Experimental Investigation on Isolation and Purification Of Reactive Dyes And Water Pollutant Chemicals By Flocculation Method Using Fungal Consortium

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Abstract- *Lenziteselegans, a white-rot fungus that produces ligninolytic enzymes and is capable of decolorizing manmade dyes, was isolated from rotting wood. L. Elegans KSG32 produced laccase (363.7 IU/mL) and lignin peroxidase (5.16 IU/mL) for the first time, according to our knowledge. The capacity of Cibacron red dye to decolorize was tested in both solid and liquid environments. The use of L. elegans KSG32 in the decolorization of Cibacron red dye and the chemical pollution of water. The capacity of a microorganism to cause dye discoloration has gotten a lot of press. Microbial decolorization of dyes and water pollutant compounds is thought to be a cost-effective way to remove these pollutants from the environment. There has been a lot of research in recent years on the use of fungus to remove dye from dye effluent. It is proving to be a viable alternative to or addition to conventional treatment methods.*

Keywords- white-rotfungi, laccase, lignin peroxidase, Cibacron red dye, water pollutant chemical, decolorization.

I. INTRODUCTION

Synthetic dyes are aromatic chemicals that are employed in a wide range of industries, including the dyeing industry (22%), textile industry (54%), paper and pulp industry (9%), tannery and paint industry (9%), and dye manufacturing industry (6%), as well as effluents containing chemicals from those industries.

1. Cibacron red dye is one of the most common synthetic dyes. They have been demonstrated to be cancer-causing, and their protected delivery into the climate addresses a general well-being hazard. Substrate unbound colors from the industry are released into streams without treatment in sums going from 10% to 15%.
2. Discharging this wastewater into aquifers hurts all biological systems.
3. Both physical and chemical procedures may be used to remove or degrade this color and the substances that cause water pollution. However, secondary pollutants such as sludge and hazardous chemicals are produced, discouraging the adoption of such expensive technologies.
4. Biological methods can be used to overcome these disadvantages. The various microorganisms and degrading enzymes can be used, the most cost-effective and ecologically friendly dye removal methods

Lignin peroxidase (LiP), manganese peroxidase (MnP), and laccase are all used by ligninolytic fungi in their enzyme system. The extracellular hemeproteins are MnP and LiP. In LiP, the redox potential is very high, and the pH is inadequate. Substrates including polymers and non-phenolic aromatic compounds may be reduced by LiP 6–9 via oxidation. One of the most active oxidants, 10MnP'S, transforms aromatic amines and their phenolic counterparts into organic radicalsand cacatalyzeses the conversion of Mn2+ to Mn3+. Extrinsic multicopper oxidoreductase Laccaoxidizeizes various phenols and phenolic compounds, as well as diamines, aromatic amines, and benzenethiols (iodine). These are uncommon features seen in ligninolytic enzymes that help decolorize and degrade synthetic colors. Ligninolytic enzymes are abundant in white-rot fungus (14-16), which belbelongs the basidiomycetes family. The pharmaceutical industry employs the majority of these enzymes.

Many harmful compounds, including organochlorine-based heavy metals, pigments, and dyes, are found in wastewater collected from textile businesses. Because of the great development in industrialization man's need for color, and dyestuff consumption has expanded day by day. In the textile and dyeing industries, chemical dyes have grown more popular owing to their simplicity of use and cost-effectiveness

in a wide variety of compositions, as well as their durability and color when compared to natural dyes. Color is one of the most visible indications of water contamination, and the discharge of brightly colored synthetic dye effluents may harm recipient water bodies. Dyes may be found in sewage from the medical, textile, printing, and cosmetics industries. When it comes to BOD to COD removal, dyes often have a poor rate of removal (BOD/COD less than 0.1).

The goal of this study is to find a fungus that can manufacture ligninolytic enzymes and then test those enzymes for decolorization and water pollution removal capacities.

II. MATERIALS AND METHODS

Culture and purification of fungal strains:

A sum of 32 obvious parasitic fruiting bodies was gathered from rotting wood and soil litter in the encompassing region (Denkanikottai, Shoolagiri).Pure cultures may be prepared by detaching a tiny portion of the inner meat of the fruiting body and plating it onto potato dextrose agar (PDA). PDA plates are sterilized with 70 percent C2H5OH (ethanol). Mycelium was transferred to fresh agar trays and kept at 4°C in PDA trays to achieve pure cultures.

