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Eco-Friendly Dye Removal: Impact of Dyes on Aquatic and Human Health and Sustainable Fungal Treatment Approaches

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ABSTRACT

Water is an absolute need for all forms of life and human progress. However, the rapid industrialization and increasing global demand for textile products have led to a substantial rise in wastewater discharge from the textile industry, primarily due to dye production and application operations. Without adequate treatment, this effluent has the potential to become toxic, endangering the environment and all forms of life. Dyeing textiles have countless negative effects on aquatic environments, including altering their visual appeal, increasing biological and chemical oxygen demands (BOD and COD) levels, inhibiting photosynthesis and plant growth, and contributing to bioaccumulation and resistance. Traditional wastewater treatment methods, such as membrane separation, electrochemical processes, coagulation, adsorption, and activated sludge, have notable limitations, including high costs and inefficiencies. Developing an effective and sustainable technique for treating dyed wastewater and removing color is essential to address this issue. This review highlights the potential of microorganisms, particularly fungi, as innovative sustainable tools for treating dye-contaminated wastewater. Fungi offer multiple advantages, including low operational costs, environmental compatibility, and versatility through biosorption, bioaccumulation, and biodegradation. It also emphasizes the potential of hybrid and integrated treatment approaches to overcome the limitations of standalone methods. The findings underline the promising role of fungal systems as a sustainable and scalable solution for mitigating the environmental impact of textile dye pollution and preserving water resources.

INTRODUCTION

The incorrect disposal of wastewater from textile companies is one of the world's most pressing problems today. The textile industry is a major contributor to environmental deterioration and worldwide economic decline in several nations **(Olisah *et al.*, 2021)**. A significant environmental polluter that also affects human health is dye-containing wastewater, which is generated by the textile sector in large quantities and includes persistent contaminants **(Almroth *et al.*, 2021; Ali *et al.*, 2022)**. Approximately 7,107 tons of synthetic dyes are produced worldwide every year; more than 10,000 tons of these hues are used by the textile sector **(Chandanshive *et al.*, 2020)**.

Wastewater from the textile industry includes many kinds of harmful dyes, aromatic compounds, and heavy metals that are essential for making the dye-color pigments used in textiles. Color pigments for textile dyes are made using heavy metals including lead, chromium, arsenic, and cadmium **(Singha *et al.*, 2021)**. The harmful substances are carried in wastewater for long distances, and they stay in the water and soil for long periods. They endanger all kinds of life, reduce soil fertility, inhibit photosynthetic activity in aquatic plants, cause anoxic conditions for aquatic plants and animals, enter the food chain and cause resistance and bioaccumulation, and potentially increase toxicity, mutagenicity, and carcinogenicity **(Dutta & Bhattacharjee, 2022; Patil *et al.*, 2022)**. Large amounts of wastewater with high concentrations of dissolved solids, organics, metals, salts, and persistent colors are produced due to the fabric production process's heavy water demand **(Ismail & Sakai, 2021)**. Synthetic dyes are highly soluble and persistent in water, rendering traditional treatment methods ineffective **(Shindhal *et al.*, 2021)**. This calls for the implementation of innovative sustainable strategies to tackle secondary pollution and inefficient removal of organic loads following discoloration **(Samsami *et al.*, 2020)**. Consequently, before dye-containing wastewater is disposed of into the environment, it must be adequately treated using cost-effective and ecologically friendly treatment technologies. The use of microorganisms to break down and digest harmful substances to fewer toxic ones offers an environmentally safe and sustainable extensive technology, with design flexibility as well as simple operating conditions **(Ghosh *et al.*, 2017)**. Fungal species are regarded as a promising tool among microorganisms due to their adaptability to harsh environmental conditions, low cost of cultivation and upkeep, and lack of need for costly or specialized equipment. The study aimed to provide a thorough analysis of the detrimental impacts of textile wastewater, particularly dyes, on ecosystems and living creatures. It also focused on the use of fungi as innovative sustainable technology to manage dyes containing wastewater effluents as well as the techniques for immobilizing fungal biomass or their enzymes to maximize dye decolorization.

1. Dyes, uses, and their classification

According to **Ahmad *et al*. (2015)**, textiles may be colored by attaching organic chemicals called dyes to their surfaces. The molecules of dye compounds have three significant groups: matrix, auxochrome, and chromophore. The chromophore, on the other hand, is responsible for providing color **(Benkhaya *et al*. 2020)**, as shown in Fig. (1). An auxochrome can be added to the chromophore to make it more water-soluble and to increase its affinity for attaching to fibers. According to **Hunger (2003)**, the remaining atoms in the molecule make up the matrix.

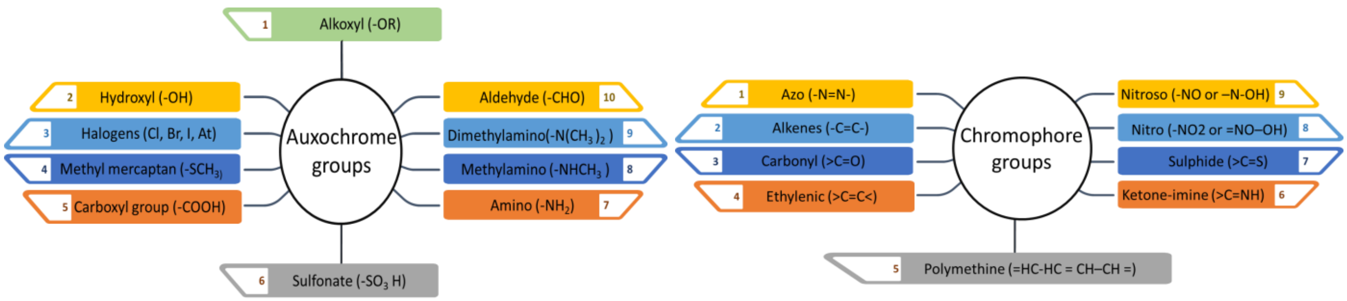

Fig. 1. Auxochrome and chromophore groups of textile dyes

Textile fibres, paper, food, tannery, leather, and medicinal items are just a few of the many objects that may be colored with dyes. Fig. (2) displays a variety of synthetic colors and their uses. The food and beverage, printing, textile, and pharmaceutical sectors are the primary consumers of these dyes. Most of the dyes that end up in the environment come from the textile sector. Chemical dyes such as azo, dispersion, reactive, mordant, acid, basic, and sulfide are often used in the textile industry. According to **Silva *et al.* (2021)**, the textile business makes use of a wide variety of fibers, including wool, cotton, silk, polyester, polyamide, acrylic, and many more. In addition, finishing agents, whitening, de-sizing, softening, and sizing are only a few of the steps in the textile industry's process that include the usage of several very dangerous chemicals and dyes **(Kishor *et al*., 2021)**.

