

Comparative Insights Into Bacterial and Fungal Textile Dye Effluent Decolorization Mechanisms

Rena Erlianisyah Putri¹, Riska Kurniawati¹, Nia Rossiana^{1*}, Dede Heri Yuli Yanto² and Febri Doni¹

¹Department of Biology, Faculty of Mathematics and Natural Sciences, University of Padjadjaran, Jatinangor 45363, West Java, Indonesia.

²Research Center for Applied Microbiology, National Research and Innovation Agency (BRIN), Jl. Raya Jakarta-Bogor No. KM. 46, Cibinong, Bogor Regency, West Java 16911, Indonesia.

*Corresponding author

Nia Rossiana, Department of Biology, Faculty of Mathematics and Natural Sciences, University of Padjadjaran, Jatinangor 45363, West Java, Indonesia.

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ABSTRACT

Textile industry can produce liquid waste from the dyeing process which generally contains complex structured of dyes. Biological processing of textile dye waste is considered to have advantages over physical and chemical methods, one of which is decolorization. Decolorization using biological agents such as bacteria and fungi turns out to have good effectiveness in degrading the structure of textile dyes, so it is necessary to study the decolorization mechanism to match the expected target. The mechanism of decolorization between bacteria and fungi has many similarities in term of mechanisms, which rely on two main processes, namely biosorption and biodegradation. Waste treatment with the help of microbes has been widely used, this process is often referred to as the biodegradation process. The process of biodegradation of waste generally utilizes a population of microorganisms or other products.

Keyword: Bacterial, Fungal, Decolorization, Azo Dyes, Textile, Effluent

Introduction

The textile industry sector has experienced significant growth from year to that the waste can meet quality standards. Wastewater that is simply dumped year, thereby encouraging a positive climate for market prospects. Despite this potential growth, however, the textile industry is the second most polluting sector in the world, contributing 10% of the world's total carbon emissions. In practice, the textile industry contributes a sizeable volume of waste, which includes liquid waste and emissions which are non-product outputs from textile industry activities. Especially for the textile industry, which in its production process has a finishing-dyeing unit, has the potential to cause water pollution with a high ammonia content [1].

The textile industry in general is still making efforts to manage the environment by treating waste (treatment) by building a waste treatment, but processing this waste requires a lot of capital and then the industry into the environment causes pollution, including contamination of the water sources such as rivers and

lakes. Liquid waste receives more serious attention than other forms of waste because liquid waste can cause environmental pollution in the form of physical pollution, chemical pollution, biological pollution and radioactive pollution. Textile waste is considered a dominant liquid waste produced by the textile industry because there is a dyeing process which, apart from requiring chemicals, also requires water as a solvent medium. The textile industry is an industry engaged in the garment sector by processing cotton or synthetic fibres into cloth. Almost all textile products use textile dyes to add color as an aesthetic value and hue. Textile dyes are all dyes that have the ability to be absorbed by textile fibers and are easily removed from the color (chromophore) and groups that can bind to textile fibers (auxochrome). These dyes include pigmented chemicals to give color or tint to textiles which can be added through absorption, diffusion or temperature control processes.

Synthetic textile dyes were chosen because they have several advantages over natural dyes, which are more stable, inexpensive, easy to apply, durable, and available in a variety of colors. These dyes have been proven to pollute the environment. Proper and correct waste management requires relatively large

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costs. In the process of water treatment there are several ways so that water meets the specified quality standards. The process is based on aspects of physics, chemistry and biology. The process includes filtering (physical aspect), adding chemicals (chemical aspect) and adding bacteria (biological aspect). There are several chemicals that are commonly used, easily available, and inexpensive, including chlorine and activated carbon. Chlorine is widely known by the public as a water purification agent; besides that, it also functions as a disinfectant. Activated carbon can absorb toxic substances and as a purification agent. While the addition of Bacillus bacteria can play a role in reducing the levels of COD, BOD, and ammonia in waste. Biological methods as an alternative that is considered cheap and environmentally friendly, one of which is by using bioremediation techniques. Bioremediation is defined as the process of biological recovery of polluted environmental components into a non-toxic form. Waste treatment with the help of microbes such as bacteria, fungi, and algae has been widely used, this process is often referred to as the biodegradation process. Biodegradation is defined as the process of oxidation of organic compounds by microbes as a result of metabolism of organic substances through enzymes to produce carbon dioxide, water and energy for use in synthesis, mortality and respiration.