Production of ligninolytic enzymes by screening method:

Lignin-containing basal agar (PDA) medium was used to culture the isolates. Secondary screening, including qualitative and quantitative approaches, was performed on the L. Elegans KSG32 strain growing on the basal agar medium. Potato dextrose agar (PDA) plates containing 0.0025% w / v azure B were used to detect lignin peroxidase production and 0.02% glycol qualitative technology to detect lacquer synthesis.

SSF was used to conduct the quantitative screening. The substrate for SSF was chosen to be leaves from pineapple plants. The leaves were chopped into 5x1 cm squares and autoclaved for 15 minutes at 121°C. With the use of a 0.1M citrate buffer, the moisture content was reduced to 90%. (pH 5). As an inoculum, we utilized three 1.1 cm2 mycelial plugs from a six-day-old PDA plate culture.Incubation was done for 5 days at NTP condition using inoculated flasks.

Extraction of crude enzyme:

After incubation, 50 mL 0.15 M citrate solution (pH 5-6) was added to each beaker to remove the enzyme from the ore. The culture supernatant was collected and stored at the same temperature after centrifugation at 10,000 rpm for 10 minutes at 4 C.

Enzyme assays:

The crude extracts were checked by LiP, MnP, and laccase activity. The technique of H2O2-dependent oxidation of veratrine alcohol to veratraldehyde was used to determine LiP activity. Increasing absorption was checked at 310nm. The technique of determining MnP activity utilising phenol red as a substrate. At 610nm, the absorption was examined once again. Used to detect oxidative (2, 2'-and-bis (3-ethyl-benzothiazoline-6-sulfonic acid) lactase activity of ABTS. At 422nm, the absorbance was measured.

Flocculation method for fungi consortium:

This work aims to provide an alternative technology for fungal-assisted biocompatibility to extract microalgae from municipal sewage sludge using spores produced on fungal strain (L. Elegans KSG32).The model species was the facilitative heterotrophic microalgae Chlorella Vulgaris UMN235. Important parameters Likespore inoculum, the organic carbon content and pH change in the medium have shown significant positive effects on the formation of fungal and algal particles under different growth conditions.

Chemicals and Culture medium:

All factors, FeCl2-4H2O, FeCl3-6H2O, NaOH, HCOOH (88% w / v) and low molecular weight chitosan (decryption degree 84.5%, molecular weight 50-190 kDa) and cypacron brilliant red (Mw-10) -Must: 517 nm) from Aldrich Corporation. The yeast extract contains glucose (OYG) medium (g / L): glucose (DEFCO), 1.25, peptone (DEFCO), 5.0 and yeast extract (DEFCO), 3.0, pH 7 [14]. Mineral salt medium (MSM) (g / L) used in the decomposition study: K2HPO4, 1.73; KH2PO4, 0.68; MgSO4.7H2O, 0.1; Sodium chloride, 0.1; FeSO4.7H2O, 0.03; NH4NO3, 1.0; CaCl2.2H2O is associated with 0.02 glucose (3 g / L), with pH adjusted to around 7.5 in the medium.

The activity of fungal consortium on Cibacron red dye:

On both PDA and PDB, Lenzites Elegans KSG32 performed decolorization investigations on numerous dyes. Growing L. Elegans KSG32 on dye-incorporated PDA plates was used to test their capacity to grow in the presence of Cibacron dye. Cibacron is the dye that was utilized. The dye is filtered and sterilized before being introduced to the sterilized PDA at a concentration of 0.05g/L. A 0.5cm2 mycelial plug from a 6-day-old culture was used to inoculate the integrated

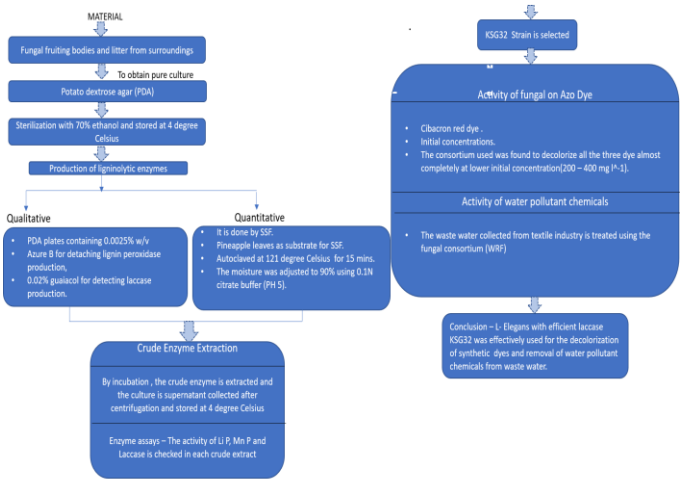
dye plates. The trays were sealed for 10 days at 28°C. When color is changed in PDB, the dye is supplied to the medium in one of two ways: a) at the time of vaccination or b) at the final concentration of 5-day-old cultures from 0.05 g / L.