Basic chemistry, material source, industrial use, nuclear structure, chromophores' origin, color index, and specific techniques (such as strong bonds or physical pressures) are among the many variables bonding that might affect the dye's ordering. According to **Holkar *et al.* (2016)** and **Akpomie and Conradie (2020)**, dyes are often classified into several classes based on their origin, structure, and use. Dyes may be classified as either synthetic or natural, depending on where they come from. Natural colors might come from plants like berries, wood, fungi, lichens, bark, minerals, or animals. Synthetic dyes are manufactured by humans from chemicals, petroleum byproducts, and minerals found on Earth **(Ben Slama *et al*., 2021)**. Chemical structure, on the other hand, determines the classification of synthetic colors such aryl methane, indigo, azo, phthalocyanine, and heterocyclic dyes. Dye types are classified as cationic, anionic, or non-ionic according to their solubility in solutions with different particle charges. Acid, basic, direct, mordant, metal complex, and reactive dyes are examples of soluble dyes, whereas azoic, sulfur, vat, and dispersion dyes are examples of insoluble dyes **(Yagub *et al*., 2014)**. Nevertheless, as shown in Figs. (2, 3), the chemical structures, application procedures, and sources of dyes are the main factors that define their classification.

Fig. 2. Various types of dyes and their applications **(Ahmad *et al.*, 2015)**

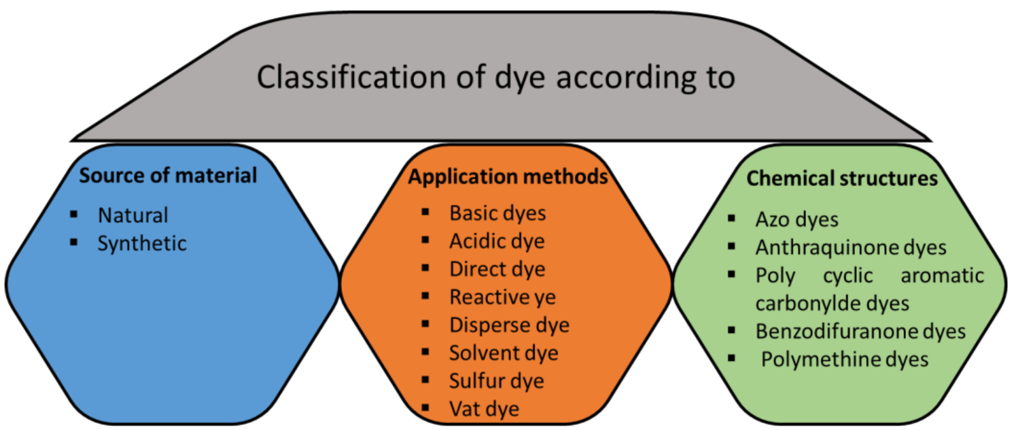

Fig. 3. Classification of dye according to their application methods, chemical structures, and source

2. Environmental impacts of dyes

The dyeing, printing, and finishing industries contribute to significant environmental impacts including the following pollution:

i. According to **Hasanbeigi and Price (2015)**, gaseous emissions from wastewater treatment facilities, dyeing processes, resin finishing, and fabric preparation and dyeing are the second most important sources of air pollution.

ii. Excessive dye consumption causes subsurface leakage and inadvertent release of harmful substances, which in turn causes soil contamination. In addition, there will be a decrease in plant growth rate, erosion, productivity, and soil quality **(Lellis *et al.* 2019)**.

iii. Another problem is water pollution; yearly textile manufacturing uses 94 billion cubic meters of water, or around 4% of the world's freshwater supply **(BBC News, 2021)**. Soil, underground water, pools, and plants are negatively impacted by the raw effluents released into nearby rivers during the wet processing of textiles. Dyeing textiles increases toxicity, mutagenicity, carcinogenicity, and oxygen consumption; it also limits plant growth, impedes photosynthesis, and reaches the food chain **(Lellis *et al.*, 2019)**.

3. Sources of dyes to the aquatic ecosystem

Synthetic organic dyes are manufactured all over the world and used extensively, which is a major problem for the environment. Dye chemicals are released into aquatic environments by industries including paper, tannery, and textile, which use a lot of water and release wastewater **(Carmen & Daniel, 2012; Pereira & Alves, 2012)**, as shown in Fig. (4). Dyeing operations produce wastewater with different dye compositions based on cloth type and dye class. The textile sector releases 200 billion liters of colored effluents yearly, accounting for about half of all industrial effluents containing dyes **(Carmen & Daniel, 2012; Kant, 2012)**. The disposal of unwanted or expired prescriptions containing colorants, the use of chemicals around the home, and hair coloring are all sources of wastewater that may introduce synthetic organic colors into the environment **(Eriksson *et al.*, 2003)**. Sewage treatment plants (STPs) handle both municipal and commercial wastewater **(Zhou, 2001)**.