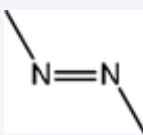
Bacteria are important biological agents that have the ability to degrade waste. Bacteria capable of decolorizing dyes can generally be found in places exposed to dye waste. Besides bacteria, fungi can also be used as an excellent waste-decolorizing agent. Waste biodegradation processes generally utilize populations of microorganisms or other products. There are three kinds of biological waste treatment, namely aerobic, anaerobic, and facultative. The choice of treatment depends on the characteristics of the wastewater, the conditions and the purposes and objectives of the treatment. Although in principle, bacteria and fungi have the same working principle in decolorizing textile dyes, it is necessary to know in detail the specific steps carried out by bacteria and fungi in decolorizing a type of dye. Therefore, this review discusses the mechanism of decolorization and how the similarities and differences in the mechanisms carried out by bacteria and fungi are carried out so that their use can be carried out correctly on the intended dye targets.

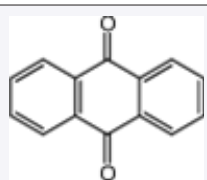
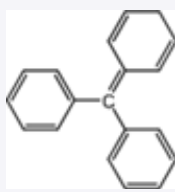
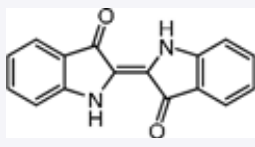
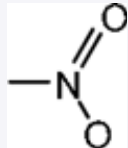

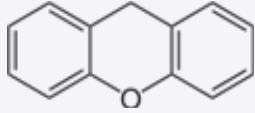
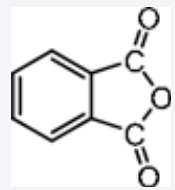
Textile Dye

Classification

Dyes that are often used in the textile industry can be classified according to their chromophore groups which can be seen in table 2. [Harus ditambah lagi diskusi disini].

Table 1: Classification of Types of Textile Dyes Used in the Textile Industry

Dye Type	Chromophore Example	Structure
Azo		Congo red

Anthraquinone		Remazol Brilliant Blue R
Trimethylmethane		Crystal violet
Indigo		Indigo blue
Nitro		Naphthol yellow 5
Nitroso		Mordant green 4
Xanthene		Fluorescein
Phthalein		Pigment blue 15:3

Toxicity

In general, dyes are not fully attached to the fibers of textile products during the dyeing process so that about 20-25% is discharged as waste into waters such as rivers, lakes and streams. This is very concerning because textile dyes have a complex chemical structure and are resistant to all conditions, making it difficult to degrade naturally in the environment. The negative impacts of the presence of dyes in waters include reducing dissolved oxygen levels, increasing chemical oxygen demand (COD), and biochemical oxygen demand (BOD). Waters containing dyes will get a little sunlight and reduce the solubility of the gas so that it will interfere photosynthetic activity.

Most dyes have toxic effects on living organisms, causing toxicity and carcinogenic and/or mutagenic effects on humans and other animals. Some benzidine dyes, such as direct black 38, direct blue 6 and direct brown 95, cause cancer in humans. Azo dyes are considered the most dangerous because the chemicals have a stable structure containing azoic linkages, aromatic and amino rings so that they are more stable to water, light, bleach, heat and detergents.

Treatment Type of Textile Dye Effluent

Physical Treatment

Physical processing of textile dye waste is a method that utilizes the principle of mass transportation. Several methods that are

often used include adsorption, coagulation/flocculation, ion exchange, and membrane-based filtration.

