

## Experimental Investigation on Isolation and Purification of Reactive Dyes and Water Pollutant Chemicals by Flocculation Method Using Fungal Consortium

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**Abstract:** *Lenzites elegans*, a white-rot fungus that produces ligninolytic enzymes and decolourize manufactured 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 decolourize was tested in both solid and liquid environments. The use of *L. elegans* KSG32 in the decolourization of Cibacron red dye and the chemical pollution of water. The capacity of a microorganism to cause dye discolouration has gotten a lot of press. Microbial decolourization 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 fungus to remove dye from dye effluent. It is proving to be a viable alternative to conventional treatment methods.

**Keywords:** white-rot fungi, laccase, lignin peroxidase, Cibacron red dye, water pollutant chemical, decolourization.

### 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]-[5]. 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 wellbeing hazard. Substrate unbound colours from industry are released into streams without treatment in sums going from 10% to 15% [6]-[12]. Discharging this wastewater into aquifers has a negative impact on all biological systems. Both physical and chemical procedures may be used to remove or degrade this colour and the substances that cause water pollution [13]. However, secondary pollutants such as sludge and hazardous chemicals are produced, discouraging the adoption of such expensive technologies. Biological methods can be used to overcome these disadvantages [14]. The most cost-effective and ecologically friendly dye removal methods [15]-[23]. Lignin peroxidase (LiP), manganese peroxidase (MnP), and laccase are all used by ligninolytic fungi in their enzyme system [24]-[27]. The extracellular hemoproteins are MnP and LiP. In LiP's, 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 radicals catalyzing the conversion of Mn<sup>2+</sup> to Mn<sup>3+</sup> [28-[33]. Extrinsic multicopper oxidoreductase Laccases may oxidize various phenols and phenolic compounds, as well as diamines, aromatic amines and benzenethiols (iodine) [34]-[38]. These are uncommon features seen in ligninolytic enzymes that help decolourize and degrade synthetic colours. Ligninolytic enzymes are abundant in white-rot fungus (14-16), which belong to the basidiomycetes family. The pharmaceutical industry employs most of these enzymes [39]-[42].

Many harmful compounds, including organochlorine-based heavy metals, pigments, and dyes, are found in wastewater collected from textile businesses [43]. Because of the great development in industrialization and man's need for colour, dyestuff consumption has expanded day by day [44]. 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 and their durability and colour compared to natural dyes [45]. Colour is one of the most visible indications of water contamination, and the discharge of brightly coloured synthetic dye effluents may harm recipient water bodies. Dyes may be found in sewage from the medical, textile, printing, and cosmetics industries [46]. When it comes to BOD to COD removal, dyes often have a poor removal rate (BOD/COD less than 0.1). This study aims to find a fungus that can manufacture ligninolytic enzymes and then test those enzymes for decolourization and water pollution removal capacities [47]-[51].

## 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) [52]-[55]. 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 C<sub>2</sub>H<sub>5</sub>OH (ethanol) [56]-[61]. Mycelium was transferred to fresh agar trays and kept at 4°C in PDA trays to achieve pure cultures [62]-[67].

### *Production of ligninolytic enzymes by the screening method*

Lignin-containing basal agar (PDA) medium was used to culture the isolates. Secondary screening, including qualitative and quantitative approaches, were 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 [68]-[71]. 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 a 0.1M citrate buffer, the moisture content was reduced to 90%. (pH 5). We utilized three 1.1 cm<sup>2</sup> mycelial plugs from a six-day-old PDA plate culture as an inoculum. Incubation was done for 5 days at NTP condition using inoculated flasks [72]-[74].

### *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 [75]-[97]. The culture supernatant was collected and stored at the same temperature after centrifugation at 10,000 rpm for 10 minutes at 4 C .[98-125]

### *Enzyme assays*

The crude extracts were checked by LiP, MnP, and laccase activity. The H<sub>2</sub>O<sub>2</sub>-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 utilizing phenol red as a substrate [126-149]. 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 [150-175].