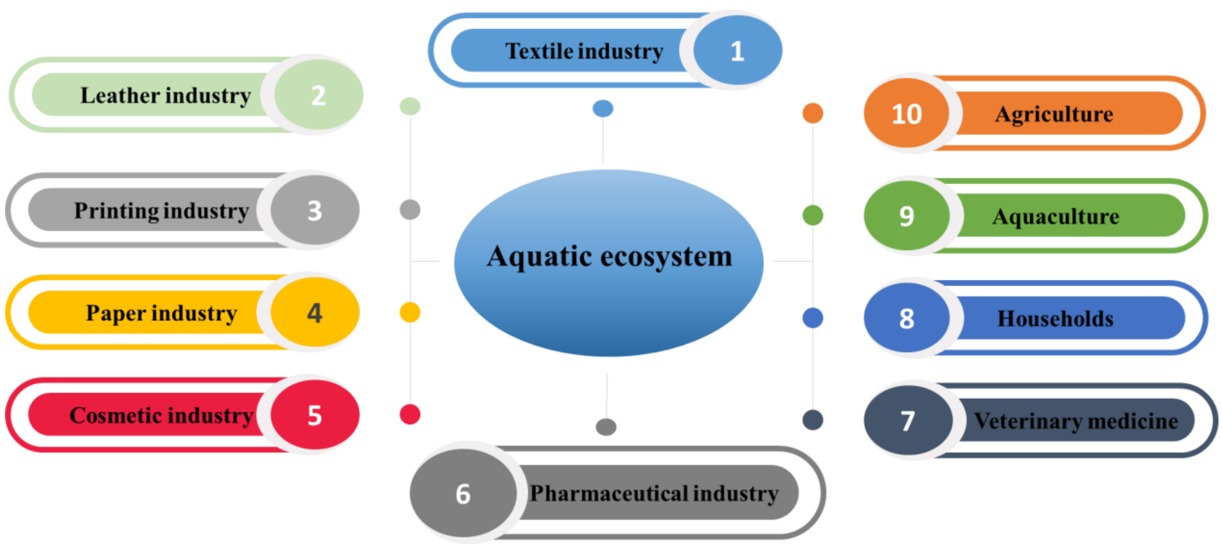

Fig. 4. Sources of dyes to aquatic ecosystems

4. Impact of dye on the aquatic environment and human health

The use of synthetic organic dyes is a major problem for aquatic environments. A loss of biodiversity in aquatic habitats is a direct result of the discharge of dyes into these environments. One further obvious natural problem with dye is that it may absorb and reflect light in water. According to **Liang *et al*. (2017)**, it blocks light from reaching the aquatic environment's photic zone. Changes to aquatic ecosystems and reduced photosynthesis relative to aquatic vegetation are two major ecological outcomes **(Khan *et al*., 2015)**. **Ghaedi *et al*. (2015)** found that when textile dyes are present in water in high quantities, they reduce oxygen levels, block sunlight, and hinder the biological activity of aquatic flora and wildlife. In addition, 60–70% of azo dyes are toxic, carcinogenic, and resistant to traditional treatment methods since they do not biodegrade and do not undergo typical physicochemical degradation **(Rawat *et al*., 2011)**. The long-term dangers caused by the unchecked release of mineral elements caused eutrophication, which in turn hindered photosynthesis in marine plants.

Dyes may cause metabolic changes and possibly carcinogenic aromatic amines; fish are very susceptible to these chemicals and are used as bioindicators for water pollution **(El-Naggar *et al.*, 2024; Gouda & Taha 2024)**. When it comes to biochemical, physiological, and histological factors, textile effluents are bad for fish biota. Physical factors such as color, turbidity, temperature, and total solids may be changed by the buildup of hazardous pollutants and textile effluent. This, in turn, can affect the food chain, restrict the availability of light, and induce permanent structural abnormalities in the DNA of fish **(Athira & Jaya, 2018)**. Dye toxicity may alter DNA structures irretrievably, even at toxin concentrations normally deemed safe for fish to survive **(Parmar *et al*., 2016)**.

Research conducted by **Soni *et al*. (2006)** examined the effects of treated and untreated textile industry effluent on freshwater fish *Gambusia affinis*. The results showed that the treated water had a lower death rate and less cytotoxic effects on blood cells. The presence of sixteen different dye residues found in textile industry products makes it clear that edible fish are no longer fit for human consumption if exposed to these harmful colors. **Amer *et al*. (2022)** and **Sharma *et al*. (2022)** found that symptoms such as cramping, fever, and hypertension may be caused by consuming contaminated fish and aquatic creatures. Dye and textile pigment contamination of wastewater may lead to a wide range of pH levels, particles in suspension, high biological oxygen demand (BOD), chemical oxygen demand (COD), total organic carbon (TOC), and a plethora of colors. When these particles in the water block the fish's gills, they can't exchange gases as easily, which might slow their development rate or possibly kill them. Adult fish may have genotoxic effects when exposed to reactive azo dyes, which can increase the creation of erythrocytic micronuclei and the generation of gill micronuclei in fingerlings. The hypoxia detrimental effects on human immunological responses and physiological processes make contaminated fish a major threat to human health **(Zheng *et al*., 2021)**, as depicted in Fig. (5).

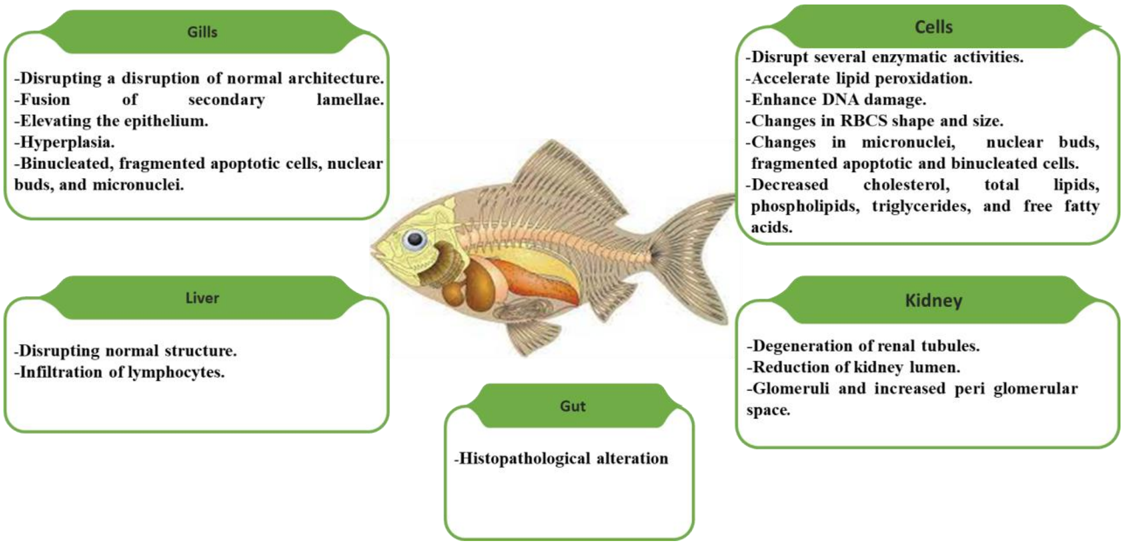

Fig. 5. Negative impact of dyes on fish

According to **Khattab *et al*. (2018)**, textile finishing effluents may cause under-oxygenation, bad taste, bacterial proliferation, pestilential smells, and wrong coloring since they discharge organic materials into aquatic ecosystems, which include dyes and pigments. **Cardoso *et al*. (2012)** found that these compounds may block light transmission, which means that aquatic plants won't be able to undergo photosynthesis. The massive amounts of textile dyes dumped into rivers, lakes, oceans, and seas pose a threat to microalgae, which play an essential role in aquatic ecosystems as producers. Dye pollution, on the other hand, may impede microalgae development and energy transfer. Pigment, protein, and other nutrients are among the growth characteristics of algae that are impacted by this pollution.