Adsorption

Adsorption is one of the most commonly used physical treatment methods for the decolorization of dyes which is influenced by several factors such as the surface area of the sorbent, particle size, dye-sorbent interaction, pH, and temperature. Some examples of economical adsorbents are activated carbon, silica gel, peat, wood chips, and agricultural lignocellulosic residues.

Coagulation

Coagulation is the process of aggregation or accumulation of colloidal particles to precipitate as precipitate. This method is inexpensive and energy efficient, but produces large volumes of concentrated sludge, is pH dependent, incompatible with soluble dyes (such as reactive, azo, acidic, and basic dyes), poses a risk of neurotoxicity, and is carcinogenic to humans.

Ion Exchange

Ion exchange is a procedure in which a strong bond is formed between the solute and the resin resulting in the separation and generation of high quality water. The disadvantages of this system are that it cannot be used for highly concentrated wastes that are easily soiled by the gel matrix, and the high sensitivity to waste [2].

Membrane-Based Filtration

Membrane filtration can separate dye compounds so they can be concentrated. This procedure uses small pores by principle membrane selective permeability. This method can be classified based on the pore size of the membrane, namely microfiltration, ultrafiltration, nanofiltration and reverse osmosis. The advantage of this technique is that it can be used for all types of dyes, high separation efficiency, safe, easy to use, resistant to temperature and microbial attack, and environmentally friendly. However, this technique is still not suitable for large-scale use due to high capital costs, concentrated dye discharge, the possibility of serious clogging, requires special equipment, and limited lifespan due to membrane fouling [3].

Chemical Treatment

Chemical treatment mechanisms can be used independently or in synergy with other processes. However, in the case of real wastewater, most of the systems are chemically ineffective at decomposing complex chemicals and so cannot be used for wastewater treatment handle large volumes of waste, requiring special equipment, and a lot of energy.

Ozonation

Ozonation is an AOP that allows various organic compounds present in untreated water to be oxidized, via the formation of hydroxyl radicals (OH). This method is classified as effective for double bonds of dye molecules, does not increase the sludge volume, can degrade recalcitrant dyes, but the time is short and its stability is affected by the presence of salt, pH, and temperature.

Chemical Oxidation

This method uses an oxidizing agent such as ozone (O₃) and hydrogen peroxide (H₂O₂) to form radicals that are highly

reactive at high pH values so as to break the double bond of the conjugated dye chromophore as well as other functional groups, such as aromatic ring dye complexes.

Advanced Oxidation Process (AOP)

The AOP method consists in the formation and subsequent reaction of hydroxyl radicals, which are very strong oxidizing species capable of reacting with organic complexes and inorganic compounds in textile wastewater that cannot be oxidized by traditional oxidizing agents. Common AOPs are photocatalytic oxidizers and are based on the Fenton reaction for total mineralization of reactive dyes or their transformation into simpler ones.

Biological Treatment

Biological waste treatment methods can generally be done by bioremediation. Bioremediation is a process in which biological organisms are used to remove or neutralize environmental pollutants through metabolic processes. The biological organisms in question include microscopic organisms, such as fungi, algae and bacteria. Some of the advantages of biological processing are environmentally friendly, low use of chemicals, low energy consumption, economy, and low sludge production. There is a lack of use of physical and chemical methods for wastewater treatment, hence biological treatment considered as the cheapest and safest alternative to remove dyes and pollutants that can harm the environment. One example of bioremediation related to the removal of dye waste is decolorization.

Decolorization

Decolorization is the act or process of removing color; The bleaching process uses biological agents such as bacteria, fungi or algae. Textile Dye Decolorizing Bacteria Bacteria that can be used as decolorizing agents for waste dyes can be obtained from various sources, including from the textile waste itself or indigenous bacteria. This is shown by the research that has been carried out in the form of bacterial isolation from textile wastewater, which obtained four superior bacterial isolates belonging to the *Bacillus* genus as shown in Figure 1.