The jars were maintained and sealed at 28°C for up to 12 days with occasional shaking. At regular intervals, 1 mL flasks were sampled and centrifuged at 10,000 rpm for 10 minutes. Making use of a UV-visible spectrophotometer, the supernatants were examined for changes in absorption at the die absorption maximum (Shimadzu). Cibacron dye has a maximum wavelength of 365nm. The following equation was used to get the percentage of decolorization: (Initial absorbance - Observed absorbance/ initial absorbance)/100

The activity of fungal consortium oneffluents:

White rot fungi have also been used to remove many textile dyes, and fungal strains capable of decolorizing several types of dyes have been researched. Bio-decolorization of lignin-containing pulp and paper effluents utilizing white-rot fungus L. Elegans KSG32., as assessed by a reduction in color absorption, was first reported in 1980. Both are obvious instances of color removal from polymeric lignin molecules by the microbial breakdown. P. hrysosporium, a wood-rotting fungus, has been the focus of extensive study into the breakdown of a variety of resistant xenobiotic chemicals, including dyes. Lignin peroxidase and Mn-dependent peroxidase or laccase enzymes are involved in color removal42. Many fungal species have been identified as degraders of dyes. The fungus KSG32 of L. elegans. According to their life circumstances, these fungi may be separated into two groups: live-cell biodegradable dyes and dead cells (fungal cells) absorbing colors. Unlike oxidation by aerobic or anaerobic metabolism, biosorption refers to several metabolic-independent activities (chemical and physical absorption, complexity, ion exchange, electrostatic reaction, chelation, and micro-deposition) that occur within the cell wall. To remove hazardous organic chemicals, live and dead biomass can be used (dried, heat-killed, acid-treated,and/or chemically treated).

The capacity to decolorize was evaluated and isolated using species such as L. Elegans KSG32. The findings revealed that after 7 days of culture, the isolated strain reduced the lignin color by up to 20%. The capacity of the fungus L. Elegans KSG32 to decolorize was found to be 80 and 70 percent, respectively 47-50. White-rot fungi's lignin-degrading system is made up of extracellular enzymes such as las laccases, peroxidases, and oxidases51. They may decompose phenolic chemicals and synthetic dyes, among other organic pollutants47,52,53. It has been reported that Elegansan's KSG32 can destroy a wide range of synthetic colors.



III. RESULT AND DISCUSSION

L.elegans KGS32 strain is most effectively degraded on both dye and water pollutant chemicals. It has been the most effective are comparatively other strains on white-rot fungi its degradation of the variable composition of wastewater from the textile industry in the modern-day are several research institutions and groups are developing some fungi for more efficient on processed. A complete aromatic compound such as red dye is possible, this is the cumulative effect of di-degrading enzymes. The degradation potential of Cibacron red dye is investigated. Each color is only effective at low concentrations. The result after the screening of L. elegans KGS32 for this aromatic and enzymatic profile is possible the decolorization of wastewater and red dye.

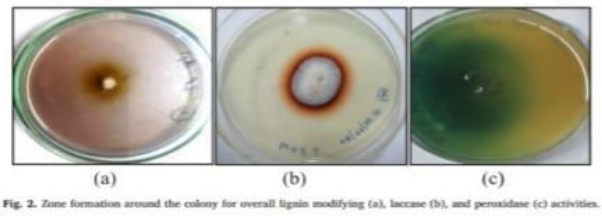


Fig. 2. Zone formation around the colony for overall lignin modifying (a), laccase (b), and peroxidase (c) activities.

