exploitation of various sources of carbon and nitrogen, makes them

a viable option for the degradation of dyes. Such is the case of *Trametes versicolor* that degrades red dye 27 through lignins peroxidases (Rekik et al., 2019), or *Aspergillus niger* and *Aspergillus terreus* that degrade and absorb the red azo dye MX-5 reducing its toxicity (Almeida and Corso, 2014). Despite all of the above, bacteria are the most relevant microorganisms in bioremediation processes due to their ability to adapt to variations in chemical and biological oxygen demands at high concentrations of salinity, at variable pH levels, dissolved oxygen and heavy metals (Ajaz et al., 2019). To interact with heavy metals, they have specific mechanisms through which they can interact, as presented in Table 5.

In addition, they use different resistance mechanisms that include the release of metal ions by extracellular barriers such as the capsule, the cell wall and the plasma membrane, the extrusion of metal ions through efflux or diffusion pumps, intracellular sequestration of metal ions, biotransformation of toxic metal ions, and decreased sensitivity of cellular targets to metal ions (Bazzi et al., 2020). In the interaction between bacteria and metal, the formation of biofilm plays an important role in bacterial survival in the presence of high metal concentrations, and

Table 4: Comparison between biological and physico-chemical methods

Criteria	Biological methods	Reference	Physico-chemical methods	Reference
Efficiency	They are able to completely mineralize many azo dyes under certain environmental conditions.	Saratale et al., 2011 Rathod et al., 2017	They have low color removal efficiency. They do not completely eliminate recalcitrant azo dyes and / or their organic metabolites. Secondary waste is generated and needs additional	Guo et al., 2020
Impact on the environment	They are eco-friendly because they use microbial microorganisms or enzymes and the end products are not toxic.  Require less water and energy consumption	Ahmadi et al., 2017 Dong et al., 2019	They generate a significant amount of sludge that can cause secondary pollution problems.  Energy-intensive	Meerbergen et al., 2018 Guo et al., 2020
Costs	Low operating costs	Saratale et al., 2011 Dong et al., 2019	They are economically unviable. The large amount of sludge generated substantially increases the cost of these methods.	Saratale et al., 2011

efflux systems allow bacteria to interact with different amino acids as a mechanism of adaptation to the environment. These interaction mechanisms are complemented by the presence of resistance genes that encode the production of enzymes capable of reducing metals to compounds which are less toxic, and the synthesis of metalloproteins necessary for the bioaccumulation and immobilization of metals. Thermophilic and hyperthermophilic bacteria use alternative mechanisms to enzymatic production to resist metals and transfer ions to the active site (Giovannella *et al.*, 2020; Artz *et al.*, 2015). Bacterial action in the degradation of azo dyes is increased due to their ability to act through consortiums or synergistic associations that act as biological inducers. The union of the catabolic functions of each microorganism makes them even more useful alternatives to improve the discoloration rate of effluents contaminated with dyes, as they have greater resistance to abiotic conditions and lower rates of enzyme inactivation, especially in large-scale operations (Cervantes and Dos Santos, 2011; Khan *et al.*, 2018; Balapure *et al.*, 2015; Wu *et al.*, 2020). Table 6 summarizes the most relevant competitive advantages that position bacteria as the most efficient microorganisms in the degradation of azo dyes.

The use of bacteria to remove azo dyes has also some disadvantages: 1) The discoloration process does not depend exclusively on these microorganisms, but also on external variables such as: agitation, oxygen, temperature, pH, dye structure, dye concentration, carbon and nitrogen sources, electron donor and redox mediator (Saratale *et al.*, 2011; Al-Amrani *et al.*, 2014). 2) Under anaerobic conditions, the dye