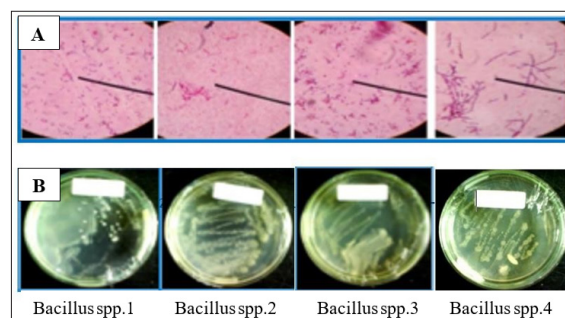


Figure 1: (A) Colony of *Bacillus* spp. (B) Microscopic of *Bacillus* spp.

Source : Putri from four bacterial isolates, the highest percentage value of Remazol Blue dye decolorization was found at a dye concentration of 100 mg/L using *Bacillus* spp1 bacteria. which reached 90.88%. The efficiency of decolorization at a dye concentration of 200 mg/L was lower than the effectiveness of decolorization at concentrations of 50 mg/L and 100 mg/L. Decolorization efficiency at a concentration. of 100 mg/L can be

seen in Figure 2. This is because the concentration of dyes that are too high will affect the ability of bacteria to grow and decolorize to reduce the levels of dyes. In addition, the bacteria *Bacillus* spp. the results of isolation from textile waste were able to reduce the COD content contained in Remazol Blue by 30.38-65.29%.

