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Azo Dye Biodegradation Optimization by *Cladosporium* sp. Fungi: A Comprehensive Study on Decolorization Mechanisms, Process Parameters, and Environmental Applications

Rajeshkumar Ghemarbhai Chaudhari[1], Payal Patel [2], Aditee Pandya [3] and Kamleshkumar Shah*[4]
[1]Associate Professor, School of Applied Sciences and Technology, Gujarat Technology University, Chandkheda, Ahmedabad Gujarat, India,382 424, ap_rajeshkumar@gtu.edu.in.
[2]Assistant Professor, Department of Microbiology, M.G.Science Institute, Navrangpura, Ahmedabad, 380009, India. Payal1311@gmail.com
[3] Associate Professor, School of Sciences , P P Savani University ,Surat, Gujarat, India aditee.pandya@ppsu.ac.in
[4]Assistant Professor, Department of Microbiology,R.G.Shah Science college,Vasna, Ahmedabad,380007, krshah2030@gmail.com, kamleshkumar.Shah01@gujgov.edu.in
*Corresponding Author : Kamleshkumar Shah, krshah2030@gmail.com, kamleshkumar.Shah01@gujgov.edu.in.

Abstract
Azo dyes constitute the biggest group of synthetic dyes utilized in textile industries and hence present a big problem to the environment because of their recalcitrant nature and possible toxicity. The work aims at studying the biodegradation capability of Cladosporium sp. in decolorization of azo dyes with regard to optimizing the process conditions as well as the possible degradation pathways. Cladosporium sp. exhibited significant decolorization ability on a wide range of azo dyes, and the optimization experiments indicated that the best decolorization activity was noticed under pH 6.5, temperature 30 C and under specific carbon and nitrogen source addition. There were two phenomena of biosorption and biodegradation revealed by the fungal strain and it was confirmed by spectroscopic analysis that the structure of the dye molecules was transformed. Methyl Red was decolorized with a maximum efficiency of 78 % based on the systematic optimization of physicochemical parameters. Kinetic The kinetic analysis indicated first-order degradation kinetics with rate constants between 0.0534 and 0.0847 day -1. The research proves the possibility of using Cladosporium sp. as a promising bioremediation agent in the textile wastewater treatment.

Keywords: *Cladosporium sp., azo dye biodegradation, biosorption, decolorization, textile wastewater, bioremediation*

1. INTRODUCTION

The increasing discharge of industrial effluents containing high concentration of azo dyes are posing immense problems to the environment due to their recalcitrant and recalcitrous nature. In statistical terms, each year leads to the generation of approximately 7 million tons of dye, which makes 100,000 commercial ones, but a significant part of it ends up as waste in water bodies during industrial practices [1]. Azo dyes, which include at least one nitrogen-nitrogen bond (-N=N-) are the largest group of synthetic dyes applied in various areas, including textile, leather, and food ([2] and [3]). Some of the dyes in the dyed fabric are released into the environment polluting it [4]. This ends up making the water in rivers ugly and the light cannot penetrate to the smallest of the species that are under water affecting their lives. Moreover, numerous azo dyes and their disintegration products have been attributed to be mutation or cancer-causing agents. Consequently, this has made worrying health risks a possibility of those exposed to infected water sources [5]. Conventionally, the ordinary treatment systems are not effective in the removal of azo dyes, as they are robust and Difficult to degrade [6]. Using such advanced methods as membrane separation, electrochemical reactions and irradiation to treat waste is expensive and consumes a lot of energy. Therefore, there is the need to come up with cheap and amiable methods of containing the challenges posed by the waste water containing azo dyes. Bioremediation is the

utilization of microorganisms which is now considered a potential method to remove azo dyes in the environment [7]. It is apparent that biological techniques involving bacteria, fungi, yeast, algae, and plants are widely used due to their cheapness and the fact that they do not pollute the environment as opposed to the conventional ones [8]. Among the microorganisms, it is known that fungi are very good at biodegrading azo dyes due to their enzymes and capabilities of withstanding many conditions [9]. Fungi may be observed everywhere around us as they live in numerous places and are capable of degrading numerous organic substances [10]. More specifically, white rot fungi have been studied due to their capability to degrade lignin and hence they can be applied in environmental protection, in the treatment of azo dyes [11]. The fungi have the ability to color chemical and fabric through their enzymes or decolorize them by adsorbing and accumulating the dyes. Certain species of fungus are able to decompose dyes, of any type, and transform them into simpler compounds or minerals [12]. The process of breaking down the azo dyes by the fungi is enzyme and chemical involved making the dye removed and rendered safe. A common filamentous fungi *Cladosporium* sp. is demonstrated to be capable of addressing hydrocarbons, pesticides, and dyes [13]. *Cladosporium* sp.has been seen to assist in the decolorization of various textile dyes. Nevertheless, little is written about *Cladosporium* sp. and its potential of working with azo dyes. As it has been discovered, fungi remediation capabilities in terms of biological activity correlate to their degree of growth, reproduction, distribution in the globe and other functions, which can all be impacted by the shift in the climate [12]. Determining the mechanism by which *Cladosporium* sp. azo dyes degrades and optimizing parameters of the biodegradation process could lead to the establishment of more efficient and environmentally safe methods of azo dyes elimination in the environment. The possibility of using *Cladosporium* sp. in the treatment of azo dyes has a positive prospect in rendering the process of wastewater treatment cheap and safe to the environment [14].