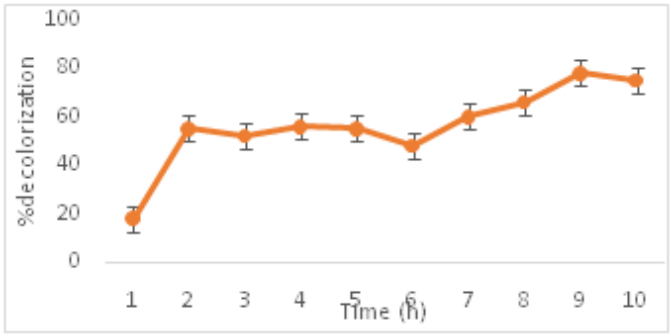


Figure 1:The relationship between time and decolorization percentage of Cibacron red dye

The decolorization effect on Cibacron red dye when added at the inoculation time to the L Elegans culture.

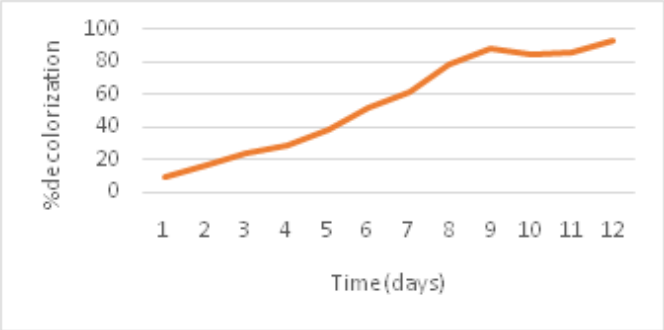


Figure 2:Relationship between days and decolorization of Cibacron red dye

The following graph shows the decolorization of Cibacron red dye when it is added to 5 days old LElegans KSG32 culture.

Figure 3: Laccase and LiP activity

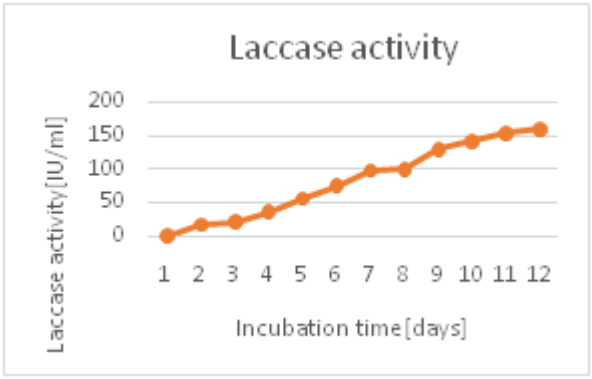


Figure 3(i)

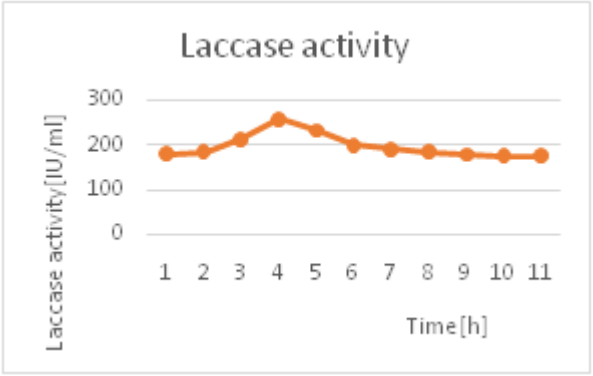


Figure 3(ii)

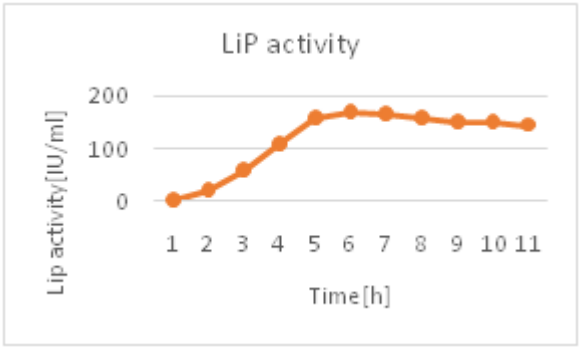


Figure 3(iii)

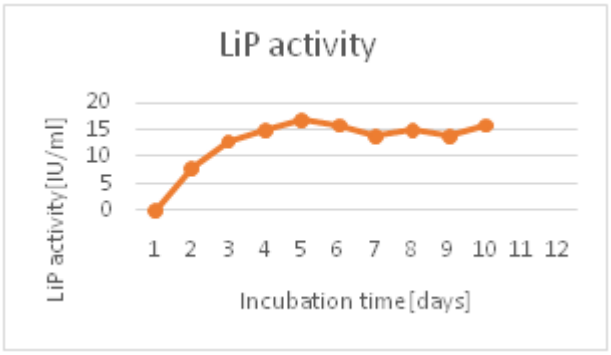


Figure 3(iv)

Figure 3 depicts the enzyme activity during the organism's development on Cibacron red dye-containing agar media.