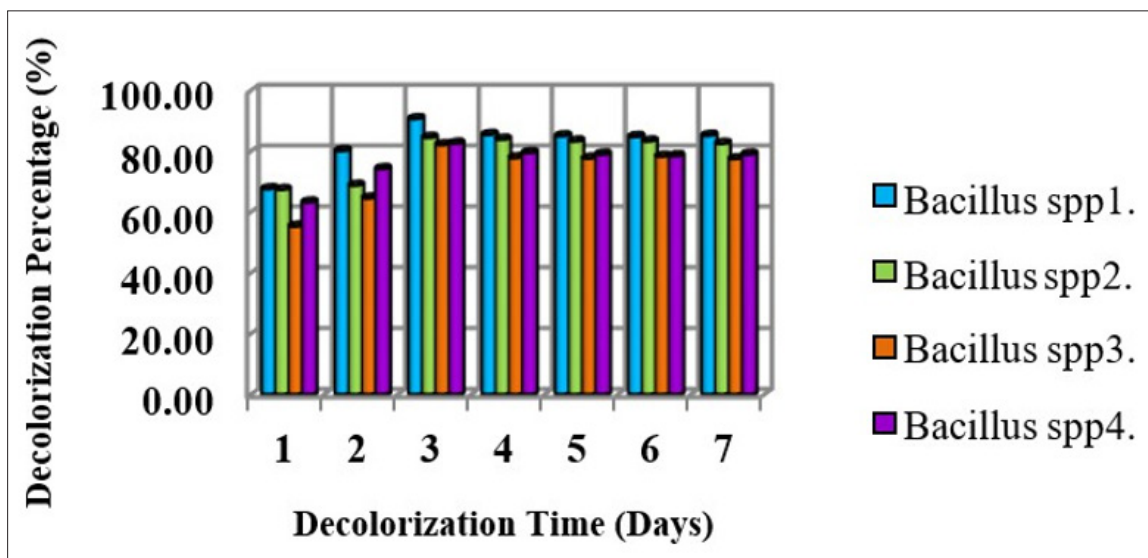


Figure 2: Decolorization Efficiency Isolates at a Concentration of 100 mg/L

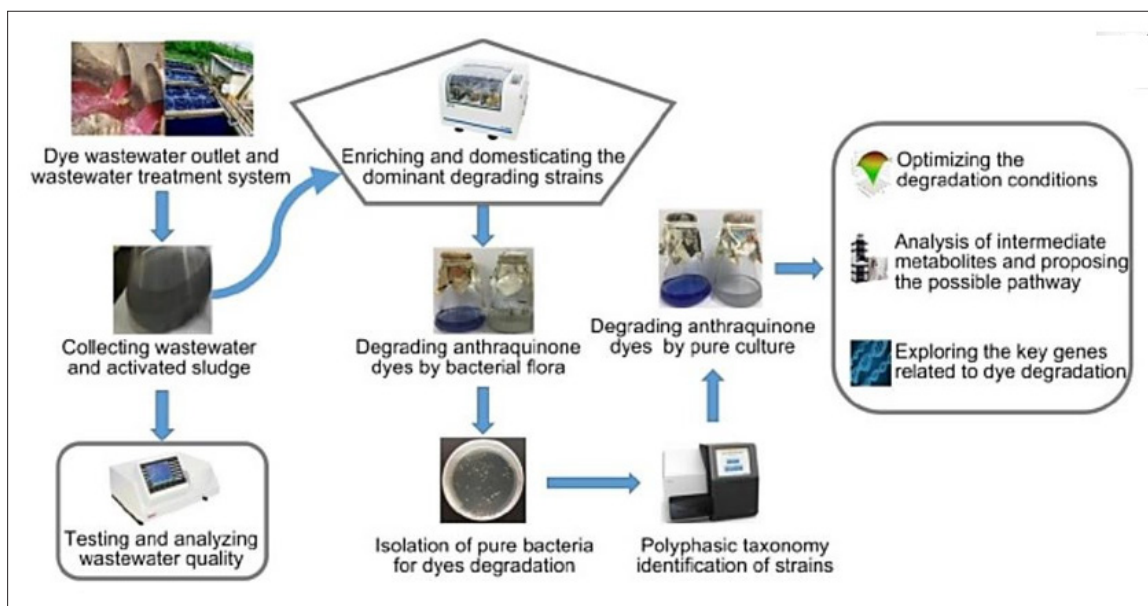


Figure 3: Procedure for isolating dye-degrading strains under laboratory conditions

Apart from bacteria from the genus *Bacillus*, there are other species of bacteria that can be used as decolorizing agents for waste dyes. A range of indigenous bacteria, including *Bacillus* sp., *Pseudomonas* sp., *Shewanella* sp., *Aeromonas* sp., *Rhodococcus* sp., and *Klebsiella* sp. (have been isolated to degrade dyes [4].

Some data on bacterial strains taken from various studies that have the potential as decolorizing agents for textile dyes can be seen in Table 2.

Table 2: Decolorization by Bacteria in Period 2017-2023

No	Bacteria Strain	Mechanism	Dye	Degradation Rate (%)	Time	Reference
1	<i>Streptomyces rimosus</i>	Biosorption	Methylene Blue	86	5 min	Dalei and Sri (2017)
2	<i>Pseudomonas cepacia</i> 13NA	Azoreductase	p-Aminoazobenzene	60-80	10 h	Dalei and Sri (2017)