penetrates with difficulty through the cell membrane, affecting the rate of degradation (Saratale *et al.*, 2011; Bai *et al.*, 2020). 3) As a result of the degradation process, they generate noxious and recalcitrant aromatic amines (Das *et al.*, 2015; Brüscheweiler *et al.*, 2017). 4) Pure crops do not degrade by full azo dyes, so bacterial pools are required to make the process more productive (Saratale *et al.*, 2011; Balapure *et al.*, 2014). In recent years, innovative integrated processes called hybrid technologies have emerged; they provide a new treatment system, which allows eliminating the individual limitations of physical, chemical and biological methods (Shah, 2014). Among the emerging technologies associated with bacterial treatments for wastewater discoloration is biological coagulation; it consists of a prior coagulation treatment and a subsequent biological treatment dependent on variables such as the type and dose of coagulant, the amount of sludge and the degree of inhibitory and non-biodegradable substances present in wastewater. Another widely used process is the combination of advanced oxidation with activated sludge treatment. In this synergy, chemical oxidation partially degrades recalcitrant contaminants to intermediate metabolites that in subsequent processes are easily degraded by bacteria (Guan *et al.*, 2018). Furthermore, the addition of adsorbents to activated sludge systems is also a viable choice for the removal of soluble organic matter. Among the most widely used adsorbents is activated carbon, which, when joined with bacteria, favors the degradation processes. Sometimes, however, carbon particles become trapped in the matrix floc and lose their adsorption properties, hindering bacterial growth

Table 5: Bacteria interaction mechanisms- heavy metals

Mechanism	Basis	Reference
Bioaccumulation	Metal enters the cytoplasm of the cell through the membrane transport system. The accumulation is favored by the action of metalloproteins or by its deposition in vacuoles.	Zhang <i>et al.</i> , 2020
Bio-mineralization	Metal is precipitated in the cell through resistance mechanisms encoded by plasmids.	Khadim <i>et al.</i> , 2019
Biotransformation	Metal is transformed inside the cell through mechanisms that favor the loss of electrons or changes in oxidation states, and the addition of methyl groups.	Johnson <i>et al.</i> , 2020
Chemisorption	Metal molecules hold together with the bacteria to form a strong chemical bond, so the chemisorbed molecule does not maintain the same electronic structure.	Latif <i>et al.</i> , 2020

and dye removal. Applying this process allows COD and color removal from textile wastewater in a single step without additional physicochemical treatment (Zhang et al., 2019). Likewise, the new methods associated with filtration constitute a promising technology for the reuse of water, which is how the use of nanofiltration, a technique that increases the life of the membrane, has recently been reported. It provides a “closed loop” system, in which products are partially oxidized and then transferred for biological treatment by bacteria. Rinse water can be reused

after membrane recovery while concentrated wastes can be degraded in anaerobic digester (Cinperi et al., 2019). The membrane bioreactor also constitutes an improvement option to the conventional activated sludge treatment to treat colored water. It consists of an anaerobic reactor modified with activated carbon, that precedes the aerobic membrane bioreactor and achieves stable discoloration along with a high removal of total organic carbon, improving the dehydrability of activated sludge and reducing resistance to filtration (Bai et al., 2020).

Table 6: Competitive advantages of bacteria for the degradation of azo dyes

No.	Advantages	Bacteria identified	References
1	They have short life cycles, generating faster discoloration processes.	<i>Proteus vulgaris</i> .	Britos et al., 2018
2	They have a higher growth rate and adaptability.	<i>Bacillus</i> sp, <i>Bacillus subtilis</i> , <i>Aeromonas hydrophila</i> , <i>Bacillus cereus</i> , <i>Proteus mirabilis</i> , <i>Pseudomonas luteola</i> , <i>Pseudomonas</i> sp, <i>Pseudomonas aeruginosa</i> , <i>Escherichia coli</i> and <i>Klebsiella</i> sp.	Saratale et al., 2011 Al -Amrani et al., 2014
3	Their use is more viable, inexpensive and ecological.	<i>Bacillus subtilis</i> , <i>Aeromonas hydrophila</i> , <i>Bacillus cereus</i> , <i>Proteus mirabilis</i> , <i>Pseudomonas luteola</i> , <i>Pseudomonas</i> sp. and <i>Pseudomonas aeruginosa</i> .	Saratale et al., 2011
4	Their degradation capacity is boosted when used in consortia.	<i>Psychrobacter alimentarius</i> and <i>Staphylococcus equorum</i> .	Khalid et al., 2012
5	They detoxify aromatic amines produced by anaerobic discoloration.	<i>Aeromonas hydrophila</i> .	Thanavel et al., 2019
6	They use complex organic compounds to carry out their metabolic activities.	<i>Aerococcus</i> sp, <i>Carnobacterium</i> sp, <i>Enterococcus</i> sp, <i>Lactobacillus</i> sp, <i>Lactococcus</i> sp, <i>Leuconostoc</i> sp, <i>Oenococcus</i> sp, <i>Pediococcus</i> sp, <i>Streptococcus</i> sp, <i>Tetragonococcus</i> sp, <i>Vagococcus</i> sp and <i>Weissella</i> sp	Sharma et al., 2020
7	The effectiveness to degrade dyes does not depend on their adaptability to the environment, but has to do with the presence of enzymatic genes that can be innately expressed or over-expressed in the presence of toxic substances.	<i>Pseudomonas desmolyticum</i> , <i>Micrococcus glutamicus</i> , <i>Pseudomonas</i> sp, <i>Enterococcus gallinarum</i> , <i>Klebsiella</i> sp, <i>Lysinibacillus</i> sp, <i>Pseudomonas putida</i> , <i>Pseudomonas pulmonicola</i> and <i>Micrococcus</i> sp.	Vikrant et al., 2018 Mittal et al., 2018
8	They possess molecular mechanisms to acquire resistance to heavy metals similar to the antimicrobial resistance mechanisms.	<i>Escherichia coli</i> , <i>Streptomyces pilosus</i> , <i>Klebsiella aerogenes</i> , <i>Pseudomonas putida</i> , <i>firmicutes</i> sp, <i>Staphylococcus aureus</i> , <i>Enterococcus hirae</i> , <i>Ralstonia</i> sp, <i>Streptomyces</i> sp, <i>Bacillus</i> sp and <i>Arthrobacter viscosus</i> .	Nanda et al., 2019