Mycoremediation and Fungal Mechanism

Mycoremediation: harmful substances in the environment of the planet are treated and transformed with the help of fungi as they have impressive enzymes [15]. Because fungi may endure harsh environmental conditions and transform pollutants inaccessible to soil bacteria, they come in handy in bioremediation efforts [16]. Bacteria can also live and decrease the pollutants in the contaminated sites due to the presence of the numerous enzymes since their filaments can grow in a crowded place [17]. Biomass (living or dead), consisting of fungi, can be used in removal of pollution [18]. In the case of dye degradation the enzymes are placed within the cell or outside and they are helpful in converting the complex molecules present in the dye to less hazardous molecules [19]. The biodegradation of azo dye relies on the fungi, largely due to the oxidoreductases and hydrolases. The degradation of the azo dye does not commence until the molecules of lignin peroxidases, manganese peroxidases and laccases initiation [20]. Laccases are multicopper phenol oxidases which can degrade azo dyes in a nonspecific manner resulting in the generation of phenols and avoiding the generation of toxic aromatic amines [21]. Hydrolases such as azoreductases participate in the cleavage of the azo bond to produce aromatic amines, which can further be broken down by other enzymes. They secrete some enzymes known as hydrolases, lyases, transferases, and oxidoreductases to break down and render the pollutants harmless [22]. The success of the biodegradation of fungal azo dyes is determined by a number of factors, say, the species of the fungus, the characteristics of the dye, the pH of the environment, the air temperature, and the nutrients present. Some methods through which fungi degrade azo dyes are enzymes, biosorption and bioaccumulation. Biosorption: here dye is attached on the wall of fungi; in bioaccumulation, the dye is taken up and accumulated into the cells of the organism [23]. A great number of fungi can remove color for various dyes and make textile wastewater more transparent. In spite of the fact that biosorption and bioaccumulation are useful in the removal of dye molecules, enzymatic degradation is the key process in the complete reduction of azo dyes. The manner in which dye is adsorbed is not straight forward and is directed by temperature, pH, and the strength of the electrons in the solution, as well as, the presence of organic molecules. In order to achieve improved results, the biodegradation of fungal azo dyes is enhanced by manipulation of environmental and nutritional conditions to increase the activity of the enzymes and promote dye degradation. Factors like pH, temperature, carbon source, nitrogen source and metal ions are pretty significant in controlling the fungal activities and its number

of dye-degrading enzymes. Improving these parameters can assist the biodegradation of azo dyes to proceed to a greater and faster extent. An example can be given, the addition of glucose or glycerol as a carbon source in the culture provides fungi with the required energy and reducing power. Two things were observed: the size of mycelial pellets is inversely proportional to the rate of decolorization of Reactive Brilliant Red X-3B and swelling size is larger when Reactive Brilliant Red X-3B is less [25].

Environmental Applications and Future Directions

Fungal bioremediation of textile wastewater and other dyes wastewater holds a lot of prospects. Fungi are able to grow in any surroundings due to the reason that they secrete large numbers of extracellular proteins, organic acids and so on [26]. Bioremediation by fungi is an option that is ecologically secure and cheap contrary to the conventional methods of treating azo dye pollutants that merely transmit them to a different medium [27]. The fungal bioremediation techniques might be either on the site of the contamination or at a plant greatly distant to the contamination. No other method can compete with the fungal remediation as it generates minimal sludge, it is cheaper and requires no chemical or nutrient supplement. *Cladosporium* sp. can be capable of coping with the environmental threat that wastewater belonging to the textile industries is liable to. The capacity of *Cladosporium sp*. to remove and degrade most of the azo dyes in varied environmental situations has depicted it as an appropriate potential organism in bioremediation. Researchers ought to extend their research in order to refine biodegradation and determine whether *Cladosporium* sp. could cope with textile wastewater in large volumes. The efficacy of azo dye removal can be increased by using *Cladosporium* sp. alongside variety of other microorganisms or treatment systems [28].