#### *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 [176-181]. Important parameters Like spores 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 [182-189].

#### *Chemicals and Culture medium*

All factors, FeCl<sub>2</sub>·4H<sub>2</sub>O, FeCl<sub>3</sub>·6H<sub>2</sub>O, NaOH, HCOOH (88% w / v) and low molecular weight chitosan (deacetylation 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: K<sub>2</sub>HPO<sub>4</sub>, 1.73; KH<sub>2</sub>PO<sub>4</sub>, 0.68; MgSO<sub>4</sub>·7H<sub>2</sub>O, 0.1; Sodium chloride, 0.1; FeSO<sub>4</sub>·7H<sub>2</sub>O, 0.03; NH<sub>4</sub>NO<sub>3</sub>, 1.0; CaCl<sub>2</sub>·2H<sub>2</sub>O 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 decolourization 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 [190-199]. The dye is filtered and sterilized before being introduced to the sterilized PDA at a concentration of 0.05g/L. A 0.5cm<sup>2</sup> 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 the colour changes 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 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. Using a UV-visible spectrophotometer, the supernatants were examined for changes in absorption at the dye absorption maximum (Shimadzu). Cibacron dye has a maximum wavelength of 365nm. The following equation was used to get the percentage of decolourization: (Initial absorbance - Observed absorbance/ initial absorbance)/100

#### *The activity of fungal consortium on effluents*

White rot fungi have also been used to remove many textile dyes, and fungal strains capable of several decolorizing 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 colour absorption, was first reported in 1980. Both are obvious instances of colour 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 various resistant xenobiotic chemicals, including dyes. Lignin peroxidase and Mn-dependent peroxidase or laccase enzymes are involved in colour removal<sup>42</sup>. 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 live cell biodegradable dyes and dead cells (fungal cells) absorbing colours. 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) within the cell wall. Live and dead biomass can be used (dried, heat-killed, acid-treated and / or chemically treated). The decolourization capacity 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 colour by up to 20%. The capacity of the fungus *L. Elegans* KSG32 to decolourize was found to be 80 and 70 percent, respectively 47-50. White-rot fungi's lignin-degrading system comprises extracellular enzymes such as laccases, peroxidases, and oxidases<sup>51</sup>. They may decompose phenolic chemicals and synthetic dyes, among other organic pollutants<sup>47,52,53</sup>. It has been reported that *L. elegans* KSG32 can destroy a wide range of synthetic colours (figure 1).