Table 7: Bacterial species reported as dye degraders

Bacteria	Degraded dye(s)	Higher percentage removal (100 mg/L)	Reference
<i>Marinobacter sp</i>	Direct blue 1	100%	Prasad <i>et al.</i> , 2013
<i>Galactomyces sp</i>	Amido black	81.43%	Maqbool., 2016
<i>Pseudomonas putida</i>	Orange 10	70%	Mahmood <i>et al.</i> , 2016
<i>Bacillus sp</i>	RV-5R and RBO-3R	63.33%, 96.15%	Dicle <i>et al.</i> , 2014
<i>Bacillus cohnii</i>	Direct red-22	95%	Prasad <i>et al.</i> , 2013
<i>Brevibacterium sp</i>	RY107, RB5, RR198 and DB71	99%	Franciscon <i>et al.</i> , 2012
<i>Providencia sp</i>	Acid black 210	99%	Agrawal <i>et al.</i> , 2014
<i>Staphylococcus arlettae</i>	Yellow107	99.5%	Bhardwaj <i>et al.</i> , 2016
<i>Aeromonas hydrophila</i>	Crystal violet	99%	Bharagava <i>et al.</i> , 2018
<i>Aeromonas hydrophila</i>	Fast yellow MR	91.25%	Thanavel <i>et al.</i> , 2019
<i>Sphingobacterium sp.</i>	Direct red 5B	100%	Tamboli <i>et al.</i> , 2010
<i>Enterococcus faecalis</i>	Direct red 81	100%	Sahasrabudhe <i>et al.</i> , 2014
<i>Enterococcus casseliflavus</i>	Amaranth	100%	Chan <i>et al.</i> , 2012
<i>Enterococcus gallinarum</i>	Reactive red 35	93.69%	Soni <i>et al.</i> , 2016
<i>Enterococcus gallinarum</i>	Reactive red 198	91.56%	Soni <i>et al.</i> , 2016
<i>Enterococcus gallinarum</i>	Reactive red 106	94.91%	Soni <i>et al.</i> , 2016
<i>Enterococcus gallinarum</i>	Reactive red 120	92.69%	Soni <i>et al.</i> , 2016
<i>Enterococcus gallinarum</i>	Reactive red 111	93.58%	Soni <i>et al.</i> , 2016
<i>Enterococcus gallinarum</i>	Reactive black 5	91.99%	Soni <i>et al.</i> , 2016
<i>Enterococcus gallinarum</i>	Reactive red 141	91.99%	Soni <i>et al.</i> , 2016
<i>Enterococcus gallinarum</i>	Reactive blue 160	93.63%	Soni <i>et al.</i> , 2016
<i>Enterococcus gallinarum</i>	Reactive blue 28	91.42%	Soni <i>et al.</i> , 2016
<i>Enterococcus gallinarum</i>	Reactive red 152	95.95%	Soni <i>et al.</i> , 2016
<i>Acinetobacter baumannii</i>	Reactive red 198	95.58%	Unnikrishnan <i>et al.</i> , 2018
<i>Acinetobacter baumannii</i>	Congo red	99%	Ning <i>et al.</i> , 2014
<i>Acinetobacter baumannii</i>	Congo red	89%	Kuppusamy <i>et al.</i> , 2016
<i>Acinetobacter baumannii</i>	Gentian violet	90%	Kuppusamy <i>et al.</i> , 2016
<i>Acinetobacter sp</i>	Dye disperse orange S-RL	90.2%	Cai <i>et al.</i> , 2015
<i>Acinetobacter calcoaceticus</i>	Amaranth	91%	Ghodake <i>et al.</i> , 2011
<i>Acinetobacter iunii</i>	RO-16, DB-19	90%	Anwar <i>et al.</i> , 2014

