Biodegradation Of Iron Industry And Gold Industrial Wastewater By White Rot Fungi – *Calocybe Indica* & *Agaricus Bisporus Comparative Study*

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ABSTRACT

Increasing discharge and improper management of liquid and solid industrial wastes have created a great concern among industrialists and the scientific community over their economic treatment and safe disposal. Hence, there is a growing need for the development of novel, efficient, eco-friendly, and cost-effective approach for the remediation for these industries released into the environment and to safeguard the ecosystem. In this regard, recent advances in wastewater of heavy metal have propelled bioremediation as a prospective alternative to conventional techniques. Heavy metals are toxic and dangerous to the ecosystem. White rot fungi (WRF) are versatile and robust organisms having enormous potential for oxidative bioremediation of a variety of toxic chemical pollutants due to high tolerance to toxic substances in the environment. The decolorization and detoxification potential of WRF can be harnessed thanks to emerging knowledge of the physiology of these organisms as well as of the bio catalysis and stability characteristics of their enzymes. This knowledge will need to be transformed into reliable and robust waste treatment processes.

Keywords: Industrial pollutants; Biodegradation; White rot fungi (WRF); Wastewater treatment; Biodegradation studies.

INTRODUCTION

Industrial wastewater is a major source of heavy metal contamination in our environment. Heavy metals are of economic significance in industrial use and the most important pollutants in the environment. Environmental pollution by heavy metals has become a serious threat to living organisms in an ecosystem. Metal toxicity is of great environmental concern because of their bioaccumulation in nature (1). Several inorganic metals like magnesium (Mg), nickel (Ni), chromium (Cr3+), copper (Cu), calcium (Ca), manganese (Mn), and sodium (Na) as well as zinc (Zn) are vital elements needed in small quantity for metabolic and redox functions. Heavy metals such as aluminium (Al), lead (Pb), cadmium (Cd), gold (Au), mercury (Hg), and silver (Ag) do not have any biological role and are toxic to living organisms (2).

Several factors which influence, and limit biodegradation efficiency include temperature, pH, redox potential, nutritional status, moisture, and chemical composition of heavy metals. The use of microbes alone has shown limited efficiency owing to various factors including poor competitiveness as well as excessive heavy metal concentrations. Effectiveness can be enhanced by several amendments with inorganic nutrients, biosurfactants, bulking agents, and compost as well as biochar (3).

WRF are a physiological group comprising fungi that are capable of biodegrading lignin and the name white rot derives from the white appearance of the wood attacked by WRF, where lignin removal gives ableached appearance. Taxonomically, WRF is mostly basidiomycetes, although few ascomycetes are also capable of white-rot decay (4). The extracellular and non-specific lignin mineralizing enzymes (LMEs) and low-molecular mass mediators enhance the bioavailability of pollutants to WRF. Property of WRF to withstand a wide range of pH further enhances their pollutant degradation capabilities (5).

The white rot fungus is milky white to pale yellowish white in colour. Cap is 40-70 mm in diameter, broadly convex, becoming flat when grows; surface initially white to

yellowish white, becoming greyish to greyish orange, smooth, glabrous; margin entire. Gills sinuate to narrowly adnate, white, crowded with numerous lamellulae. Stipe 45-95 × 10-21 mm, fleshy, often sinuate, expanding towards base; surface smooth. Spore print is white (6).

Calocybe indica is studied for its effect in decolorization of industry effluents and proved to be a promising potential for effluent decolourization and remediation. Thus, it can help in improving the quality of wastewater released into streams and rivers. This was not much explored option before; however, it can be now used for biodegradation (7). *Agaricus bisporus* (Lange) Sing commonly known as mushroom, is the most widely cultivated and one of the most economically important mushrooms in the world, which accounts for about 25% of the world's total output of edible fungus. The biodegradation processes that could justify the removal of heavy metals when *Agaricus bisporus* is used as a bioremediating agent (8).

