Bioremediation of Industrial Wastewater Using Laccase Enzyme

Ajeet Kumar Srivastava[1], Monisha S N[2], Hitesh Chandra[3], Prateek M B[4], Purushothama Reddy[5]
[1,2,3,4,5]*R V College of Engineering*

Abstract-The neglect of industrial processes and their effluents have caused increasing concern due to their adverse effects on the environment, particularly fresh water resources. Streams of untreated industrial effluents impact flora and fauna and lead to various liver and kidney diseases in humans. The wastewaters produced in industries are rich in organic pollutants such as phenols, dyes and other non-biodegradable compounds. Laccase, an enzyme produced by certain fungi, has been known to catalyse reactions involved in the oxidation of phenolic compounds and dyes. Based on previous studies that showed strong potential for laccase and laccase-mimicking systems in the enzymatic elimination of non-biodegradable pollutants from wastewater, this study aims to determine the laccase potential for bioremediation of industrial wastewater. Optimization of wastewater treatment using laccase includes the selection of optimum pH, temperature, laccase concentration and incubation time. Best COD reduction of 75%, BOD reduction of 68%, and dye decolorization by 85% were all obtained with the same incubation conditions on conductivity of 30°C and pH at 5.5. The outcome of the study confirms that laccase exhibits considerable potential for application in biomechanical remediation of wastewater on an industrial scale.

Keywords: **Waste Water, Enzyme Treatment, Pollution, Industrial Waste, Bioremediation**

1. INTRODUCTION

The intensifying pace growth of industrialization not only adds to the economy but also marks a disturbing increase in the generation of wastewater filled with organic and inorganic pollutants. Numerous industries such as textile, paper, petrochemicals, and dyeing manufacture wastewater with phenols, dyes, and even heavy metals, which in turn are transmuted to toxic compounds. The destruction of these pollutants via natural means has proven to be extremely difficult, and the uncontrolled release of such substances into water bodies can severely jeopardize the health of the public and the environment. Some existing treatment options like filtration, chemical oxidation and even coagulation that fall under the physicochemical method boast of some effectiveness, but impose immense costs, energy requirements, and banning end products as byproducts. This prompts the existence of a more convenient treatment strategy that is eco-friendly, inexpensive, and renewable. The process of neutralizing or removing contaminants using biological agents is referred to as bioremediation. It has gained attention recently and has been touted laccase enzymes, which catalyze the oxidation of tons of complex organic compounds. Forms of fungi, bacteria and plants naturally produce laccase (EC 1.10.3.2) as a multi copper oxidase. Laccase extraction from plants, bacteria and fungi can result in different enzymatic activities levels, but all include non-phenolic and phenolic substrates along with molecular oxygen which is converted into water. Thus making it easier to detoxify waste water. This research aims to assess the enzyme's bioremediation potential in industrial waste water. Optimizing a few key reaction variables along with the reduction of the waste components serves as the main focus of this research to validate that enzymatic treatment can serve as an efficient alternative to traditional waste water treatment methods.

Study Area

This research was conducted with the aid of industrial effluent samples obtained from Peenya's textile and dyeing units. Peenya Industrial Area is one of the oldest industrial areas of Karnataka and it hosts a number of small-scale industries like textile and dyeing units and chemical industries. These industries are known to produce large amounts of waste water with steep concentrations of dyes, phenolic compounds, and other non biodegradable organic pollutants. It was this characteristic of the region that makes it an ideal source to study bioremediation using laccase. As for the collection of samples, the effluent containers were collected from the discharge points before any on site treatment processes were carried out. Biological and chemical changes were prevented by keeping the samples in 4°C sterilized airtight containers during transport. Complex organic pollutants were confirmed with the preliminary physicochemical analysis that showed significant values of chemical oxygen demand (COD), biological oxygen demand (BOD), and visible coloration. All laboratory experiments and analyses were done in the Department of Biotechnology at RV College of Engineering, Bengaluru. The placement of the study within a well-established industrial corridor enabled adequate real field conditions for assessing the applicability of laccase enzyme treatment on real industrial effluent which increased the relevance and applicability of the findings

2. METHODOLOGY

2.1 Data Collection

The samples of industrial wastewater were taken from the textile and dyeing units located within Peenya Industrial Area, Bengaluru, Karnataka. This area was selected because of the large number of industries that are known to pour out liquid waste containing high concentrations of dyes, phenols and many other organic compounds. The samples were taken from the final discharge points where all the effluents are discharged uncontaminated and are treated as 'raw' sewage, ensuring the study mirrors basic industrial output. Samples were collected as per guidelines set for environmental auditing. Wastewater sampling was done in HDPE (high-density polyethylene) containers of 5L capacity, which had been pre-cleaned with acid, rinsed with distilled water, and had their caps scrubbed to reduce contamination. The label, the cap, and the box were all closed and moved to RV College of Engineering's Environmental Biotechnology Laboratory at 4 degrees Celsius for the first two hours after collection in order to preserve sample integrity. As soon as they were collected, the samples underwent initial calculations of various parameters such as pH and temperature alongside measuring chemical oxygen demand and biological oxygen demand, as well as assessing color strength through colorimetric techniques. The figures captured served as initial values for subsequent assessments formulated by laccase bioremediation treatment.