Methylene Blue and other textile dyes may release aromatic amines, some of which have cancer-causing properties. According to **Ali and Saleh (2012)**, when *Spirulina platensis* and *Chlorella vulgaris* were exposed to methylene blue, their growth rate, protein content, and pigment content were decreased in a concentration-dependent manner. Additionally, chlorophyll synthesis was reduced, which had an impact on their development and photosynthetic rate. According to **Sharma *et al*. (2021)**, toxicological studies often use algae as indications of contamination. To determine if textile dyes are phytotoxic, aquatic macrophytes are being employed as an ecological indicator. The growth rate and inhibition of aquatic macrophytes are greatly affected by these dyes **(Lobiuc *et al*., 2018; Hocini *et al*., 2019; Adomas *et al*., 2020; Singh *et al*., 2021)**. **Sree *et al*. (2015)** found that this is often caused by oxidative stress or a shift in the plant's photosystem as a result of reduced electron transport in the chloroplasts. One such organism that has been the subject of research is *Lemna minor*, an essential link in the food chain. According to **Adomas *et al.* (2020)**, the growth, biomass production, and chlorophyll biosynthesis of *L. minor* are all inhibited by the dye Gentian Violet, with Congo Red being the less hazardous of the two. The phytotoxic effects of these colors may be sensitively assessed by measuring decarboxylase activity and biogenic amine concentrations **(Adomas *et al*., 2020)**.

Textile dyes are highly carcinogenic and have been linked to several diseases in people and animals **(Tounsadi *et al*., 2020; Jin *et al*., 2021)**. Dermatitis and problems affecting the central nervous system are among the many ailments linked to textile dyes. One possible explanation for these problems is the inactivation of enzymes caused by their cofactors being replaced **(Wu *et al*., 2021)**. Ingestion or inhalation of textile dyes, especially when they are dusty, may irritate the skin and eyes **(Clark, 2011)**. Reactive dye workers risk developing various allergic responses, including rhinitis, allergic conjunctivitis, contact dermatitis, and occupational asthma **(Hunger, 2003)**, as shown in Fig. (6).

It has been suggested that some chemicals used in textile production could affect spermatogenesis and ovulation **(Suryavathi *et al*., 2005)**. Benzidine and its derivatives are the major sources of azole dyes, which find widespread use in the paper, textile, and leather industries. Human bladder cancer has been associated with its toxicity, according to a comprehensive review **(Tounsadi *et al*., 2020)**. Animals' gut flora convert azo dyes into their parent amines, which the stomach readily absorbs, hence it's no surprise that these colors end up in animal and human urine **(Amin *et al*., 2016)**. Dyeing textiles with Reactive Green 19, Disperse Red 1, and Reactive Blue 2 may have long-term genotoxic consequences on human health. Unlike Reactive Blue 2 and Disperse Red 1, which are not genotoxic, **Leme *et al*. (2015)** found that Reactive Green 19 is genotoxic in a dose-dependent way. **Hossain *et al*. (2018)** discovered that freshwater flora, such as fish and algae, may collect dyes in their food chains, a consequence of the pervasive usage of these colors in the environment. Compared to when these chemicals were first put into water, reports show that individuals currently have organic chemical levels in their systems that are one thousand times more harmful **(Korpi *et al*., 2009)**.

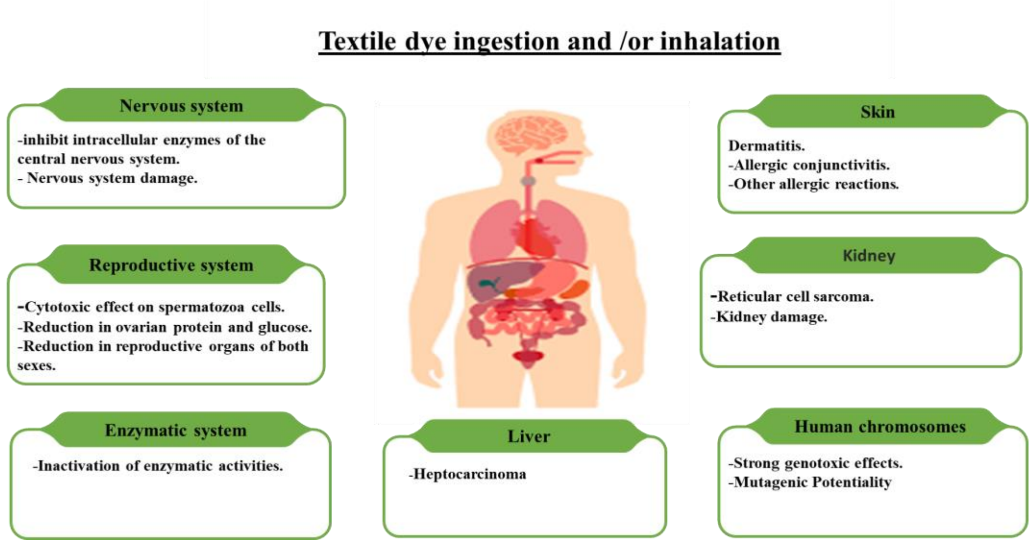

Fig. 6. Negative impact of dyes on human health

5. Treatment technology of dyes removal

Dye removal procedures are essential for preventing the contamination of water sources and ecosystems caused by wastewater. Some examples of these methods include physical and, chemical approaches, based on nanoparticles, and biological procedures.

5.1. Physical treatments

They remove different colors from wastewater by using physical forces. Adsorption, sedimentation, filtration, coagulation-flocculation, reverse osmosis, ultra-filtration, and nano-filtration are all part of this category **(Ledakowicz & Pazdzior, 2021)**.

5.1.1. Filtration technology

It encompasses microfiltration, ultrafiltration, nanofiltration, and reverse osmosis, and is a crucial physical treatment approach for wastewater purification **(Marmagne & Coste, 1996; Gunatilake, 2015)**. On the other hand, nanofiltration and ultrafiltration are great for removing various colors, but microfiltration isn't a good fit for treating wastewater because of its big pore size **(Chidambaram *et al*., 2015)**.

5.1.2. Reverse osmosis

In terms of salt removal efficiency, reverse osmosis is superior; nevertheless, it is not without its drawbacks, including a need for high pressure, a lot of energy, and a short lifespan **(Perera, 2019)**.

5.1.3. Adsorption

Another technique, adsorption, adheres dissolved molecules to the surface of an adsorbent material (such as activated carbon 86) by use of physical and chemical forces.

5.2. Chemical treatments

The removal of dyes from wastewater is a critical environmental concern, prompting the development of various chemical treatment methods. Each technique exhibits unique advantages and limitations, influencing their applicability in industrial wastewater treatment.