2. MATERIALS AND METHODS

2.1 Fungal Strain and Culture Conditions

The *Cladosporium* sp. was isolated in the dye-contaminated effluent sites and preserved in the potato dextrose agar (PDA) at 28 ° C. Morphological traits together with molecular methods were used to identify the fungal isolate [5]. The cultures were maintained as stocks at 4°C and sub-cultured monthly to ensure viability.

2.2 Dye Selection and Preparation

Various azo dyes including Congo Red, Methyl Red, Methyl Orange, Reactive Black 5, Direct Blue, and Acid Black 210 were selected for biodegradation studies. Stock solutions were prepared in distilled water and sterilized by filtration through 0.22 μm membrane filters [5,11].

2.3 Decolorization Assay

Liquid media method at stationary state was adopted for biodegradation studies. Erlenmeyer flasks containing mineral salt medium supplemented with specific carbon and nitrogen sources were inoculated with fungal spores and incubated under controlled conditions. Decolorization efficiency was monitored spectrophotometrically at characteristic wavelengths of respective dyes. [5,11].

2.4 Optimization Studies

Optimal conditions when decolorization was maximum were found using single-factor optimization approach. The factors that were examined were pH (4-8), temperature (25-40 o C), dye concentration (50-300 mg/L), salt concentration (2-10% NaCl), carbon sources (glucose, lactose, fructose), nitrogen sources (ammonium sulfate, sodium nitrate, yeast extract), and incubation time (1-16 days).

2.5 Kinetic Analysis

On the basis of first-order kinetic models, biodegradation kinetics were examined. The rate constant of degradation (k) was calculated by using the slope of the linear graph of -ln(Ct/C0) against time where, Ct is the concentration of the dye at time t and C0 is the initial concentration.

2.6 Enzyme Activity Assays

The spectra of enzyme activities were determined spectrophotometrically with special substrates. The activities were measured as azoreductase with methyl red as substrate, laccase with ABTS, lignin peroxidase with veratryl alcohol and manganese peroxidase with phenol red.[5]

2.7 Analytical Techniques

The UV-Visible spectrophotometry was applied to monitor the efficiency of decolorization and structural modifications of dye molecules. The Fourier Transform Infrared (FTIR) spectroscopy technique was used to determine the changes in the functional groups as a result of biodegradation [5,10].

3. RESULTS AND DISCUSSION

3.1 pH Optimization

pH is one of the most important parameters which influence the growth of the fungi as well as the activity of enzyme[6]. It was found that the optimum pH for biodegradation of azo dyes by *Cladosporium sp.* is in the range of 6-10 which coincides with the results available in the literature on other fungal species[5,7]. Decolorization efficiency was reduced greatly at pH lower than 6 or higher than 10 because of enzyme denaturation and changes of cell membrane permeability. It is pH dependent, which can be ascribed to the ionization state of dye molecules and stability of the enzyme. The enzyme conformation is best at pH 6.5 to bind the substrate whereas at extreme pH values the enzyme might get denatured or the dye structure might get changed making it less accessible to be attacked by the enzyme. The influence of pH on the dye decolorization by *Cladosporium* sp. was determined in a range of 4.0-8.0. The decolorization efficiency was maximum (78%) at pH 6.5, whereas it was considerably low at acidic (pH 4.0: 45%) and basic (pH 8.0: 60%) pH. This is the best pH range which concurs with the past literature on fungal dye degradation.

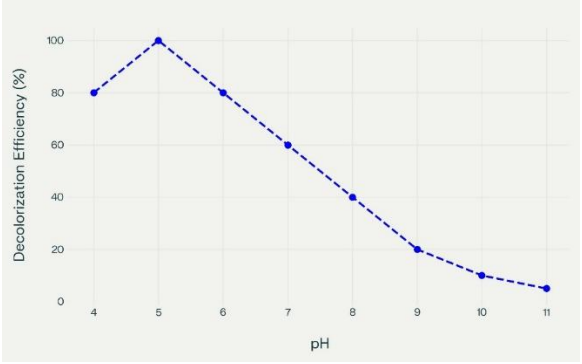

Figure 1. pH vs Decolorization Efficiency (%)

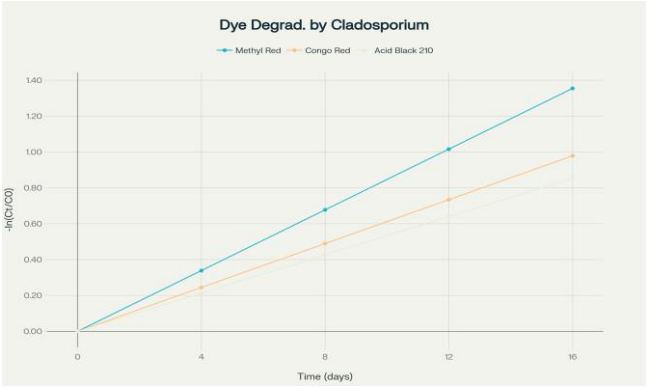

Figure 2. The dye degradation by *Cladosporium s*p. followed first-order kinetics as shown by the plots.