3rd diagram

Figure 3(i) depicts the incubation activity by day.

Figure 3(ii): This shows the because activity on Cibacron red dye addition to the 5-day old culture

Figure 3(iii):This graph depicts the activity of the Lips on each day of incubation.

Figure 3(iv) depicts lip activity after the addition of Cibacron red dye to a 5-day-old culture.

Enzyme activity during decolorization:

Figure 3 depicts how enzyme activity plays an important role in organism development. The current research reveals that there is an effect on visual appearance in the adsorption of colors on mycelium laccase, and the presence of lipase enzymes was seen and it is controlled and monitored daily in chromosomal fungal cultures. Laccase activity increased when Cibacron red dye was present, but Lip activity decreased. Cibacron red dye was added to the 5-day old culture. After stabilizing the activity, laccase activity climbed to the maximal decolorization. The enhanced laccase activity in the presence of Cibacron red dye is confirmed.

Decolourization of Cibacron red dye:

In decolorization research, the toxicity of dye fungal growth plays a key role. In comparison to the control PDA plate, the L. Elegans KGS32 strain contains the red dye on the PDA plate of the dye. The toxicity of dye is measured in dye decolorization tests by comparing the biomass content of L. elegans KSG32 after 12 days of development in PDB with and without dye. If dye concentration is increasing the fungal growth is affected by studies using Cibacron red, it can reduce biomass to toxicity. According to the average concentration, 0.4g/l has been reported in the textile effluent. The dyes employed for their investigation did not have any hazardous effluent L. Elegans KSG32 development, according to the results. Instead, the color might be promoting fungal mycelium development as a carbon source. Because Ligninolytic enzymes have lower substrate specificity, the white-rot fungus may successfully bioremediate dyes like Cibacron red dye. On substrates such as wood, nylon, and silk, the dye was generally employed since it had excellent wash characteristics. To get sophisticated Cibacron red dye, the white-rot fungus may mineralize the dye ingredient and decolorize it.

Waste water Fungalconsortium:

The fungal strain is elegans KSG32 it is capable of dye decolorization and was isolated from different niches. These fungi are using the decolorized and bioremediation on water pollutant effective analysis can textile effluent. The bioremediation potential of the fungal strain has been determined to be quite good. In the wastewater, the collaboration decreased solids by 93 percent and 73 percent, respectively. All the white-rot fungi are decolorized ability but we used higher efficiency strain to reduce the decolorizationfor two days 48hours.The growth of the fungi is not affected by water. These fungi are found to have a capacity of decolorized 4 g/l concentrates of these dye
Decolourized rate and kinetics were monitored by spectrophotometry. Parameter of the process:PH, dye concentration, and inoculum size by dye decolorization rate were all investigated. When the inoculum size is increased, the process slows down, and the dye decolorization rate drops by 93 percent.The fungus is capable of isolating a decolorizing and microbial isolate from textile waste polluted sites, This waste can be used in aerobic treatment before being released into the environment as chemicals that contaminate water.

IV. CONCLUSION

1. Dye decolorizations

L. Elegans KSG32, which has laccase and lignin peroxidase activity, is utilized to decolorize synthetic dyes.The dyes are not toxic when induced on organisms. This property is used in bioremediation.

2. Textile waste effluents

Many fungal strains are there, which are capable todecolorize dye wastewater. These were created using a simple, low-cost media and had a high production rate as well as a high biosorption and degeneration capability.

Living cells decolorize through a variety of processes, including intracellular, extracellular oxidase, and biosorption. It is connected to operational circumstances such as nutritional needs, concentration, and toxicity. Decoloration employs dead biomass, which is simple to operate and has a high biosorption capability, allowing it to function as an effective biosorbent. Fungal decolorization is an alternative to the current treatment procedure.

White–rot fungi are a valuable microorganism for bioremediation of apparent pharmaceutical pollutants. They can degenerate a broad range of interactable compounds, and their ease of handling makes them ideal biological agents for wastewater treatment.

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