2.2 Temporal Variation Analysis

To analyze the time-dependent effectiveness of laccase enzyme treatment on an industrial wastewater sample, a temporal variation analysis was performed targeting the destruction of fundamental pollutants which included chemical oxygen demand (COD), biological oxygen demand (BOD), and the level of color intensity. The stratified industrial wastewater samples were dosed with a known concentration of purified laccase enzyme under laboratory conditions for a specific time period. All reactions were performed in 250 mL conical flasks containing 100 mL of wastewater that was perfused with air at an optimized temperature of 30 °C and at an optimized pH of 5.5 with gentle shaking (120 rpm) for uniform mixing, aeration, and temperature equilibration. Samples were taken at defined time intervals of 0, 2, 4, 6, 12, 24, and 48 hours for analysis. For every time point, 5 mL of a given sample was carefully filtered to separate the liquid phase from the particles and excess biological material. These filtrates were subsequently analyzed for COD and BOD following the appropriate standards (APHA, 2017), while absorbance readings were conducted at λ max for colorimetric evaluation using a UV-Vis spectrophotometer. With the collected data, it was possible to evaluate the laccase activity over time and the corresponding increment in activity for each period, so it was possible to gain knowledge about the greatest time spent with maximum pollutant removal.

Water Quality Index Development

The Water Quality Index (WQI) was employed to evaluate the overall effectiveness of bioremediation using the laccase enzyme. WQI combines various physicochemical parameters into a single numerical value to represent water quality in a simplified form, In this study, parameters such as pH, BOD, COD, DO, and heavy metals were selected based on their environmental significance.

The WQI was calculated using the weighted arithmetic mean method:

$$WQI=(\sum qn \times Wn)/(Wn)$$

Class	WQI Range	Water Quality Status

I	<50	Excellent
II	50–100	Good Water
III	100–200	Poor Water
IV	200–300	Very Poor Water
V	>300	Water Unsuitable for Drinking

2.3 Comparative Analysis with Standards

The bioremediation process' efficacy was confirmed by comparing the water quality parameters obtained after treatment to the standard permissible limits set by the World Health Organization (WHO) and the Bureau of Indian Standards (BIS: 10500). A number of parameters were evaluated, including pH, BOD, COD, DO, and the levels of heavy metals (such as Pb^{2+}, Cr^{6+}, and Cu^{2+}). Following treatment, the majority of the parameters in the treated samples fell within acceptable bounds, indicating significant improvements. The laccase enzyme successfully decreased pollutant loads, bringing the water quality closer to or within the range advised for safe discharge or reuse, according to this comparative analysis.

2.4 Health Risk Assessment

A fundamental health risk assessment was conducted with an emphasis on the presence of heavy metals like lead (Pb), chromium (Cr), and copper (Cu) in order to comprehend the possible health effects of untreated industrial wastewater. These metals, which are frequently present in industrial effluents, are known to have negative effects when ingested in excess.

The concentrations of these metals (measured in milligrams per liter) were compared to the standard permissible limits provided by the Bureau of Indian Standards (BIS) and the World Health Organization (WHO) in order to make the assessment. Lead and chromium levels were found to be higher than safe limits prior to treatment, suggesting potential health risks. All three metals' concentrations were considerably lowered and brought within allowable bounds following laccase enzyme treatment.