3	<i>Pseudomonas luteola</i>	Azoreductase	Red G	37.4	2 d	Dalei and Sri (2017)
		Azoreductase	RBB	93.2	2 d	Dalei and Sri (2017)
4	<i>Corynebacterium glutamicum</i>	Biosorption	Reactive Black 5	94	12 h	Dalei and Sri (2017)
5	<i>Streptomyces</i> BW130	Adsorption	Azo-reactive Red 147	29	14 d	Dalei and Sri (2017)
		Adsorption	Azo-copper Red 171	73	14 d	Dalei and Sri (2017)
6	Bacterial consortium (mixture of <i>Ochrobactrium</i> sp., <i>Salmonella enterica</i> , and <i>Pseudomonas aeruginosa</i>)	Bioaccumulation	Reactive Black-B	99	2-4 d	Dalei and Sri (2017)
7	<i>Bacillus subtilis</i> IFO 3002	Azoreductase	p-aminoazobenzene	80-90	30 h	Dalei and Sri (2017)
8	<i>Pseudomonas putida</i> (NCIMB 9776)	18% Biosorption	Tectilon Blue (TB4R)	50	24 h	Dalei and Sri (2017)
9	Mixed anaerobic culture	Azoreductase	Diazo-linked chromophores	85	2 d	Dalei and Sri (2017)
10	<i>Bacillus</i> . sp. VITAKB20	Biodegradation	Reactive Orange 16	97.57	48 h	Pandey et al. (2019)
11	<i>Lysinibacillus</i> sp. KPB6	Biodegradation	Reactive Blue 250	94.03	48 h	Pandey et al. (2019)
12	<i>Salinivibrio kushneri</i>	Azoreductase	Congo red	90	48 h	John et al. (2020)
13	<i>Staphylococcus aureus</i>		BY HP-2R	79	5 d	Aktar et al. (2019)
14	<i>Bacillus cereus</i>	Biodegradation	Orange 3R	91.69	-	Khaled et al. (2022)
15	<i>Pseudomonas parafulva</i>	Biodegradation	Orange 3R	89.21	-	Khaled et al. (2022)
16	<i>Klebsiella pneumoniae</i> MW815592	Biotransformation	Reactive red 195, reactive blue 214, and reactive yellow 145	-	42 h	Sennaj et al. (2023)

Textile Dye Decolorizing Fungal

Many fungi can be used as decolorizing agents, but wood decay fungi are widely recognized as the most effective agents. The wood decay fungi group consists of white rot fungi (WRF), soft rot fungi (SRF), and brown rot fungi (BRF). However, of the three groups of fungi, the most effective are the white rot fungi. White rot fungi are a group of fungi that degrade cellulose and lignin, and brown rot fungi are fungi that are only able to break down cellulose and do not degrade lignin. White rot and brown rot are the two major groups of rot, while softening fungi only damage the surface of the wood.

White Rot Fungi

White rot fungi are the most efficient ligninolytic organisms described to date. Owing to the extracellular nonspecific and nonstereoselective enzyme system in white rot fungi, the ability to degrade lignin is more efficient. White rots break down lignin and cellulose, and commonly cause rotted wood to feel moist, soft,

spongy, or stringy and appear white or yellow. Mycelia colonize much of the woody tissues. White rots usually form in flowering trees (angiosperms) and less often in conifers (gymnosperms). Fungi that cause white rots also cause the production of zone lines in wood, sometimes called splatted wood. This Partially rotted wood is sometimes desirable for woodworking [5].

Soft Rot Fungi

Soft rots are caused by both bacteria and fungi. These organisms break down cellulose, hemicellulose, and lignin, but only in areas directly adjacent to their growth. Soft rot organisms grow slower than brown or white rot organisms, and therefore damage occurs to the host tree more gradually. Given enough time, however, any rot can cause extensive structural damage.

Brown Rot Fungi

Brown rots primarily decay the cellulose and hemicellulose (carbohydrates) in wood, leaving behind the brownish lignin.

Wood affected by brown rot usually is dry, fragile, and readily crumbles into cubes because of longitudinal and transverse cracks occurring which follow cellular lines, or across cells, respectively. The decay commonly forms columns of rot in wood. Brown rots generally occur in conifers as heart rots. Hardwood trees are more resistant to decay by brown rot than to white rot fungi. Some data on fungal strains taken from various studies that have the potential to be decolorizing agents for textile dyes can be seen in Table 3.