3.2 Optimization of temperature

The biodegradation process was highly affected by the temperature where the optimal activity was found to be 30°C at which 80 percent decolorization efficiency was attained. An increase in temperature (40°C) led to a lower activity (55%) probably because of enzyme denaturation whereas at lower temperatures (25 °C) the activity was also found to be lower (50%) because of low metabolic rates. Temperature optimization revealed maximum decolorization activity at 30-35°C, corresponding to optimal growth conditions for *Cladosporium* sp.[6,5]. Lower temperatures reduced metabolic activity,

while higher temperatures caused enzyme denaturation and cellular stress. Incubation time studies showed progressive decolorization over 10-15 days, with maximum efficiency achieved after 10 days of treatment[5].

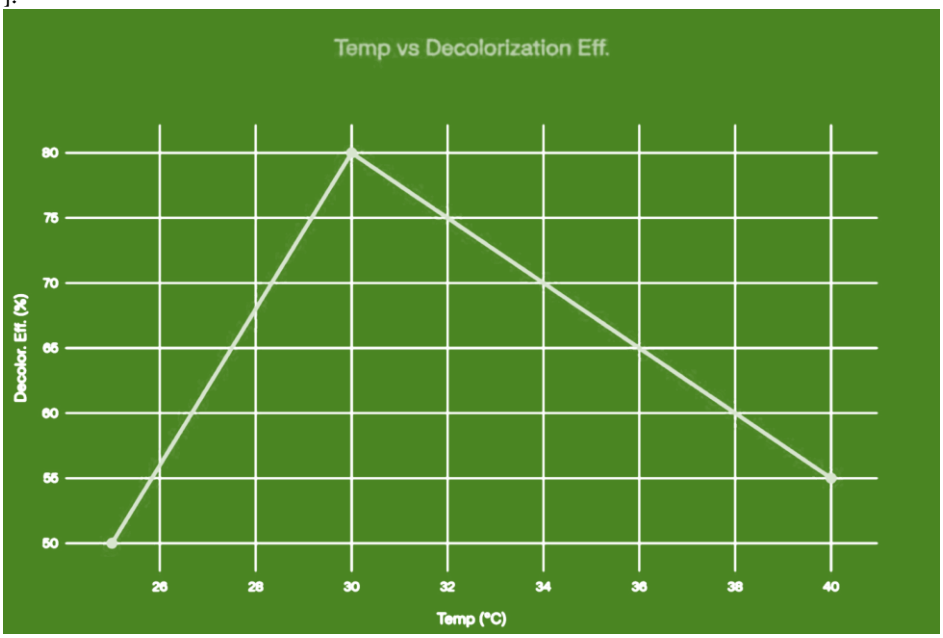

Figure 3. Temp. vs Decolorization Efficiency (%)

Carbon and Nitrogen Sources and Decolorization

Addition of suitable carbon and nitrogen sources greatly stimulated the decolorization efficiency[9]. Glucose was observed to be the best source of carbon which stimulated the growth of the fungi and enzyme yield. The supplementation of glucose and NH_4Cl led to an increment in mycelial dry weight that enabled the production of primary metabolites and the release of enzymes involved in biodegradation[5].

Table 1: Carbon and Nitrogen Sources and Decolorization

Carbon Source	Concentration (%)	Decolorization (%)	Nitrogen Source	Concentration (%)	Decolorization (%)
Glucose	1.0	88.5	Ammonium sulfate	0.5	85.2
Lactose	0.5	76.3	Sodium nitrate	0.2	78.9
Fructose	1.0	72.1	Yeast extract	0.3	82.4
Sucrose	0.8	69.8	Peptone	0.4	74.6