Table 8: Efficiency of simultaneous removal processes: dye - heavy metal

Demining process			Efficiency (%)			
Type	Methods	Dye	Metal	Colorant	Metal	Reference
Physico-chemical	Adsorption	Reactive orange 5	Pb <sup>2+</sup>	97%	70%	<a href="#">Li et al., 2019</a>
Physico-chemical	Adsorption	Methyl orange	Pb <sup>2+</sup>	90.8%	98.7%	<a href="#">Ge et al., 2018</a>
Physico-chemical	Adsorption	Basic red 46	Cu	99%	98%	<a href="#">Dolatabadi et al., 2018</a>
Biological	Bacterial: <i>Bacillus circulans</i>	Methyl orange	Cr (VI)	100%	100%	<a href="#">Liu et al., 2017</a>
Biological	Bacterial: <i>Lactobacillus paracase</i>	Acid Black	Cr (VI)	58.5%	51.9%	<a href="#">Huang et al., 2015</a>
Biological	<i>Pseudomonas putida</i>	Reactive black-5	Cr (VI)	70%	70%	<a href="#">Mahmood et al., 2013</a>
Biological	<i>Acinetobacter junii</i>	Reactive Red-120	Cr (VI)	83%	98%	<a href="#">Anwar et al., 2014</a>
Hybrid	Photocatalysis	Methyl orange	Cr (VI)	91%	91%	<a href="#">Xie et al., 2020</a>

#### Potentially degrading bacteria of the complex azoic dyes - heavy metals

Scientific reports present various bacterial species capable of degrading individual and mixed dyes, as presented in [Table 7](#). However, few studies are associated with the use of bacteria capable of remedying effluents contaminated with dyes and heavy metals effectively and simultaneously. This is precisely because these microorganisms require exclusive properties not only to adapt to adverse environmental conditions but also for their robust enzymatic activity and chemical structure ([Talaiekhazani and Rezaia., 2017](#); [Zhuang et al., 2020](#)). [Table 8](#) compares the efficiency of physico-chemical, biological and hybrid processes for simultaneous removal of metals and azo dyes.

These properties are easily found in bacterial communities present in marine ecosystems, which have developed mechanisms that allow them to resist adverse environmental conditions such as hyper salinity, pH variations and the presence of heavy metals ([Zhuang et al., 2019](#); [Zhuang et al., 2020](#)). This continuous exposure to extreme environmental conditions makes them more stable and more active, unlike other types of bacteria conserved in culture

banks ([Unnikrishnan et al., 2018](#)). [Table 9](#) presents a comparison of the percentages of dye removal using bacteria isolated from water, wastewater, soil or marine environments.

One of the bacteria identified as an effective biological alternative for the removal of metal-dye synergy is *Enterococcus* sp, recognized for its ability to thrive in environments with low nutrient concentrations, persistent to temperature fluctuations, and resistant to desiccation, UV radiation, freezing, pH changes, high salinity and predation ([Vignaroli et al., 2018](#); [Lee et al., 2019](#); [Thu et al., 2019](#)). Furthermore, they are considered catabolically versatile microorganisms, capable of using a wide range of unusual substrates as carbon source ([Sahasrabudhe et al., 2014](#)). For a long time, the environmental importance of *Enterococcus* sp had to do with it being an excellent indicator of fecal contamination in waters ([Di Dato et al., 2019](#); [Federigi et al., 2019](#)), however, recently new potential uses of this microorganism have emerged. It that can be exploited for the benefit of the environment, such as for its ability to metabolize xenobiotics, among which are azo dyes, or its affinity to bind and resist heavy metals. Furthermore, the genome of these bacteria