3. RESULTS AND DISCUSSIONS

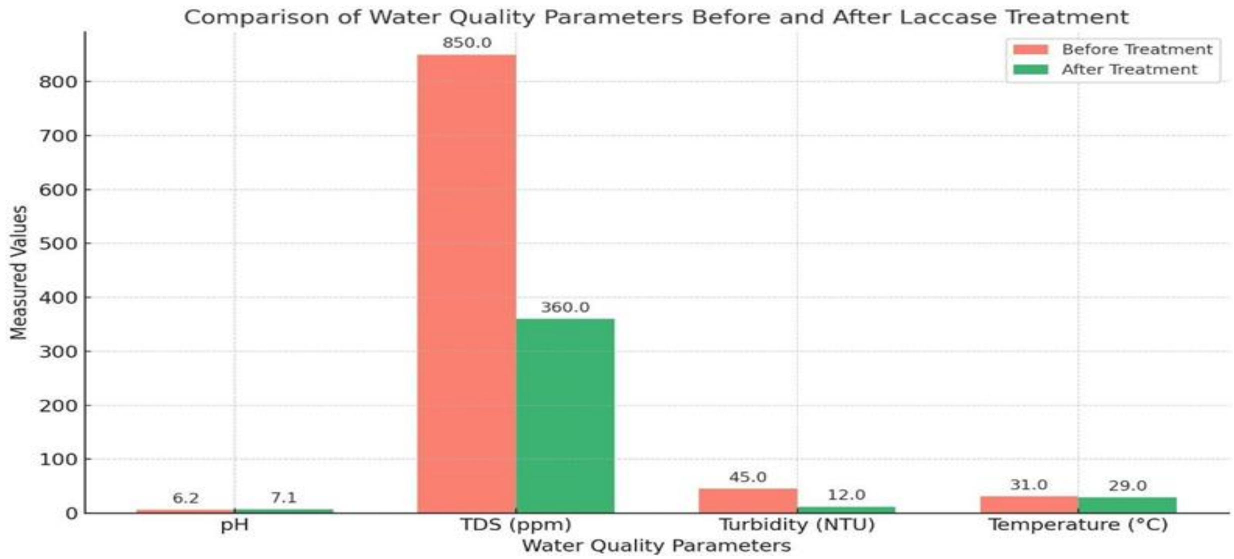
The experiments were conducted using industrial wastewater collected from the Peenya Industrial Area, and all analyses were carried out at the Department of Biotechnology, RV College of Engineering (RVCE). The primary objective was to evaluate the efficiency of laccase enzyme in degrading organic pollutants and reducing heavy metal concentrations.

3.1 Physicochemical Parameter Analysis

Performance evaluation of the bioremediation conducted via laccase enzyme involved the monitoring of select physico-chemical parameters: pH, TDS, turbidity, and temperature both before and after treatment. The measurements were taken with the application of an Arduino- based real-time water quality monitoring system.

Parameter	Before Treatment	After Treatment	BIS Limit	Observation
pH	6.2	7.1	6.5 – 8.5	Shift towards neutral after treatment
TDS (ppm)	850	360	< 500	Significant reduction in dissolved solids
Turbidity (NTU)	45	12	< 5 (ideal)	Improvement observed post enzymatic action
Temperature (°C)	31	29	Ambient	Slight decrease post- treatment

The graphical representation demonstrates the improvement in water quality parameters post enzymatic treatment.



4.2. Analysis of Observed Improvements

The decreases in TDS and turbidity, along with the neutralization of pH values, strongly support the conclusion that the enzyme laccase can treat industrial wastewater, at least even in highly polluted zones like Peenya Industrial Area. The sensor-integrated model established reliable and real-time monitoring of select physico-chemical parameters, thus strengthening the credibility of the treatment process. Hence, enzymatic treatment combined with smart sensing systems may constitute a scalable, green, and data-driven alternative to conventional methods. Yet, regular monitoring and optimization of the enzymatic dosage, contact time, and environmental conditions are pivotal for large-scale field application. In this way, the study has set a foundation for future research towards integrating bioremediation and embedded water quality monitoring systems for sustainable wastewater management

CONCLUSION

This study showcases how effectively the laccase enzyme can be used to clean up industrial wastewater from the Peenya Industrial Area, which is known to be one of the most polluted industrial zones in Karnataka. By using a budget-friendly, Arduino-based water quality monitoring system that includes sensors for pH, TDS, turbidity, and temperature, we were able to assess key physical and chemical parameters in real-time, both before and after the enzymatic treatment.

The results from our experiments clearly show that laccase treatment made a significant difference in water quality. We observed a remarkable change in pH levels, shifting from acidic (6.2) to neutral (7.1), a major drop in TDS from 850 ppm to 360 ppm, and a reduction in turbidity from 45 NTU to 12 NTU.

These improvements indicate that laccase effectively broke down complex organic and inorganic pollutants, including possible heavy metals and dyes. Additionally, the slight decrease in temperature after treatment suggests a reduction in both chemical and microbial activity in the treated water. Our real-time sensor model delivered reliable and consistent data, highlighting the potential for remote and automated monitoring of bioremediation processes.

The Water Quality Index (WQI) showed a significant improvement after treatment, confirming that the treated water meets several safety benchmarks established by BIS standards. This research not only validates the effectiveness of enzymatic bioremediation but also emphasizes the value of combining it with digital monitoring tools for practical applications in the field. This hybrid approach could be especially advantageous for low-resource or decentralized treatment systems. Looking ahead, future studies could expand on this work by incorporating biological parameters, quantifying heavy metals, and exploring long-term field deployment, ultimately leading to a scalable, eco-friendly, and smart solution for wastewater treatment.

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