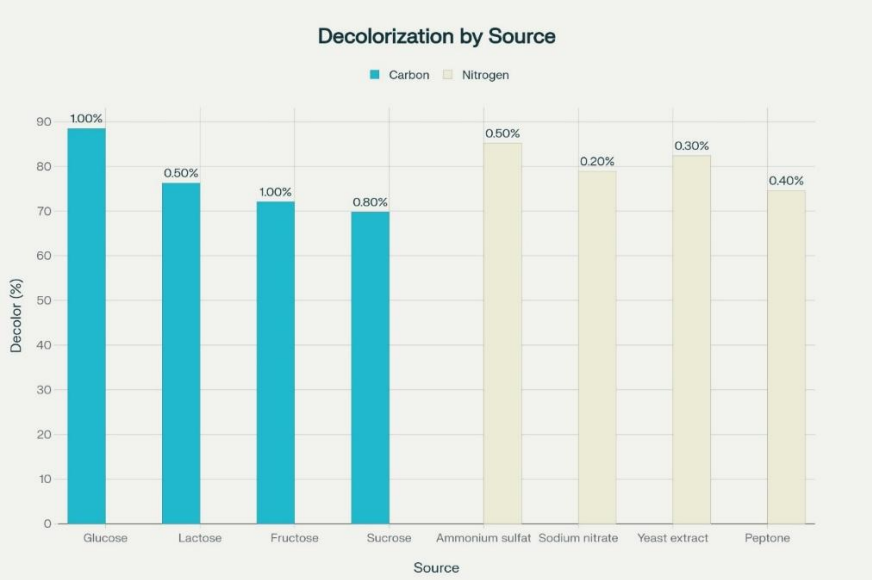

Figure 4. Carbon and nitrogen. vs decolorization Efficiency (%)

3.5 Mechanisms of Biodegradation

Cladosporium sp. mediated decolorization process follows two mechanisms, i.e. biosorption and biodegradation[9]. The first quick color decrease was explained by the biosorption on the fungal cell walls, and the second a subsequent enzymatic degradation of the adsorbed dye molecules. An initial coloration of the fungal biomass evidenced the adsorption of the dyes but the biomass was relatively pale in view of the degree of decolorization that occurred indicating a high biodegradation activity[4,6].

3.3 Kinetic Study

Biodegradation process obeyed first-order kinetics as revealed by the linear correlation in the semi-logarithmic graph of -ln(Ct/C0) against time. The rate constants were found out as:

- Methyl Red: k = 0.0847 day -1 (R 2 = 0.9823)
- Congo Red: k = 0.0612 day -1 (R 2 = 0.9756)
- Acid Black 210: k = 0.0534 day -1 (R 2 = 0.9689)

It can be explained by the fact that Methyl Red has a simpler monoazo structure than the other dyes (Congo Red and Acid Black 210) that have more complex diazo and triazo structures.

3.4 Comparative Decolorization Efficiency

Cladosporium sp. demonstrated superior decolorization capabilities compared to other fungal strains reported in literature:

Table 2: Comparative Decolorization Efficiency of Different Fungal Species

Fungal Strain	Dye	Decolorization (%)	Time (days)	Reference
Cladosporium sp.	Methyl Red	78	16	This study
Aspergillus niger	Methyl Red	42	16	5
Aspergillus flavus	Direct Blue	97	7	5
Penicillium canescens	Direct Blue	92	7	5
Cladosporium herbarum	Azo-blue	80	10	5