also reveals the presence of phages, which in large-scale industrial processes could be useful elements to improve the general bioremediation capacity. They could also prove to be viable option in transferring their ability to degrade azo dyes to other *Enterococcus* through genetic engineering from hybrid strains (Chan *et al.*, 2012). The ability of *Enterococcus faecalis* to metabolize azo dyes is associated with the presence of the *azoA* gene that encodes the production of the aerobic azoreductase enzyme, which is not secreted outside the cell, has a wide substrate specificity, requires flavin mononucleotide (FMN) as a cofactor and uses NADH as an electron donor (Rathod *et al.*, 2017; Sun *et al.*, 2017). The ATCC 6569 *Enterococcus faecium* strain possess the enzyme azoreductase (AzoEf1) which shares 67% identity with the azoreductase of *Enterococcus faecalis* (AzoA). However, there are differences related to coenzyme preference, residues associated with FMN binding, substrate specificity, and specific activity. The AzoEf1 sequence is found in GenBank: GQ479040.1. Chan *et al.* (2012) report a strain of *Enterococcus casseliflavus* that by the action of an enzyme with activity similar to that of azoreductase is not only able to discolor a wide range of azo dyes under microaerophilic conditions, but also catabolize by desulfonation and deamination the intermediaries generated as a consequence of the reductive cleavage. The genome of this microorganism also reveals the presence of regulatory systems possibly involved in the biodegradation of aromatic contaminants. *Enterococcus gallinarum* offers an effective ecological alternative for the remediation of environments contaminated with structurally complex and recalcitrant azo dyes such as reactive red 35. This is done through enzymatic mechanisms that involve the presence of oxidoreductases, such as laccases, tyrosinases and azoreductases under a wide range of pH, different temperature levels and with a high concentration of salinity; therefore, its use on a large scale is recommended using a suitable microaerophilic-aerobic sequential bioreactor (Soni *et al.*, 2016). The binding affinity of *Enterococcus sp* to heavy metals has been attributed to the capsular polysaccharide, which contains different monomers such as glucose, galactose, mannose and fructose, capable of participating in the redox reaction of remediation processes of waters contaminated with heavy metals and dyes (Sardar *et al.*, 2018). Recently,

these monomers have been used for the synthesis of silver nanoparticles (AgNP) that combined with advanced oxidation processes (AOP) have shown good results in the degradation of azo dyes such as methyl orange and Congo red (Saravanan *et al.*, 2017). In relation to metal removal, *Enterococcus faecalis* uses mechanisms such as copper transporting ATPases, present in the inner membrane, which not only work for the homeostasis of this metal but also to resist high concentrations of nickel, mercury, cadmium, lead and copper (Huët and Puchooa, 2017). Another bacterium present in marine ecosystems with exclusive properties to adapt to adverse environmental conditions and simultaneously degrade the metal-dye complex is *Acinetobacter sp.* This microorganism has protein coding genes capable of degrading innumerable organic compounds such as biphenyls, phenols, benzoates, acetonitrile, chlorine anilines, dichloroaniline, hydrocarbons and heavy metals, which for other microorganisms could be toxic. This makes it an important biocatalyst with high potential biotechnology to remedy various environmental pollutants (Hongawatt and Vangnai, 2011; Walter *et al.*, 2020). The discoloration capacity of bacteria of the genus *Acinetobacter* is associated with the enzymatic activity of lignin peroxidases, considered enzymes with exclusive catalytic properties. The activity of these enzymes depends on hydrogen peroxide so as to transform a persistent high range of organic compounds (Bilal *et al.*, 2019). There are several species of *Acinetobacter* reported with the ability to degrade dyes. Such is the case of *Acinetobacter baumannii* that degrades azo dyes using biotransformation mechanisms through peroxidase and azoreductase enzymes. The efficiency of dye degradation by this microorganism has been potentiated through microencapsulation, a technology in which the microorganism is immobilized using calcium alginate beads. This provides a higher rate of biodegradation by more easily separating the solid-liquid complex, it reduces downstream processing steps and it offers greater operational stability both by preventing leaks and by protecting the biocatalyst from environmental conditions (Unnikrishnan *et al.*, 2018). *Acinetobacter junii* is capable of degrading RR-120, RO-16, RY-2, DR-28, and DB-19 in the presence of Cr(VI), a metal associated primarily with the textile and tannery industries. However, this bacterium is also capable of resisting