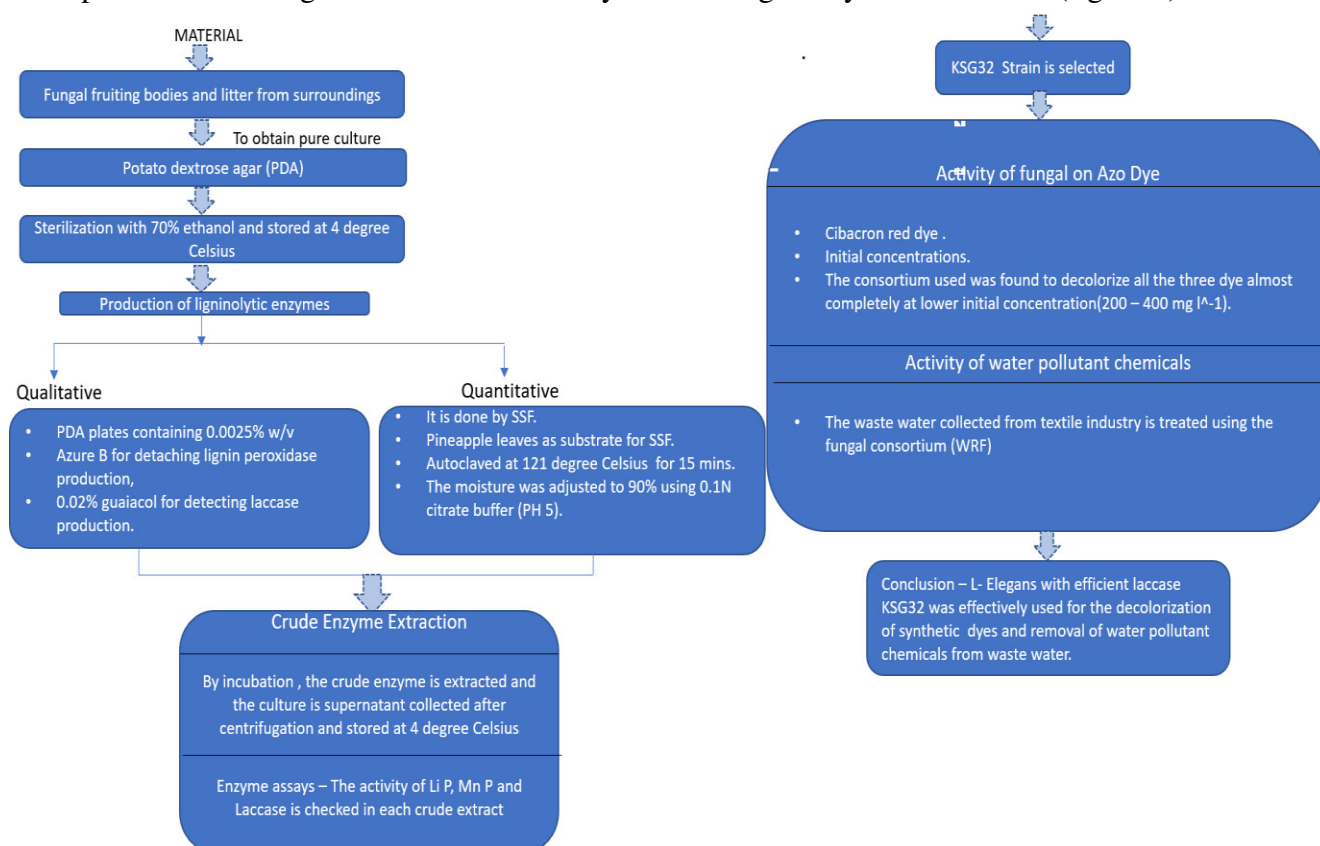


Fig. 1. The activity of fungal consortium on effluents

### 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 strain on white-rot fungi its degradation of the variable composition of wastewater from the textile industry in the modern-day are several research institution and group are developing some fungi for more efficient on processed. The complete aromatic compound such as red dye is possible, which is the cumulative effect of di-degrading enzymes. The degradation potential of Cibacron red dye is investigated. Each colour is only effective at low concentrations after screening *L. elegans* KGS32 for this aromatic and enzymatic profile, the decolorization wastewater and red dye (figure 2).