In this study, aqueous extracts of *Calocybe indica* & *Agaricus bisporus* were prepared and analysed for its degradation activity. For the prepared sample UV spectroscopic study, Thin Layer Chromatography and Lacasse study was also carried out (9).

MATERIALS AND METHOD

Chemicals

Malt Agar, meat extract powder, yeast extract powder, glucose, peptone, dihydrogen phosphate, sodium chloride, calcium carbonate, monosodium phosphate, disodium phosphate and guaiacol were the chemicals used. All the chemicals and the medium compounds were purchased from Hi-media (Mumbai) and Sigma Aldrich (USA)**.** They were of analytical grade. The chemicals were used as received, without any further purification.

Sample collection

The gold and iron effluent samples were obtained from local gold workshop and iron workshop in and around Coimbatore. The samples were collected in sterile containers and stored at room temperature. The white rot fungi samples were collected from the Mushroom Research and Training Centre, Department of Plant Pathology, Tamil Nadu Agricultural University, Coimbatore - 641 003, Tamil Nadu, India.

Sub-culturing

Sub-culturing was done using 2% Malt agar maintained at 4^{o}C (10). Malt Agar was freshly prepared and poured in sterile petri plates. The agar was let cool down. Later, to the malt agar added small pieces of the fruit body of the mushroom and kept for growth in sterile conditions

Extraction Process

Mushroom extraction was carried out using distilled water. Mushroom specimens were cut into small pieces and sun dried. 10gm of the dried mushroom pieces were mashed using a sterile mortar and pestle. To the crushed mushroom added 100ml of distilled water and centrifuged. The supernatant was used as the source for enzyme. (11).

Screening for biodegradation

200ml of Kirk's media was prepared (12). Taken the freshly prepared media as 50ml each in four Erlenmeyer flasks. To two of the flasks added 5ml of the iron effluent sample and 2ml of the aqueous extract of *Calocybe indica* and *Agaricus bisporus* in each of the flask. To the next two of the flasks added 5ml of the gold effluent sample and 2ml of the aqueous extract of *Calocybe indica* and *Agaricus bisporus* in each flask.

Production of Laccase from the Fungi

Laccase activity in the extract was determined (13). To 1ml of fermented aqueous solution, added 1ml of phosphate buffer. This is mixed well and is incubated for 5 minutes at room temperature. After 5 minutes added 0.5ml of guaiacol solution and mixed well. This

mixture was incubated at room temperature for 1 hour. After an hour the optical density was obtained at 480nm.

UV – Vis Spectrophotometer analysis

The concentration of the biodegraded water was determined by UV-Spectrophotometer with a wavelength of 520 nm respectively (14). The model used was Microprocessor UV-VIS Labtronics LT – 291.

TLC Analysis

The aqueous extracts were spotted on the commercially available thin layer chromatography (tlc) plates. The spotting's were marked using a pencil. It was spotted approximately around 25-30 times to mark the extract strongly. The plates were run in the solvent system of methanol, ammonia, n-butanol and water in the ratio of 4:3:2:1 respectively (14). The chromatograms were viewed in the iodine chamber and RF value was calculated.

RESULTS

Screening for biodegradation

The fungal spore isolates were inoculated and was transferred to a sterilized Erlenmeyer flask. It was incubated at 27^{o}C 5-7 days. They were screened for testing their ability for industrial dyes biodegradation (Figure 1).