Table 1. Chemical dye removal methods **(Joshi *et al*., 2003; Forgacs *et al*., 2004; Gupta & Suhas, 2009; Salleh *et al*., 2011; Zhou *et al*., 2018)**

Method	Description	Advantages	Disadvantages
Advanced oxidation process	Multiple oxidation processes occur simultaneously to degrade dye particles.	Eliminates toxic materials, effective under unusual conditions and efficient dye removal.	Expensive, inflexible, produces undesirable by-products and pH-dependent.
Electrochemical destruction	Electro-coagulation or non-soluble anodes degrade dye molecules.	No chemical consumption, no sludge accumulation, and effective for soluble and insoluble dyes.	Generates hazardous by-products, high electricity costs and less effective in high-flow conditions.
Fenton reaction	Fenton’s reagent (catalyst + hydrogen peroxide) removes dye particles.	Effective for soluble and insoluble dyes, eliminates toxins and suitable for solid-content wastewater.	Ineffective for disperse and vat dyes, high iron sludge generation, long reaction time and only functions at low pH.
Oxidation	Oxidizing agents break down complex dye molecules into carbon dioxide and water.	Completely degrades dyes; commonly used, short reaction time and straightforward	Costly, activation of hydrogen peroxide is challenging, pH-dependent and requires catalysts.

		application.	
Ozonation	Ozone, generated from oxygen and is used to degrade dye particles.	Gaseous application, does not increase wastewater volume, effective, no sludge formation and quick reaction.	Short half-life (20 min), high operational costs, produces toxic by-products and unstable.
Photochemical method	Fenton reaction combined with ultraviolet (UV) light for dye removal.	Highly effective and no sludge or foul odor production.	Expensive and generates numerous by-products.
Ultraviolet irradiation	UV light decomposes dye particles in wastewater.	No sludge formation, weakens foul odors and does not require hazardous chemicals.	Energy-intensive, high cost and limited treatment efficiency over time.

In summary, chemical dye removal methods vary significantly in their efficiency, cost, and environmental impact. While some methods offer rapid and effective dye degradation, their feasibility is often hindered by high costs, pH dependency, and by-product formation. Future research should focus on searching for other methods to enhance sustainability and efficiency in wastewater treatment.

5.3. Biological decolorization treatments

Looking at the systemic law of nature, comprehensive investigations and development efforts have concentrated on biological sustainable techniques as a secure and clean substitute for dye decolorization **(Kaushik & Malik, 2009)**. Various microorganisms (bacteria, fungi, or yeasts) as well as plant and animal parts are used in these techniques. The removal of dye molecules from a wide variety of microorganisms, especially fungi, has been studied. Fungi are the most studied due to their mass production, which makes it easier than with other microorganisms **(Karthik *et al*., 2016; Abbasi, 2018)**.

According to **Vargas-Gastélum and Riquelme (2020),** fungi can be found in almost every kind of habitat, from tropical rainforests to freshwater, marine, desert, and deep-sea sediment settings. Based on their unique biochemical, physiological, and metabolic profiles, different species of fungi can break down and digest a wide variety of potentially harmful or long-lasting compounds. A wide range of organic contaminants may be decomposed spontaneously by fungi due to their decomposer activity **(Routoula**

& Patwardhan, 2020). Fungal organisms, both living and dead, may be used to extract harmful contaminants from polluted environments, either *in situ* or *ex-situ* **(Gouda & Taha, 2023; Taha *et al*., 2023a)**.

Several fungal species have been employed as a feasible choice for the removal of various dyes belonging to the following genera: *Rhizopus* **(Gül, 2013)**; various types of white-rot fungi **(Gnanadoss, 2013; Dai *et al*., 2018)**; *Aspergillus* **(Asses *et al*., 2018; Salem *et al*., 2019)**; *Penicillium* **(Chen *et al*., 2020)**; *Trichoderma* **(Karthik *et al*., 2020; Argumedo-Delira *et al*., 2021)**; *Trametes* **(Pecková *et al*., 2020)**; *Sarocladium* **(Nouri *et al*., 2021)** and others. Biodegradation, biosorption, and bioaccumulation are the fundamental processes that enable dye decolorization to reduce contamination levels to less hazardous, undetectable, or acceptable levels **(Chaney *et al*., 1997)** and clean up or restore contaminated sites **(Dubey *et al*., 2011; Rawat *et al*., 2011)**, as shown Fig. (7).

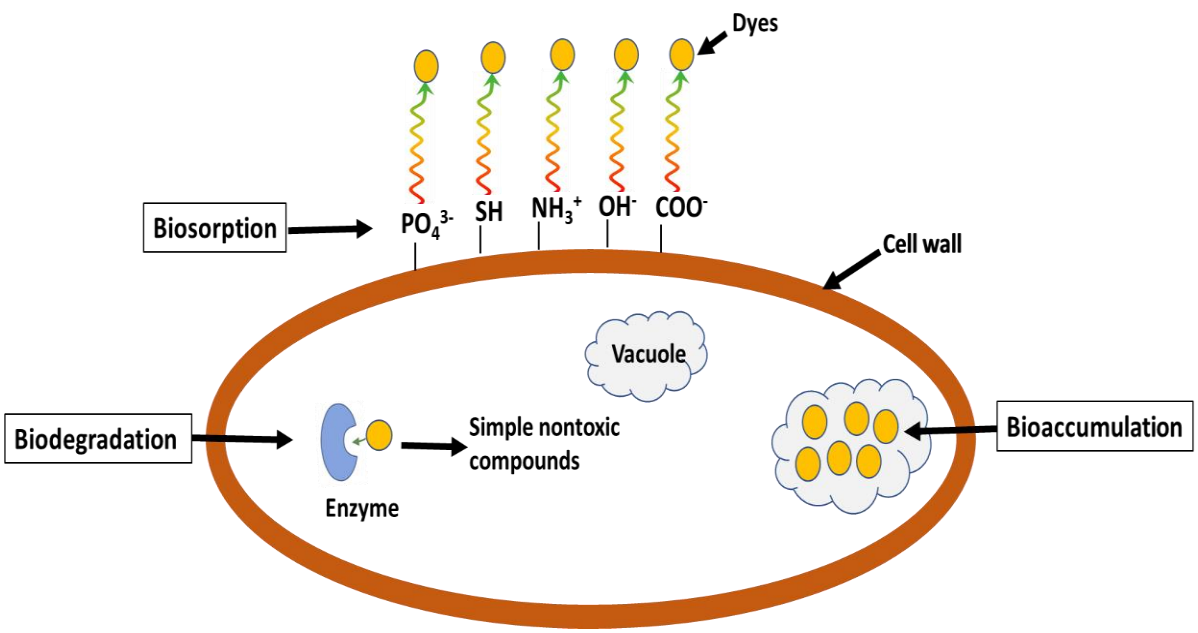

Fig. 7. Schematic diagram showing fundamental processes for dye decolorization

6. Fungal-based decolorization as a sustainable technology

6.1. Fungal biodegradation

Mineralization happens after biodegradation, which is characterized as the biologically induced breakdown of chemical molecules. In the course of dye removal, many investigations have shown that certain fungal groups may biodegrade colors. Fungi remove dyes from wastewaters via a two-step process that begins with adsorption onto their hyphae and continues with the breakdown of the chemical bonds keeping the colors together by extracellular enzymes in their hyphae **(Upadhyay *et al*., 2024)**.