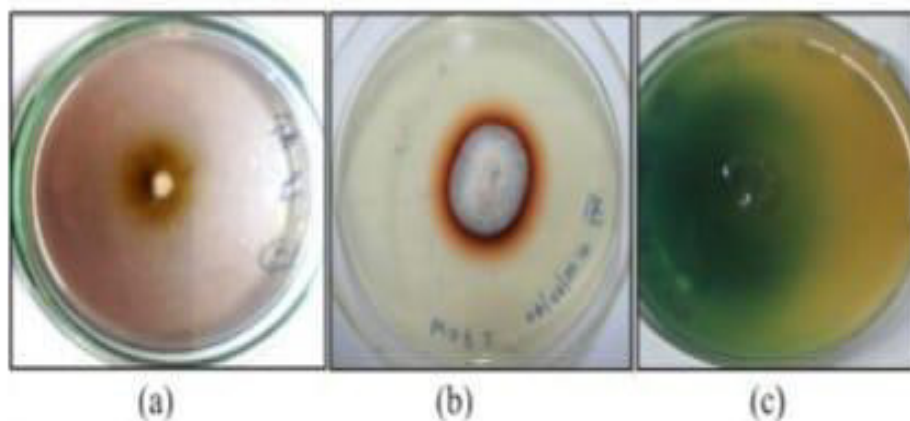


Fig. 2. The result after the screening of *L. elegans* KGS32

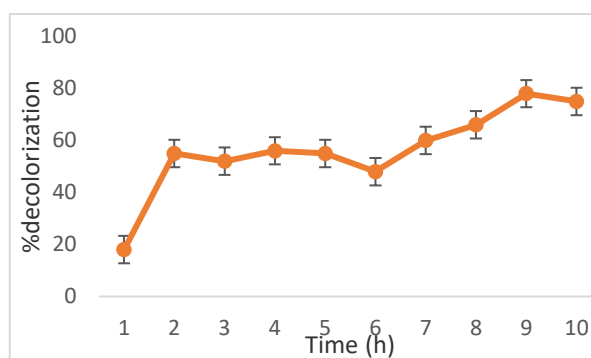


Fig. 3. The relationship between time and decolorization percentage of Cibacron red dye

The decolourization effect on Cibacron red dye when added at the inoculation time to the *L. Elegans* culture (figure 3).

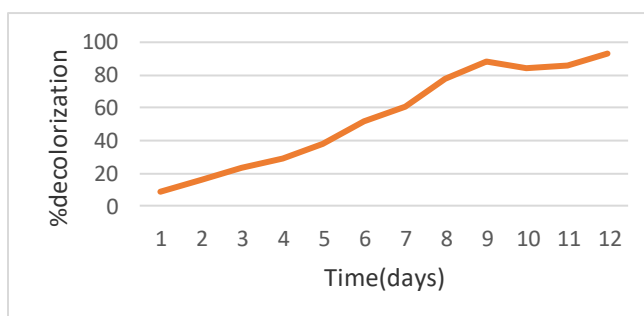


Fig. 4. Relationship between days and decolourization of Cibacron red dye

The following graph shows the decolourization of Cibacron red dye when added to 5 days old *L. Elegans* KSG32 culture (figure 4).