Table 3: Decolorization by Fungal in Period 2017-2023

No	Fungal Strain	Mechanism	Dye	Degradation Rate (%)	Reference
1	Aspergillus niger	Biosorption	Acid Blue 161 Procion Red MX 2 5B	50-60 30-40	Almeida and Corso (2019)
		Biodegradation	Congo red	97	Asses et al. (2018)
2	Achaetomium strumarium	Biosorption and biodegradation	Acid red 88	99	Bankole et al. (2018a)
3	Peyronellaea prosopidis	Biosorption	Scarlet RR	90	Bankole et al. (2018b)
		Biodegradation	Dye mixture (Congo Red, Red RBL, Rubine GFL, Scarlet RR and Scarlet GDR)	84	Bankole et al. (2018b)
4	Consortium containing Daldinia concentrica and Xylaria polymorpha	Biodegradation	Xylidine Ponceau 2R	100	Bankole et al. (2018c)
5	Corioloropsis sp	Biodegradation	Cotton blue and crystal violet	85	Muck et al. (2018)
6	Cylindrocephalum aurelium	Biodegradation and Biotransformation	Mordant Orange-1	86	Mostafa et al. (2019)
7	Trametes villosa	Biodegradation and biosorption	Acid Red 357 and Acid Orange 142	90	Ortiz- Monsalve et al. (2019)
8	Penicillium megasporum	Biosorption and Biodegradation	Congo red Orange G Rhodamine B	89.4 76.2 54	Agrawal et al. (2021)
9	Ceriporia lacerata BRB 81	Biodegradation	AB129	91.4	Nurhayat et al. (2022)
10	Leiotrametes menziesii BRB 73	Biodegradation	AB129	55.7	Nurhayat et al. (2022)
11	Phellinus noxius BRB 11	Biodegradation	Remazol Brilliant Blue R	60	Nurhayat et al. (2022)
12	Ganoderma lucidum	Biodegradation	DR5B	95.16	Sun et al. (2023)

Decolorization Mechanism by Bacterial and Fungal

Bacterial

Biosorption

Biosorption is one of the decolorization mechanisms used by several microorganisms including bacteria. The biosorption ability is attributed to the heteropolysaccharide and lipid components of the cell wall which contain a variety of functional groups, including amino, carboxyl, hydroxyl, phosphate, and other charged groups that cause strong attractive forces between the dye and the cell wall. Biosorption can be carried out by living cells or dead cells, but dead cells are more advantageous than living cells because they do not require nutrition, can be restored using organic solvents or surfactants, can be stored so they can be used for a long time. and recoverable.

Enzymatic Degradation

Enzymes are biological catalysts whose applications can be microorganisms or enzymes. Enzymes can be utilized in various

areas of life that are environmentally friendly, for example in bioremediation through the process of biodegradation of pollutant and toxic compounds such as textile dyes. Some of the enzymes secreted by bacteria in the decolorization process are as follows.

Azo Reductase

Azo reductases are enzymes that catalyze reactions that occur in the presence of reducing equivalents such as FADH and NADH. Azo dyes have a sulfonate substituent group with high molecular weight and impossible to pass through the cell membrane. This mechanism involves electron transport so bacteria must form a link with the intracellular electron transport system and high molecular weight azo dyes. The electron transport component is localized on the outside bacterial cell membrane (in the case of gram-negative bacteria) can come into direct contact with azo dyes substrates or redox mediators on the cell surface for build links like that.

Peroxidase

Peroxidases are hemoproteins that catalyze reactions in the presence of hydrogen peroxide. Lignin and manganese peroxidase (MnP) share a common reaction mechanism that starts with the oxidation of enzymes by H₂O₂ to an oxidized state during their catalytic cycle.

Laccase

Laccase is a multicopper phenol oxidase decolorization of azo dyes via a very nonspecific free radical mechanism forms phenolic compounds, thus avoid the formation of toxic aromatic amines.

Fungal

For the mechanism, there are two main processes for decolorization of dyes using fungi, which are biosorption and biodegradation.

Biosorption

Biosorption is the main dye removal process in basidiomycetes wood decay. In the process, the fungal biomass gradually becomes colorless after bioadsorption staining. Bioadsorption occurs by electrostatic attraction between the negatively charged dye and the positively charged cell wall components. Fungi biomass in decolorization plays a role in the biosorption of dye molecules. The biosorption process can be carried out by living or dead fungal cells. For live biomass, cell growth and dye decolorization were synchronous over the entire incubation period.