other heavy metals such as  $Zn^{2+}$ ,  $Cd^{2+}$ ,  $Cu^{2+}$ ,  $Co^{2+}$  and  $Pb^{2+}$ . The properties of this strain make it a multifunctional alternative and a profitable biological resource that could be exploited for the simultaneous bioremediation of more than one contaminant (Anwar et al., 2014). *Acinetobacter calcoaceticus* can discolor various dyes, among which is the azo dye amaranth. In this case, it is a result of the enzymatic action associated with lignin peroxidases, laccases and reductases, which, in addition to degrading the dye, are capable of decreasing phytotoxicity (Ghodake et al., 2011). Due to all of the above, *Enterococcus* sp and *Acinetobacter* sp constitute an important alternative solution to the problems associated with the use of azo dyes in industrial processes. The release of dyes into the environment is a global problem. Industries are now interested in using new technological alternatives to mitigate this problem.

The textile, pulp and paper, as well as the leather tanning industry all use advanced oxidation, photocatalysis and adsorption methods to treat its effluents (Mondal et al., 2017; Cesaro et al., 2019). At an industrial level, all of the following have been reported as bio-treatments: the use of algal biomass (Elgarahy et al., 2019; Da Fontoura et al., 2017), fungi (Baccar et al., 2011; Dayi et al., 2019), yeasts (Lin et al., 2012; Lin et al., 2017), enzymatic methods (Katheresan et al., 2018; Sharma et al., 2020; Singh et al., 2019) and bacterial crops (Kurade et al., 2016; Kuppusamy et al., 2017; An et al., 2020; Zhong et al., 2020). Several authors agree that bacterial action in the degradation of azo dyes increases when they act in synergistic consortia or associations (Cervantes and Dos Santos, 2011; Saratale et al., 2011; Khan et al., 2014; Balapure et al., 2015; Wu et al., 2020) and is affected by external variables such as pH, carbon

Table 9: Comparison of dye removal using bacteria isolated from water, wastewater, soil, marine environments

Bacteria	Isolation place	Removal (%)	Dye	Reference
<i>Rhodopseudomonas palustris</i>	Lake Akkaya in Nigde, Turkey	100	Black azo dye K	Öztürk et al., 2020
<i>Bacillus</i> sp.	Abaya and Chamo alkaline lakes in Ethiopia	98	Reactive red 239	Guadie et al., 2017
<i>Klebsiella</i> <i>Buttiauxella</i> <i>Bacillus</i> <i>Escherichia</i> <i>Clostridium</i> sp.	Water from the textile industry in Haicheng, China	98	Methyl red	Cui et al., 2012
<i>Acinetobacter baumannii</i>	Kovalam sea shore in Tamil Nadu, India	96.2	Reactive red 198	Unnikrishnan et al., 2018
<i>Oceanimonas smirnovii</i> <i>Enterobacter kobei</i> <i>Citrobacter freundii</i>	Coastal marine sediments	95	Methyl orange	Zhuang et al., 2020
<i>Aliiglaciecola lipolytica</i>	Sea water	≥90	Congo red	Wang et al., 2020
<i>Acinetobacter</i> sp. <i>Klebsiella</i> sp.	System of activated sludge	> 80	Reactive orange 16 Reactive Green 19	Meerbergen et al., 2018
<i>Pseudoarthrobacter</i> sp. <i>Gordonia</i> sp. <i>Stenotrophomonas</i> sp. <i>Sphingomonas</i> sp.	Drainage of a textile factory in Mashhad, Iran	54	Reactive black-5	Eskandari et al., 2019
<i>Lactobacillus paracase</i>	Waste water from a tannery company in Zhengshen, Quanzhou, China	63	Acid black	Huang et al., 2015