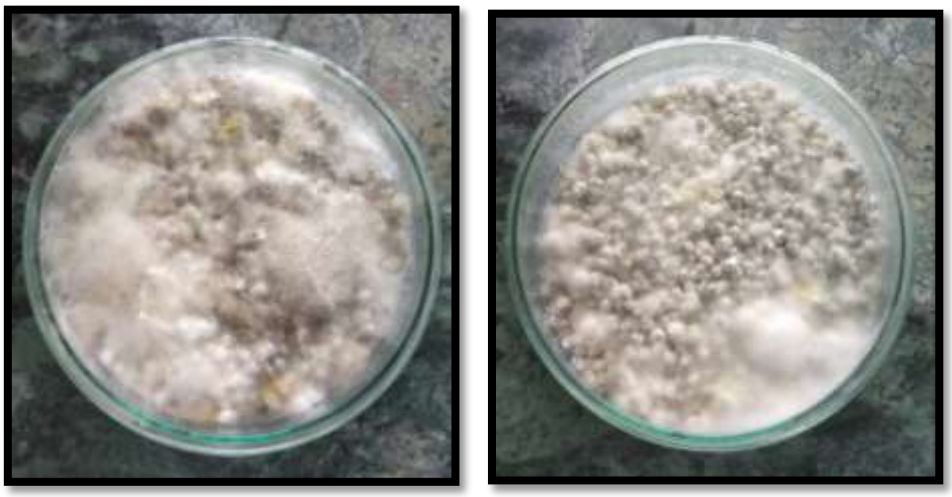

Figure 1 – Isolation of Fungi

Laccase activity

Laccase was found to be the predominant, the colour development in the extract indicated the oxidation of guaiacol, which confirms the presence of laccase (Table 1).

Table – 1 Laccase activity

Sample	***Calocybe indica* (Milky white mushroom) (U/ML)**	***Agaricus bisporus* (Button mushroom) (U/ML)**
IRON	0.820	0.768
GOLD	0.429	0.542

Mushrooms play a key role in the recycling of organic materials in the ecosystem by producing potent degradative enzymes. The degradative power of mushrooms has been exploited in the removal of various toxic and/or polluting organic chemicals of anthropogenic origin. Among the organic chemicals, synthetic dyes in industrial effluent are known to disturb aquatic environments by inhibiting photosynthesis of aquatic plants and exerting toxicity toward aquatic animals.

Ultraviolet visible (UV-Vis) analysis

The UV-Vis spectra results showed that there was a decrease in the visible range (400-800 nm) which is characteristic for the dye colour and formation of the peak at 520 nm, suggesting that decolorization occurred through breakdown of the azo bond responsible for the dye colour (Table 2).

Table 2 - UV-Vis spectra analysis

S.no	**Sample**	**Control (nm)**		**Experimental (nm)**	
		Gold	**Iron**	**Gold**	**Iron**
1	*Calocybe indica*	0.125	0.198	0.110	0.127
2	*Agaricus bisporus*	0.125	0.198	0.108	0.074

Using distilled water as a blank, Aliquots of sample 5-6ml volume of clear dye solution were prepared and absorbance was analysed using UV- Visible Spectrophotometer (Perkin Elmer Spectrophotometer). Decolorization can be determined within absorbance of wavelength 520nm (Figure 2).