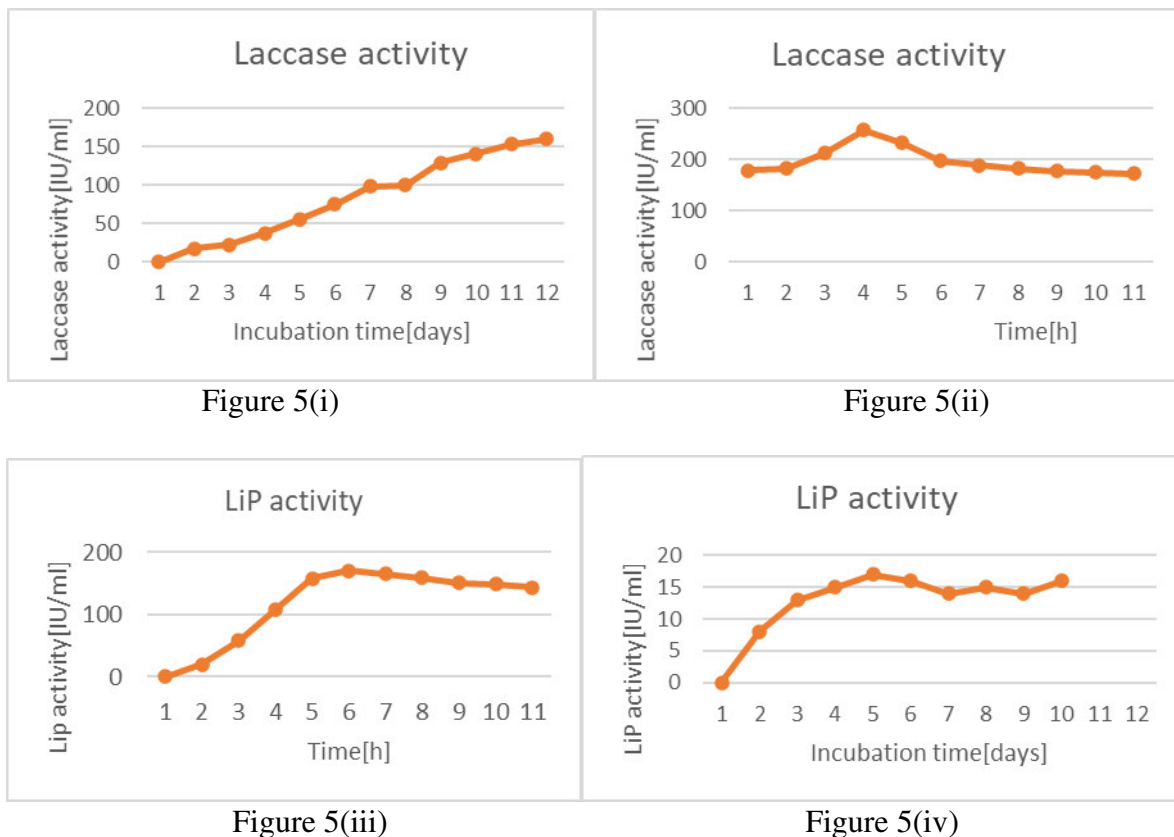


Fig. 5. Laccase and LiP activity

Figure 5 depicts the enzyme activity on Cibacron red dye-containing agar media during the organism's development.

3rd diagram

Figure 5(i) depicts the incubation activity by day.

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

Figure 5(iii): This graph depicts the activity of the Lips on each day of incubation.

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

#### *Enzyme activity during decolourization*

Figure 3 depicts how enzyme activity plays an important role in organism development. The current research reveals an effect on visual appearance in the adsorption of colours on mycelium laccase, and the presence of lipase enzymes was seen and 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 decolourization. The enhanced laccase activity in the presence of Cibacron red dye is confirmed.

### *Decolourization of Cibacron red dye*

In decolourization research, the toxicity of dye fungal growth plays a key role. Compared 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 decolourization tests by comparing the biomass content of *L. elegans* KSG32 after 12 days of development in PDB with and without dye. If dye concentration increases, the fungal growth is affected by studies using Cibacron red, and it can reduce biomass to toxicity. According to the average concentration, 0.4g/l has been reported in the textile effluent. According to the results, the dyes employed for their investigation did not have any hazardous effluent *L. Elegans* KSG32 development. Instead, the colour 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. The dye was generally employed on substrates such as wood, nylon, and silk since it had excellent wash characteristics. To get sophisticated Cibacron red dye, the white-rot fungus may mineralize the dye ingredient and decolourize it.

### *Wastewater Fungal consortium*

The fungal strain is *elegans* KSG32; it can dye decolourization and was isolated from different niches. These fungi use 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 waste water, 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 decolourization on two days 48hours. The growth of the fungi is not affected by water. These fungi are found to have a capacity of decolourized 4 g/l concentrates of these dyes. Decolourized rate and kinetics were monitored by spectrophotometry. Parameter of the process: PH, dye concentration, and inoculum size by dye decolourization rate were all investigated. When the inoculum size increases, the process slows down, and the dye decolourization rate drops by 93 percent. The fungus can isolate 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**

Dye decolorizations: *L. Elegans* KSG32, which has laccase and lignin peroxidase activity, is utilized to decolourize synthetic dyes. The dyes are not toxic when induced on organisms. This property is used in bioremediation. Textile waste effluents: Many fungal strains are capable of decolorizing dye wastewater. These were created using a simple, low-cost media and had a high production rate and a high biosorption and degeneration capability. Living cells decolourize through various 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 decolourization is an alternative to the current treatment procedure. White-rot fungi are valuable microorganisms for the 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.

**Conflicts of Interest:** The authors declare that they have no conflicts of interest to report regarding the present study.

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