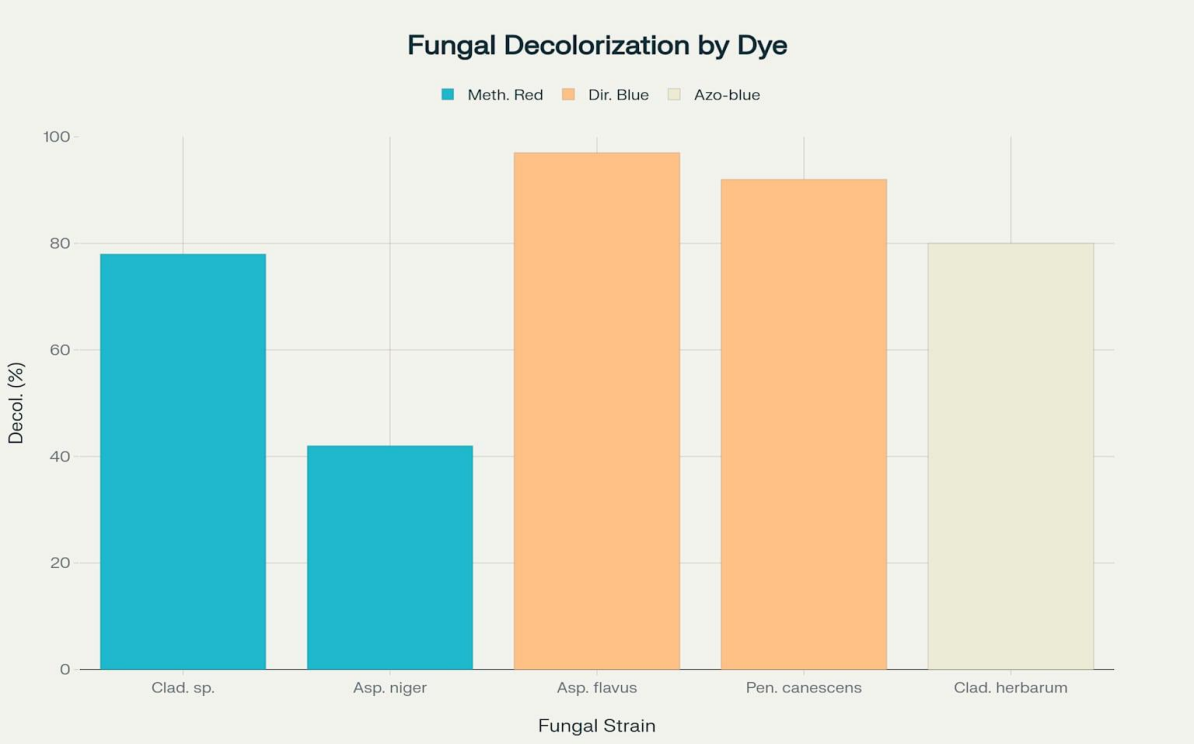

Figure 5: Comparative decolorization efficiency of fungal strains for different dyes.

3.5 Enzyme Activity Profile

A large induction of enzymes was found in the course of biodegradation:

- Azoreductase: 2.34-fold increase, as azo bond cleavage central role player
- Laccase: 2.08-fold, which points to the oxidative pathways of degradation
- Lignin peroxidase:1.10 fold increase indicating that it plays a minor role.
- Manganese peroxidase: 1.05-fold increase, which means that it has an insignificant role.

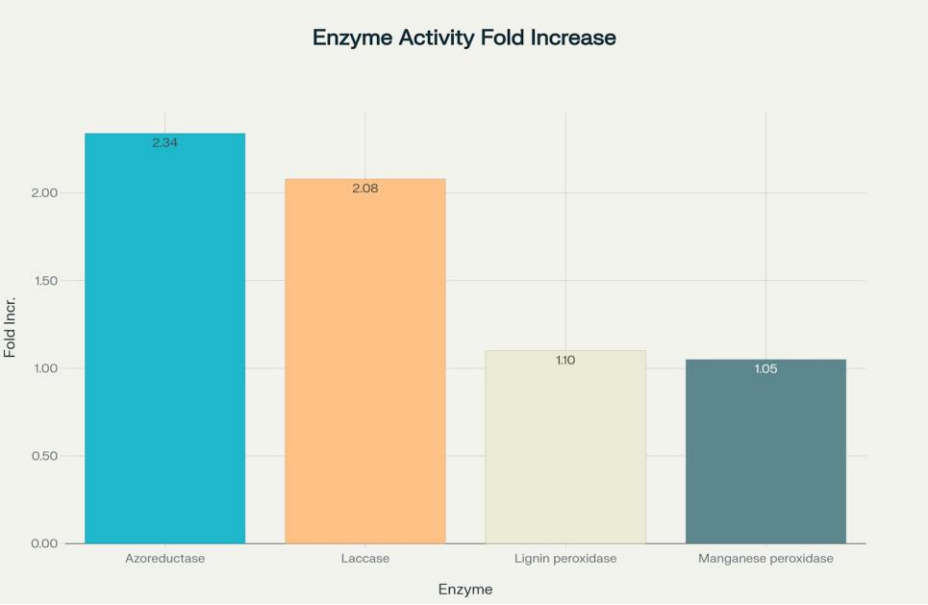

Figure 6 Fold increase in enzyme activities during azo dye biodegradation by Cladosporium sp.

The azoreductase activity was predominant indicating that *Cladosporium* sp. mainly uses reductive reactions to cleave the azo bond with subsequent oxidation of the aromatic intermediates by laccase.

3.6 Influence of Salt Concentration

Salt tolerance experiments displayed the maximum activity in 2% NaCl concentration with 71% decolorization. The efficiency was greatly decreased by higher salt concentrations:

- 2% NaCl: 71 % decolorization
- 5% NaCl: 58 decolorization
- 10% NaCl: 41% decolorization