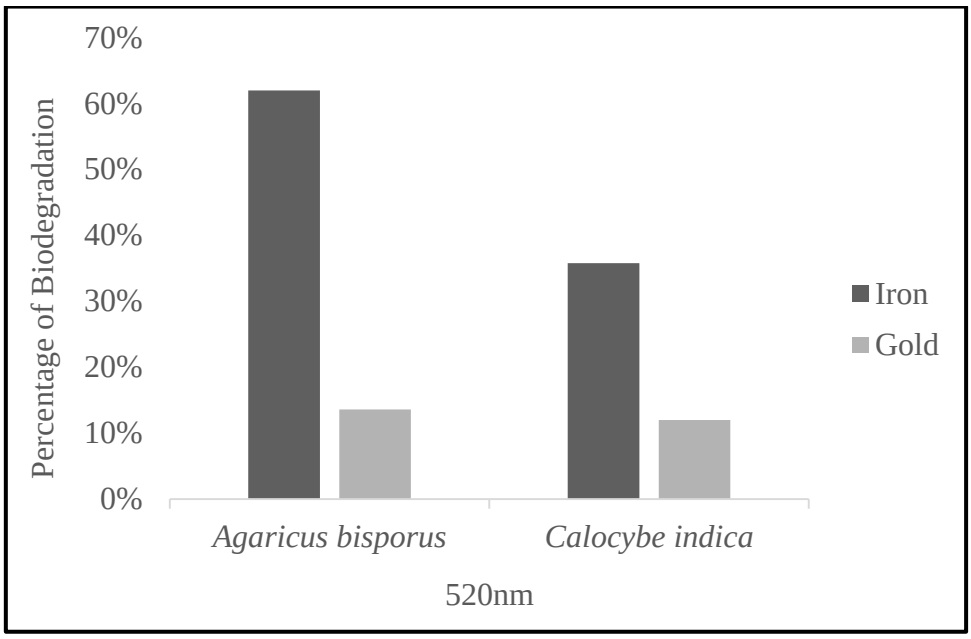

Figure 2 – UV vis analysis graph

TLC Analysis

Using TLC various degraded products were analyzed. In this study degradation products found were Nitrobenzene, 1-amino 2-hydroxyl naphthalene, 4-amino -3- hydroxy benzyl sulfonyl by comparing of retention time (Solvent - methanol, ammonia, n-butanol and water in the ratio of 4:3:2:1).

From the previous studies it exhibited that in the EAE of *Calocybe indica*, when run on chloroform: Methanol: Acetic Acid: 90:10:1 solvent system, four spots were visualized under UV 366 nm with Rf values of 0.89, 0.85, 0.80 and 0.65, along with seven spots under UV 254 nm with Rf values of 0.89, 0.85, 0.80, 0.65, 0.32, 0.15, and 0.07. The common spots with their Rf values were 0.89, 0.85, 0.80, and 0.65. Therefore, seven spots or compounds were present in the EAE of this mushroom. Four standard compounds (gallic acid, cinnamic acid,

quercetin, and ascorbic acid) were used along with the sample in this solvent system. Among the compounds, cinnamic acid gave one spot with an Rf value of 0.65 under both UV light, matching perfectly with one spot of one compound of EAE (Rf 0.65).

Therefore, we may note that of the seven compounds, one may be cinnamic acid. When EAE and standard compounds were run in chloroform: ethyl acetate: acetic acid:: 50:50:1 system, it gave four spots under UV 366 nm with Rf values of 0.77, 0.70, 0.58, and 0.25 and eight spots under UV 254 nm with Rf values of 0.77, 0.70, 0.58, 0.25, 0.83, 0.42, 0.17, and 0.11.The common spots with their Rf values were 0.77, 0.70, 0.58, and 0.25. Therefore, eight spots or compounds were present in the EAE of this mushroom. We ran four standard compounds (gallic acid, cinnamic acid, quercetin and ascorbic acid) along the sample in this solvent system. Of these compounds, gallic acid gave one spot with an Rf value of 0.17 under UV 254 nm light, matching perfectly with one spot of one compound of EAE (Rf 0.17). The Rf value of quercetin was 0.40, while one spot of one compound of EAE (Rf) (0.42 under 254 nm UV) was close to it. Therefore, EAE also may contain quercetin and gallic acid.

The data also show that seven spots (one in UV 366 nm and six in 254 nm light) with Rf values of 0.78, 0.80, 0.68, 0.64, 0.57, 0.50, and 0.41 were present in EAE in butanol: acetic acid: water:: 20:10:5 solvent system. Here, the Rf exactly with one spot of EAE under 254 nm UV light. Under these three solvent systems, EAE of *C. indica* showed eight compounds(spots) and four compounds, which might be cinnamic acid, gallic acid, quercetin, and ascorbic acid

For *Calocybe indica* (Milky white mushroom)

Table 3 – TLC analysis for *Calocybe indica*

Sample	Solvent travel	Compound travel
Aqueous treated Iron (I)	4.7 cm	0.7 cm
Aqueous treated Gold (G)	4.5 cm	3.2 cm
Control (M)	4.5 cm	0.5 cm