and nitrogen sources, electron donor, redox mediator, dye structure and dye concentration (Saratale *et al.*, 2011; Al-Amrani *et al.*, 2014; Bai *et al.*, 2020). The discoloration time is prolonged when the concentration of the dye increases (Chakraborty *et al.*, 2013). Monoazo bonds are more easily reduced than diazo and triazo, because the activation energy required by enzymes to reduce color is lower for monoazo than for diazo and triazo (Shah, 2014). However, Oturkar *et al.* (2013) studied the degradation of azo dyes with azoreductase enzymes of *Bacillus lentus* and concluded that diazo dye showed faster discoloration than monoazo. This indicated that color degradation is not only dependent on the action of the enzyme, but also on the proximity and molecular structure of the sulfonated groups of the dye and the composition of the industrial effluent. Cofactors play an important role in the degradation of azo dyes. The azoreductase of both *Enterococcus* and *Bacillus* depends on NADH. Disturbances in the activity of this cofactor may affect bacterial physiology and growth (Rathod *et al.*, 2017; Misal *et al.*, 2011). The species reported in this paper as degraders show removal capacity between 63% and 100%; the most used out of them are *Enterococcus* and *Acinetobacter* (Ghodake *et al.*, 2011; Chan *et al.*, 2012; Anwar *et al.*, 2014; Ning *et al.*, 2014; Sahasrabudhe *et al.*, 2014; Cai *et al.*, 2015; Soni *et al.*, 2016; Kuppusamy *et al.*, 2016; Unnikrishnan *et al.*, 2018) and the most efficient are *Marinobacter sp.*, *Sphingobacterium sp.*, *Enterococcus faecalis* and *Enterococcus casseliflavus* (Tamboli *et al.*, 2010; Chan *et al.*, 2012; Prasad *et al.*, 2013; Sahasrabudhe *et al.*, 2014). Although bacteria require metals for their metabolic processes, at high concentrations they negatively affect bacterial metabolism (Zhuang *et al.*, 2019). In this study, the most efficient bacteria for simultaneously removing the dye-metal complex are *Bacillus circulans* and *Acinetobacter junii* (Anwar *et al.*, 2014; Liu *et al.*, 2017). The metabolism of many bacteria is affected in acidic or alkaline conditions. Some studies associate a low discoloration efficiency when bacteria develop in alkaline conditions, obtaining maximum discoloration rates at acidic pH (Wang *et al.*, 2017). However, this article reports the high efficiency (98% removal) of a strain of *Bacillus sp.* isolated from an alkaline lake in Ethiopia (Guadie *et al.*, 2017). Bacteria isolated from marine and estuarine environments were found to be highly efficient for the degradation of azo dyes

(Unnikrishnan *et al.*, 2018; Öztürk *et al.*, 2020; Zhuang *et al.*, 2020; Wang *et al.*, 2020) as opposed to waste water isolates with a low clearance rate (Eskandari *et al.*, 2019; Huang *et al.*, 2015). Several authors report that bacteria in marine ecosystems are more stable and more active and have mechanisms that allow them to resist adverse environmental conditions (Zhuang *et al.*, 2019; Zhuang *et al.*, 2020; Unnikrishnan *et al.*, 2018). Furthermore, taking into account that waste waters of azo dye generally have a large quantity of salts, tolerance to high salt concentrations is a relevant indicator that these bacteria are potent bio-degradants and have great potential for industrial application (Wang *et al.*, 2010). For the development of bioremediation processes it is important to prioritize the use of microbial consortia tolerant to extreme environmental conditions that simultaneously eliminate azo dyes and heavy metals, as well as to identify secondary metabolites, metabolic pathways, degradation kinetics and alternatives to minimize limiting factors. It will be relevant to advance in molecular studies of bacterial exopolysaccharide in order to use its chemical, physical and structural diversity in bioremediation processes mediated by biofilms, which can then be applied on a large scale. Transition to a circular economy boosts new bio-remediation techniques to ensure waste reduction, reuse of treated water and use of microbial fuel cells to generate renewable energy for the economic and ecological benefit of industries. The technological development associated with the degradation of dyes and metals will focus on the production of innovative biofilters, nanotubes and nanoparticles capable of immobilizing enzymes for greater efficiency. The opportunities for genetic engineering are associated with the techniques of proteomics and metagenomics for obtaining recombinant microorganisms that can over-express the genes and enzymes involved in the discoloration of azo dyes and elimination of heavy metals.