Dye decolorization is facilitated by a number of enzymes, such as laccase, tyrosinase, manganese peroxidase (MnP), manganese independent peroxidase (MIP), and lignin peroxidase (LiP) **(Fu & Viraraghavan, 2001)**. Enzymes that break down lignin are the most important for dye degradation **(Abbasi, 2018; Upadhyay *et al*., 2024)**. In terms of the total decolorization of dyes for various fungi, the proportional contributions of LiP, MnP, and laccase enzymes might vary due to changes in substrate specificity **(Kaushik & Malik, 2009)**.

An example of this is the case of certain white rot fungi that possess a distinct set of extracellular enzymes such as laccase, phenol oxidase, lignin peroxidase, horseradish peroxidase (Hrp), and Mn-dependent and Mn-independent enzymes. These enzymes allow the fungus to withstand high concentrations of pollution and naturally degrade and mineralize dyes form wastewater **(Upadhyay *et al*., 2024)**.

According to **Yesilada and Ozcan (1998)**, a crude culture filtrate of the white rot fungus *Coriolus versicolor* was used to decolorize the orange II dye. **Junghanns *et al*.** (**2008)** investigated the ability of some aquatic fungi from different freshwater habitats to decolorize synthetic azo and anthraquinone dyes. A variety of aquatic hyphomycetes were found, including *Alatospora acuminate*, *Anguillospora longissima*, *Clavariopsis aquatica*, *Cylindrocarpon* sp., *Dimorphospora foliicola*, *Flagellospora curvula*, *Heliscus lugdunensis*, *Tetracladium setigerum*, *Tricladium angulatum*, and *Varicosporium elodea*.

It has been observed that siderophores, in addition to enzymes, may have a role in the decolorization of dyes. Metal complex dyes, often known as phthalocyanine dyes, are commonly used in the textile industry. One kind of chelant that forms complexes with a strong attraction for metals is the siderophores, as stated by **Duran *et al*. (1999)**. A study conducted by **Minussi *et al*. (2001)** investigated the decolorization of four different reactive dyes induced by four different fungi: *Phanerochaete chrysosporium* ATCC 24725, *Lentinus edodes* CCT 4519, *Trametes versicolor* CCT 4521, and *Trametes villosa* CCT 5567. The only fungal strain that was examined, *L. edodes*, to generate the most decolorizing siderophores across all plates. According to **Kaushik and Malik (2009)**, this suggests that siderophores might be used to clean up wastewater that contains colors.

6.2. Fungal biosorption and bioaccumulation

The two main methods in the biological process for eliminating dyes from wastewater are biosorption and bioaccumulation. **Malik (2004)** and **Vijayaraghavan *et al*. (2013)** suggested that biosorption and bioaccumulation have the potential to serve as an alternative to traditional techniques in the remediation of dyes from wastewater. Contrary to biosorption, which is the process of pollutants adhering to living and dead biological materials, bioaccumulation is the phenomenon of pollutants being taken up by living cells (a metabolically active process) **(Santaeufemia *et al*., 2016; Sintakindi & Ankamwar, 2021)**. It involves several metabolism-independent processes that primarily occur in the microbial cell wall, including complexation, chelation, ion exchange, physical and chemical adsorption, electrostatic interaction, and microprecipitation **(Gouda & Taha, 2023)**.

Biosorption is a better method due to its simplicity of design, ease of use, flexibility, and there is no production of hazardous compounds as a result of the sorption process **(Vijayaraghavan *et al*., 2013; Jureczko & Przystaś, 2021)**. In this case, using both dead and living biomass would result in the pollutant binding to the cell surface in an initial stage that would not require metabolism, or biosorption **(Gouda *et al*., 2023)**. Conversely, when using living biomass, which would require metabolism, pollutants pass through the cell membrane and enter the cell as a secondary process. It is crucial to take into account the possibility that some contaminants could now passively diffuse through the membrane **(Torres, 2020)**. **Karthikeyan *et al*. (2007)** and **Crini and Badot (2008)** agreed that biosorption is usually a fast process that employs less costly materials and needs less processing since biomass waste is abundant in nature.

Most of the time, biosorption is a quick process that uses affordable, readily available materials like agricultural and industrial fungal waste biomass **(Karthikeyan *et al*., 2007; Crini & Badot, 2008)**. The diverse functional groups, low cost, and wide range of uses of fungi in food and industrial fermentation processes make them promising candidates for dye removal **(Argumedo-Delira *et al*., 2021; Sintakindi & Ankamwar, 2021)**. Dye cleanup may benefit greatly from the morphological features of the fungal mycelium. The presence of amino, carboxyl, thiol, and phosphate groups in fungal cell walls makes dye molecules seem to absorb onto cell surfaces rapidly, sometimes in a matter of hours **(Bayramoglu *et al*., 2006; Taha *et al*., 2023b)**. Many factors, including temperature, contact duration, starting dye concentration, pH, and biosorbent dosage, affect the biosorption capabilities of fungal biomass **(Pearce *et al*., 2003; Gouda & Taha, 2023)**.

The biosorbent properties of fungal biomass facilitate dye removal, whether it is alive or dead. **Upadhyay *et al*. (2024)** state that modified structure and functional groups on the active site in autoclaved or dead mycelium are responsible for the variable biosorption capabilities of living and dead mycelium. There are several advantages of dead fungal biomass over live biomass, which include greater adaptability to many environments, zero care needs, and long storage life without degradation. In addition, this biomass may be processed and chopped into the appropriate particle size, unlike live biomass which is susceptible to harsh operating and maintenance circumstances, physiological limits, and nutritional requirements **(Santaeufemia *et al*., 2016; Kumari *et al*., 2018)**. *Aspergillus terreus* and *A. niger* biosorb and degrade Procion Red MX-5B, according to **Almeida and Corso (2014)**. Fungal biomass, whether it be alive or dead, may be used as a biosorbent. **Srinivasan and Viraraghava (2010)** and **Grainger *et al*. (2011)** found that live fungal cells can treat colored wastewaters via metabolic activities, in contrast to dead biomass which relies on physical and chemical removal processes.