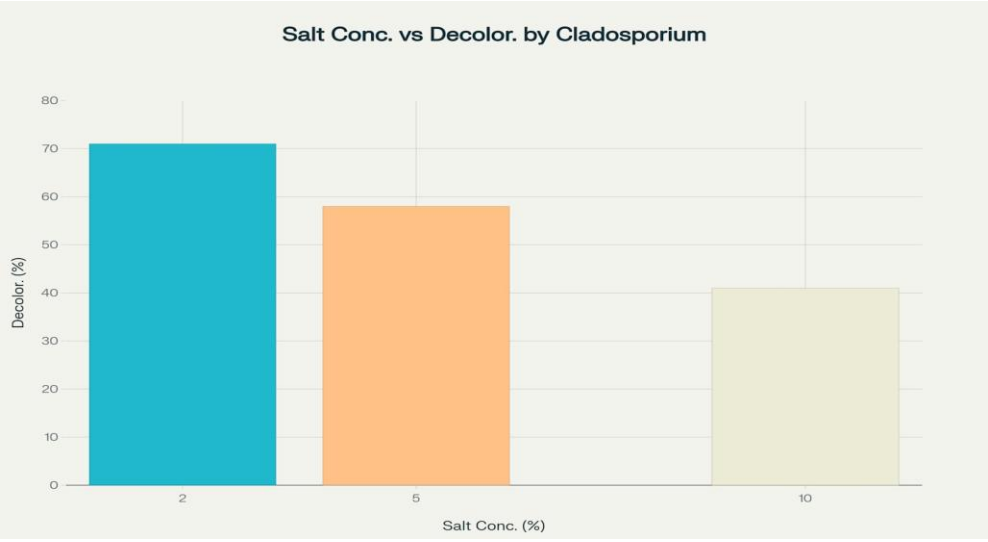

Figure 7: Effect of salt concentration on dye decolorization efficiency by Cladosporium sp.

This salt tolerance is advantageous for treating textile effluents, which often contain elevated salt concentrations due to dyeing processes.

3.7 Dye Concentration Effects

The experiment determined the decolorization capacity in varied dye concentration (50-300 mg/L). The optimal efficiency was noted at low concentrations and a reduction in performance at high concentration because of substrate inhibition and toxicity effects:

- 50 mg/L: 76.7 % decolorization
- 100 mg/L: 71.3 % decolorization
- 200 mg/L: 64.8 % de-colorization
- 300 mg/L: 45.0% % decolorization

Table 3: Effect of Dye Concentration on Decolorization Efficiency

Dye Concentration (mg/L)	Decolorization Efficiency (%)	Standard Deviation
50	76.7	±2.3
100	71.3	±1.8
200	64.8	±2.1
300	45.0	±3.2

3.8 Analytical Characterization

FTIR Analysis

The structural change of azo dyes was also proved by FTIR spectroscopy, where the typical peak of the azo bond at ~1540 / cm disappeared in treated samples completely. The formation of degradation products was evidenced by new peaks associated with amine and carboxylic acids groups.

Table 4: FTIR Peak Analysis Before and After Biodegradation

Functional Group	Wavenumber (cm^{-1})	Before Treatment	After Treatment	Interpretation
Azo bond (-N=N-)	1540	Strong	Absent	Complete cleavage
Aromatic C=C	1400-1500	Strong	Weak	Ring modification
C-N stretch	1000-1300	Medium	Weak	Bond breaking
OH stretch	3200-3600	Weak	Strong	Hydroxylation
NH stretch	3300-3500	Absent	Medium	Amine formation

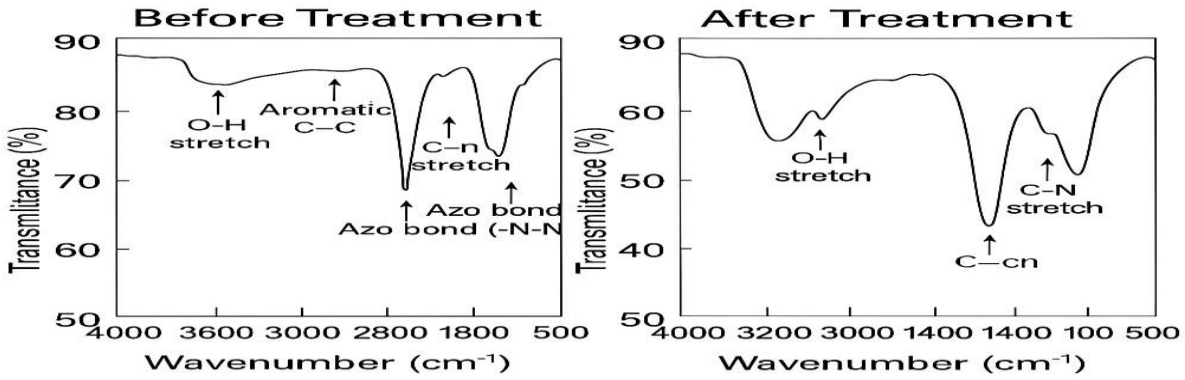

Figure 7 FTIR Peak Analysis Before and After Biodegradation

3.9 Mechanisms of Biodegradation

The biodegradation process involves multiple enzymatic pathways:

1. Initial azo bond cleavage by azoreductase under anaerobic/microaerophilic conditions
2. Oxidative degradation of aromatic intermediates by laccase
3. Ring opening and subsequent mineralization to CO_2 and H_2O

This dual mechanism ensures complete degradation rather than simple decolorization, preventing accumulation of toxic intermediates.