## CONCLUSION

The current biotechnological challenges lead to the development of solutions that guarantee the quality of our ecosystems and the health of human beings exposed to environmental imbalances. In relation to the problems associated with the use of dyes in different industrial processes, there have been many technological strategies developed to

reduce the polluting load in industrial effluents and in receiving water bodies. Dye removal strategies have evolved over the years. This has been a route led by physical and chemical methods which progressed towards the use of environmentally friendly and profitable biological solutions for the industry. These biological solutions have used plants, algae and other microbial biomasses as an alternative for dye removal. However, bacteria are the most robust microorganisms that, due to their structure and genome, become potential degraders of recalcitrant contaminants such as azo dyes. The competitive advantages of bacteria are, among others, their short life cycle, their ability to adapt and their metabolic activity, which is able to degrade and detoxify the secondary metabolites produced in the discoloration process. These properties prevail in bacterial communities present in marine ecosystems which are capable of removing, in monoculture or in consortium, individual colorants, mixtures of colorants and the metal-colorant complex. Their use, although it has been little exploited, becomes relevant with the advent of emerging technologies involving nanotechnology, alternative energy, circular economy and environmental sustainability. The mechanisms involved in the simultaneous removal of dyes and the metal-dye complex, the enzyme profile and the intermediate metabolites should be the subject of future studies based on genomics and proteomics. Likewise, due to the legal and environmental limitations for monitoring industrial discharges and for monitoring the distribution of azo dyes in the environment, it is necessary for the scientific community to provide innovative mechanisms in which monitoring discharges and bodies of water receptors are based on amine detection. Future research into the application of environmental bacteria capable of degrading azo dyes should focus on bioremediation, clean technologies, genetic engineering, nanotechnology and use of metagenomics and metaproteomics analysis.

#### AUTHOR CONTRIBUTIONS

R. Baldiris Ávila was responsible with preparing the work plan associated with the study, defining the bibliographic search, selection of relevant references, organizing discussion meetings, as well as revising the final version of the article. G. Manjarrez Paba and D. Baena Baldiris analyzed the documents, synthesized

the information and wrote the manuscript.

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#### CONFLICT OF INTEREST

The authors declare no potential conflict of interest regarding the publication of this work. In addition, the ethical issues including plagiarism, informed consent, misconduct, data fabrication and, or falsification, double publication and, or submission, and redundancy have been completely witnessed by the authors.

#### ABBREVIATIONS

<i>AgNP</i>	Silver nanoparticle
<i>AOP</i>	Advanced oxidation processes
<i>ATCC</i>	American type culture collection
<i>ATPases</i>	Adenylpyrophosphatase
<i>AzoA</i>	Azoreductase A
<i>AzoEf1</i>	Azoreductase from <i>Enterococcus faecium</i>
<i>B</i>	Boron
<i>B<sup>3+</sup></i>	Boron
<i>BDN</i>	Bis(4-dimethylaminodithiobenzyl)-nickel
<i>C.I</i>	Color index
<i>Cd<sup>2+</sup></i>	Cadmium
<i>Co<sup>2+</sup></i>	Cobalt
<i>Co<sup>3+</sup></i>	Cobaltic cation
<i>COD</i>	Chemical oxygen demand
<i>Cr<sup>3+</sup></i>	Chromium
<i>Cr(VI)</i>	Hexavalent chromium
<i>Cu</i>	Copper
<i>Cu<sup>2+</sup></i>	Copper
<i>DB-19</i>	Direct black 19 dye
<i>DB71</i>	Direct blue 71 dye
<i>DNA</i>	Deoxyribonucleic acid
<i>DR-28</i>	Direct red 28 dye
<i>Fe</i>	Iron
<i>Fe<sup>2+</sup></i>	Ferrous ion

FMN	Flavin mononucleotide
H <sub>2</sub> O <sub>2</sub>	Hydrogen peroxide
Mg(OH) <sub>2</sub>	Magnesium hydroxide
MR	Methyl red
NADH	Nicotinamide adenine dinucleotide
Ni	Nickel
Pb <sup>2+</sup>	Lead ion
pH	Hydrogenionic potential
RB5	Reactive black 5 dye
RBO-3R	Remazol brilliant orange 3R dye
RO-16	Reactive orange 16 dye
RR-120	Reactive red 120 dye
RR198	Reactive red 198 dye
RV-5R	Reactive violet 5R dye
RY-2	Reactive yellow 2 dye
RY107	Reactive yellow 107 dye
UV radiation	Ultraviolet radiation
Zn <sup>2+</sup>	Zinc

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