7. Fungal immobilization technique-based decolorization

Immobilization of the native biomass is necessary for successful decolorization to increase its mechanical strength and resistance to the different chemical constituents. For the industrial application of biosorption, immobilization is necessary for the separation of solids from liquids **(Aksu, 2005)**. Since the 1970s, immobilized cells and enzymes have gained a lot of attention. In comparison to their free-cell counterparts, immobilized cells are easier to handle, take up less space, and are more economical because the regenerated biomass is reused. These benefits can be achieved by immobilizing a variety of cells in polymeric or biopolymeric matrices. It is possible to immobilize multiple microbial cells. At first, the immobilization of bacterial cells was the focus of most research. Ultimately, there was an increased concern on filamentous fungal cells and yeasts **(Rodríguez, 2009; Dewi *et al*., 2020)**.

Immobilized fungal cells have several advantages over sparse ones. They make it easier to reuse biomass, separate liquids, and solids, and reduce clogging in continuous-flow systems. In comparison to suspension cultures, immobilized cultures are often more active and resilient to environmental stresses such as changes in pH or exposure to harmful chemical concentrations. Furthermore, the cells are protected from shear damage by immobilization **(Rodríguez, 2009)**.

Using immobilized fungi for effective dye removal has been shown in several research studies. According to **Chen *et al*. (2003)**, who used an immobilized microbial community to quickly decolorize azo dye (RED RBN) from a phosphorylated polyvinyl alcohol (PVA) gel, through flask culture, the immobilized-cell beads were able to remove 75% of the color, even at high concentrations of RED RBN, in just 12 hours. In another study, **Wang *et al*. (2008b)** found that *Aspergillus fumigatus* beads immobilized with carboxylmethylcellulose (CMC) may be used as a biosorbent to biosorb an azo color.

According to **Alam *et al*. (2021)**, immobilized *Trametes hirsuta* D7 was able to break down several anthraquinone dye types. To treat complex effluent containing Acid Blue 113, Acid Blue 225, Acid Red 111, Reactive Blue 214, Reactive Blue 41, Reactive Blue 49, Reactive Blue 19, and Reactive Red 243 at a starting concentration of 5000mg/ l, *Cunninghamella elegans* conidial suspension immobilized in calcium alginate beads was utilized. According to **Prigione *et al*. (2008**), within 30 minutes, the decolorization was partially completed (70%) and after 6 hours, it was completely achieved. **Iqbal and Saeed (2007)** found that the biosorption of Remazol Brilliant Blue R was enhanced using the immobilized *Phanerochaete chrysosporium* biomass in loofa-sponge, as compared to free fungal biomass.

7.1. Immobilization techniques

Various techniques for immobilizing cells have been used to bind fungi on artificial carriers; these techniques include covalent binding, entrapment, adsorption, and packing **(Sun *et al*., 2011)** (Fig. 8).

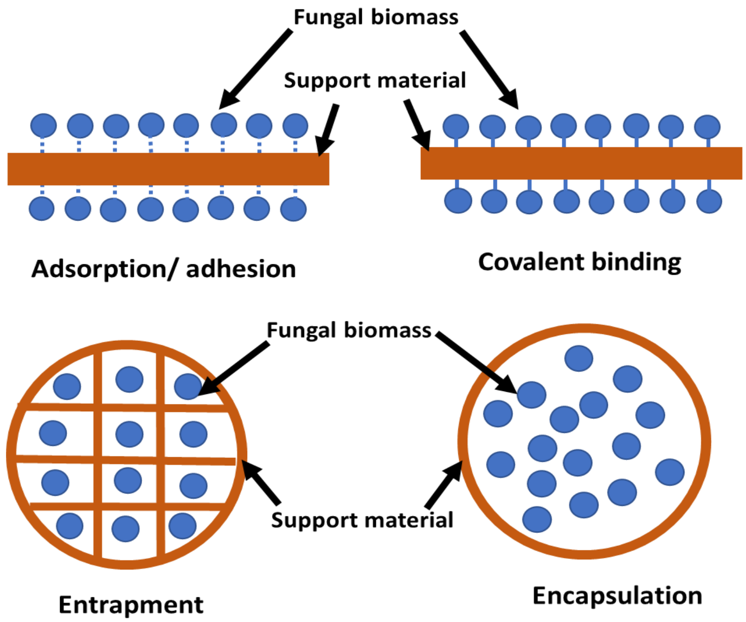

Fig. 8. Schematic diagram showing immobilization methods

7.1.1. Adsorption

Physically attaching immobilized fungus to a carrier that is insoluble in water is known as adsorption immobilization **(Martins *et al.*, 2013; Bayat *et al.*, 2015)**. The immobilized cells are prevented from altering their original structure by the creation of low-energy bonds, such as hydrogen bonding, ionic bonding, and van der Waals interactions **(Hou *et al.*, 2014; Jesionowski *et al.*, 2014)**. According to **Wang *et al.* (2008a)** and **Bayat *et al.* (2015)**, physical cell immobilization is considered the most utilized and oldest method since it is gentle, quick, easy, inexpensive, effective, and doesn't need any chemical additions. It also can provide support. In contrast, adsorption-based immobilization suffers from a weak binding force, that leads to a high rate of cell leakage from the carrier during usage. To circumvent this issue and stop microbes from desorbing from the matrix, there has to be significant adsorption between the cells and the carrier **(Wang *et al.*, 2008a)**.

7.1.2. Covalent bonding

Using chemical groups on both the support material and the cells to form covalent bonds is the basis of this inexpensive immobilization technique. According to **Bouabidi *et al.* (2018)**, two common agents utilized for immobilization are glutaraldehyde (GA) and 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC). Among the many advantages of covalent binding is the fact that it strengthens the bond between the immobilized enzyme and the carrier, which in turn increases catalytic activity **(Hashem *et***

***al*., 2016; Abdollahi *et al*., 2017)**. This process is permanent and steady, unlike adsorption. Enzyme activity is often drastically reduced due to structural changes in proteins brought about by the complicated circumstances inherent in the covalent binding immobilization process **(Garmroodi *et al*., 2016)**.