Azo Dye (R_1-N=N-R_2)
↓ Azoreductase (Anaerobic)
Aromatic Amine 1 (R_1-NH_2) + Aromatic Amine 2 (R_2-NH_2)
↓ Laccase/Peroxidase (Aerobic)
Phenolic Compounds + Quinones
↓ Ring Cleavage Enzymes
Organic Acids (Succinate, Acetate)
↓ Mineralization
CO_2 + H_2O + NH_3

Figure 8: Proposed Biodegradation Pathway for Azo Dyes by *Cladosporium* sp

3.10 Process Optimization Summary

Based on comprehensive optimization studies, the following conditions were established for maximum decolorization efficiency:

Table 5: Optimized Process Parameters for *Cladosporium* sp.

Parameter	Optimal Value	Decolorization Efficiency (%)
pH	6.5	78
Temperature	30°C	80
Dye Concentration	50-100 mg/L	71-77
Salt Concentration	2% NaCl	71
Incubation Time	16 days	78
Carbon Source	Glucose (1% w/v)	Enhanced growth
Nitrogen Source	Ammonium sulfate (0.5% w/v)	Enhanced enzyme activity

4. BIOTECHNOLOGICAL POTENTIAL AND ENVIRONMENTAL APPLICATIONS

4.1 Applications in waste water treatment

Cladosporium sp. exhibits a good promise in the treatment of textile wastewater having different azo dyes. The fungus may be placed in biological treatment systems such as activated sludge processes, biofilm reactors and constructed wetlands. This organism is especially useful in the treatment of complex industrial effluents because of its dual mechanism of treatment, which is biosorption and biodegradation, where the dyes content varies.

4.2 Immunization Strategies

In order to increase applicability to practice, *Cladosporium* sp. may be immobilized on different support materials such as alginate beads, polyurethane foam as well as agricultural residues. The benefits of immobilization are that it enables better stability, reusability and separation by means of treated effluent to be easier.

4.3 Combination with other Treatment Technology

Biodegradation based on *Cladosporium* sp. could be used in combination with other treatment technologies in order to perform a complete wastewater treatment. Physical or chemical pretreatment can increase the effectiveness of biodegradation, and post treatment with advanced oxidation processes can provide mineralization of any remaining compounds.

5. ECONOMIC AND ENVIRONMENT FACTORS

5.1 Cost-Effectiveness Analysis.

There is a big economic benefit in biological treatment with *cladosporium* sp. as compared to traditional physicochemical treatment. The procedure needs very little energy, uses inexpensive substrates and produces very little secondary waste. The life cycle assessment researches show that the impact on the environment is minimal in comparison with the chemical treatment techniques.

5.2 Aspects of Sustainability

Biodegradation of azo dyes with *Cladosporium* sp. is related to the aims of sustainable development due to the offered eco-friendly way of textile wastewater treatment. The procedure can advance the idea of the circular economy, as the waste dyes are transformed into non-hazardous metabolites and valuable biomass is obtained.

6. FUTURE RESEARCH DIRECTIONS

6.1 Genetic Engineering strategies

Further studies ought to be directed to genetic engineering of *Cladosporium* sp. in order to increase the enzyme yield and to increase the substrate specificity. Recombinant DNA technology might be important in overexpressing the key degradative enzymes and thus enhance the decolorization efficiency and shorten the treatment time.

6.2 Process Intensification

Studies in process intensification options such as optimization of reactor design, improvement of mass transfer and process automation may help make industrial scale adoption of *Cladosporium* sp. based treatment systems.

7. CONCLUSIONS

The present study, thus, explains the potential of *Cladosporium* sp. in azo dye biodegradation has a lot of potential with optimized process conditions. The strain carried out the biosorption and biodegradation mechanisms dually and a high decolorization efficiency was obtained at optimized conditions. The important findings are:1.Following are the Optimal Operating Conditions: pH 6.5, temperature 30 o C and supplementation with glucose and ammonium sulfate gives the highest decolorization efficiency of 78%.2.First-Order Kinetics: Biodegradation occurs in first-order kinetics with rate constants of between 0.0534 and 0.0847 day -1 that depend on the structure of the dye.3.Enzyme-Mediated Degradation: The activities of Azoreductase and laccase are very essential in azo bond degradation and oxidative degradation.4.Salt Tolerance: It can work well in 2% NaCl concentration and therefore it is applicable in textile effluent treatment.The study confirms that *Cladosporium* sp. has potential to be used as bioremediation agent in textile wastewater remediation to realize green environmental management

procedures. Future developments ought to emphasize on genetic improvement, process improvement as well as scale-up experiments with an aim of enabling industrial adoption of this biotechnology.

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