A comparison of the decolorization levels between the two types of biomass revealed that the decolorization of dyes by living biomass was two times greater than that of inactive biomass.

mechanism for dye removal is biosorption which is essentially specialized for dead cells. In this mechanism, physicochemical interactions such as adsorption, deposition, and ion exchange is responsible for elimination. However, use of dead cells was generally associated with their lower removal capacity compared to live cells as validated by investigations of Congo red decolorization by *Phanerochaete chrysosporium*, performing 90% and 70% removal for live and dead cells, respectively. Azo dyes have a complex.

Biodegradation

Biodegradation is generally effectively carried out by living fungal cells because living cells can produce secondary metabolites in the form of lignin converting enzymes, laccase, manganese peroxidase, and lignin peroxidase. The role of laccase, manganese peroxidase, and lignin peroxidase for dye decolorization may be different for each fungus. For example, the lignin peroxidase secreted by *Phanerochaete chrysosporium* is structure and synthetic origin, consequently, this makes it quite difficult to decolorize. Mordant Orange-1 (MO-1) is a well-characterized azo dye in the presence of at least one bearing azo bond (–N=N–). aromatic ring and has high photolytic stability and resistance to major oxidizing agents to avoid dye degradation. responsible for the decolorization of azo dyes [6].

In the decolorization process, fungi that can produce ligninolytic enzymes can produce the enzymes Laccase (Lac), Lignin Peroxidase (LiP), Manganese Peroxidase (MnP), and Dye-Decolorizing Peroxidase (DyP), but the three main enzymes are Lac, LiP, and MnP. The following differences from enzymes produced by fungi are shown in table 4 below.

E.C.	LiP 1.11.1.14	MnP 1.11.1.13	Lac 1.10.3.2	DyP 1.11.1.19
Structure	Monomer, glycoprotein up to 15	Monomer, glycoprotein	Mono-, di-, or tetramer, glycoprotein	Dimer
Prosthetic group	Heme	Heme	Four Cu atoms per active protein unit	Heme
Glycosylation	N–	N–	N–	N-Acetyl-glucosamine and mannose
Isoforms	Monomers; up to 15	Monomers; up to 11	Mono-, di-, tetramers; several	Dimeric $\alpha + \beta$ barrel structure
pH Range	2.0–5.0	2–6	2.0–8.5	3.2 (Optimum)
C–C Cleavage	Often	Often	No	Yes
H ₂ O ₂ Regulated	Yes	Yes	No	Yes
Stability	Low	Immense	Immense	Highly
Natural mediators	Unknown mediators	Mn ²⁺ ; Mn ³⁺	3-Hydroxy-anthranilic acid	Mn ²⁺
Specificity	Broad, aromatics, incl. nonphenolics	Mn ²⁺	Broad, phenolics	Non-phenolics, phenolics, veratryl alcohol
Catalytic center	Fe-protoporphyrin	Fe-protoporphyrin	Four copper atoms	Fe-protoporphyrin
Mediators	NO	Thiols, unsaturated fatty acids, organic acids as chelators, Mn ³⁺	Hydroxybenzotriazole (HBT), ABTS, syringaldazine, 3-HAA, RBB	Chelated Mn ³⁺
Cofactor	H ₂ O ₂	H ₂ O ₂	O ₂ [–]	H ₂ O ₂
Substrate	Halogenated phenolic compounds, polycyclic aromatic compounds	Lignin and other phenolic compounds	Ortho- and para-diphenols, aminophenols, polyphenols, polyamines, lignins, aryl diamines	Phenols, hydroquinones, dyes, amines, aromatic alcohols, xenobiotics

Conclusion

Biological processing of textile dye waste can be carried out using bioremediation techniques, namely decolorization. Bacteria and fungi are two biological agents that can be used effectively to remove dyes with various structures. The decolorization mechanisms of bacteria and fungi are broadly the same, namely biosorption and biodegradation [7-28].

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