7.1.3. Entrapment

Placing particles or cells inside a matrix such as a polymeric gel composed of synthetic polymers (polyacrylamide, polyurethane, polyvinyl) or naturally occurring polymeric gels (agar, carrageenan, alginate, chitosan, and derivatives of cellulose) is an irreversible immobilization technique known as entrapment. According to **Bayat *et al*. (2015)** and **El-Naas *et al*. (2017**), entrapment in natural polymeric gels has replaced synthetic polymeric materials as the technique of choice due to their toxicity concerns. The polymeric matrix, whether it is manufactured or natural, protects cells from outside aggressions **(Yoetz-Kopelman *et al*., 2016)**. Cell entrapment provides gentle and cost-effective reaction conditions, which is one of the several advantages it offers in the area of cell immobilization. Cell viability is affected by the exposure of microorganisms to temperatures below freezing that occurs with biomass entrapment during the cross-linking step **(Al-Zuhair & El-Naas, 2011)**. Furthermore, cell entrapment may reduce electron transport and substrate accessibility **(Yoetz-Kopelman *et al*., 2016)**. Important for microbial entrapment is the carrier's pore size to cell diameter ratio because leakage might happen if the size of the pores is larger than the cells that are immobilized **(Bayat *et al*., 2015)**.

7.1.4. Encapsulation

It involves a semipermeable membrane containing the biocatalyst (a cell or enzyme) and allowing immobilized cells to float freely in the core space. According to **Krishnamoorthi *et al*. (2015)**, encapsulation is the physical or chemical process of enclosing the substance to be encapsulated, also known as the core material, in a covering or shell, such that a thin, millimeter-thick capsule is formed. Unbound substrates and nutrients may flow through the semi-permeability of the membrane, but the biocatalyst is hindered by the membrane walls. The ratio of the core material's pore size to the membrane's pore size is another factor that determines encapsulation. The fundamental advantage of this technology is that it is not necessary to chemically modify the core material, hence it is anticipated that the activity of the immobilized microorganisms remains unchanged **(Burgain *et al*., 2011)**.

8. Regeneration of biomass

To regenerate fungal biomass, which reduces waste and boosts treatment efficiency, desorption is an essential step. As seen in Fig. (9), it may also be used in later biosorption procedures. Vapor, biological, thermal, acid (H_2SO_4, HNO_3, HCl, H_3PO_4, NaOH), and organic solvent (methanol and ethanol) treatments are common desorption methods, according to **Kavitha and Namasivayam (2007)** and **Hassan and Carr (2021)**. The regeneration process's energy consumption and cost have to be fair.

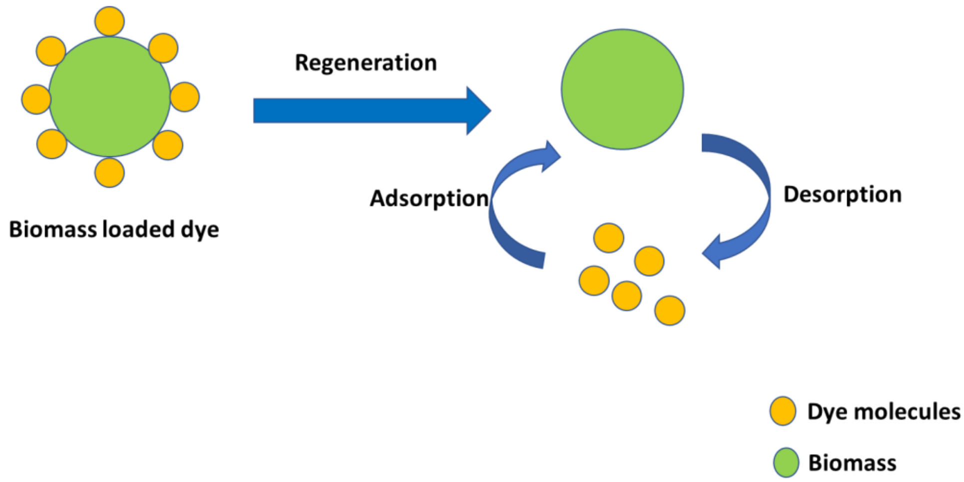

Fig. 9. Schematic representation of regeneration of fungal biomass as adsorbent

Binupriya *et al*. (2008) desorptioned 17% of Congo Red at pH 2 and 41.5% at pH 10 from *T. versicolor* with a biosorption capacity of 29.9mg/ g. Using 70% ethanol as an eluant led to complete desorption (99.5%) after 30 minutes, due to the elimination of the lipid component and the release of the attached dye. According to **Zeroual *et al*. (2006)** and **Patel and Suresh (2008)**, 90% Reactive Black 5 from *A. foetidus* and 90% Bromophenol Blue from *R. stolonifer* were desorbed using 0.1 N NaOH as an eluant. The anionic dye molecules and the negatively charged sites are electrostatically attracted to one other when an alkaline substance is added, which elevates the pH. Because of this, the dye is removed from the biomass. A more powerful alkali, on the other hand, might work against desorption. The use of 1 N NaOH resulted in only 32% desorption **(Patel & Suresh, 2008)**. According to these studies, organic substances like ethanol and mild alkalis are employed as potential dye desorption eluants.

CONCLUDING AND FUTURE CONSIDERATIONS

Numerous industries produce a large number of colored materials that pose health risks and pollute the environment. The decolorization of industrial wastewater, particularly from dye-containing effluents, remains a critical environmental challenge due to the stability and toxicity of dyes. Thus, this article comprehensively reviewed diverse decolorization assay techniques that have been employed. A variety of traditional techniques, including membrane separation, electrochemical, coagulation, adsorption, and activated sludge, are restricted by their exorbitant expenses, ineffectiveness, and time-consuming nature. In contrast, biological approaches, particularly those involving fungi, have emerged as a promising, cost-effective, and environmentally sustainable solution for dye remediation. Fungi have significant potential in decolorizing wastewater through mechanisms such as biodegradation, bioaccumulation, and biosorption. The efficiency, reusability, and stability of the fungal systems are improved by immobilizing fungal biomass through methods like adsorption, crosslinking, entrapment, and encapsulation, which further enhances decolorization. With continued research and technological advancements, fungi have the potential to revolutionize wastewater treatment, offering a reliable, competitive, and eco-friendly solution for addressing the global challenge of dye pollution. Looking ahead, the development of fungal-based dye remediation technologies is considered a promising technique in the near future through the identification of novel fungal strains, the creation of pilot and full-scale systems for treating real wastewaters that contain textile dyes, combining fungal-based approaches with complementary methods to develop hybrid systems for improved performance and conduct comprehensive life-cycle analyses to ensure the sustainability and cost-effectiveness of fungal decolorization technologies.

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