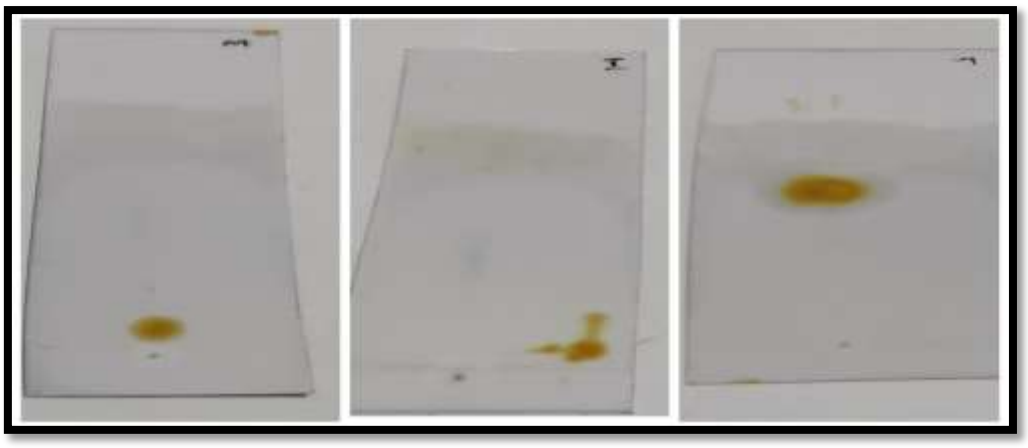

Figure 3 – TLC analysis for solvent for Iron and Gold

Table – 4 TLC analysis For *Agaricus bisporus* (Button mushroom)

Sample	Solvent travel	Compound travel
Aqueous treated Iron (I)	5.2 cm	5.0 cm, 0.7 cm
Aqueous treated Gold (G)	5.1 cm	2.5 cm
Control (M)	5.0 cm	4.7 cm, 4.5cm

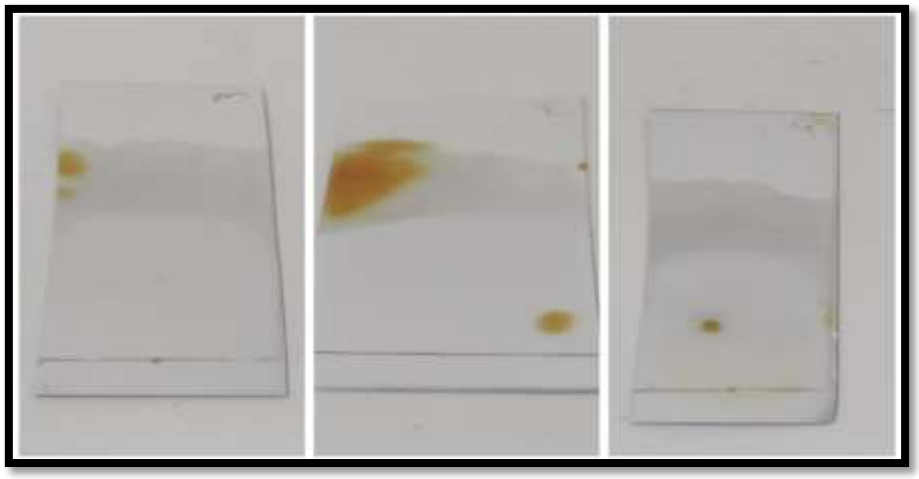

Figure 4 – TLC analysis for solvent for Iron and Gold for *Agaricus bisporus* (Button mushroom)

CONCLUSION

Our present investigation demonstrates that this edible mushroom could degrade both the effluents with enzymatic systems involved in the decolorization of these dyes in liquid medium. Further study of the mechanism of the degradation of other dyes with similar

structures by this mushroom is underway. So that this macro fungi can be used for the treatment of waste water in industries. These fungi are cheap and effective considerably.

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