



## **Nanomicelled Yeast in Adsorption of Heavy Metals and Dyes**

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### **ABSTRACT**

*Industrial activities, particularly textile dyeing and metal processing, are major contributors to environmental pollution through the release of synthetic dyes and heavy metals into soil and aquatic ecosystems. Addressing this challenge requires sustainable, cost-effective, and eco-friendly remediation approaches. In the present study, *Trichosporon asahii*, a yeast strain isolated from industrial effluent-contaminated soil, was evaluated for its dual potential in degrading the azo dye Congo Red and tolerating heavy metals such as lead ( $Pb^{2+}$ ) and iron ( $Fe^{2+}$ ). To enhance stability and reusability, yeast cells were immobilized using sodium alginate. Congo Red degradation was monitored by colorimetric analysis, while growth assays demonstrated *Trichosporon asahii*'s ability to survive under lead and iron stress. Inductively Coupled Plasma Mass Spectrometry (ICP-MS) confirmed significant biosorption of  $Pb^{2+}$  and  $Fe^{2+}$ . Laccase activity, assessed using guaiacol as a substrate, indicated the involvement of oxidative enzymes in the degradation process. The results highlight the efficiency of immobilized *Trichosporon asahii* in simultaneous dye degradation and heavy metal removal, emphasizing its potential as a sustainable bioremediation agent. This study demonstrates the promise of yeast-based technologies for integrated pollution management and lays the groundwork for advanced microbial remediation strategies.*

**Keywords:** *Trichosporon asahii*, bioremediation, Congo Red, lead, iron, laccase, immobilization

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### **INTRODUCTION**

Rapid industrial growth has led to severe pollution, with synthetic dyes and heavy metals posing major environmental and health hazards due to their toxicity, persistence, and bioaccumulation [7] [9]. Textile effluents often contain azo dyes such as Congo Red, while metal-based industries discharge Pb, Fe, Cd, Cr and other heavy metals which are associated with mutagenic and carcinogenic effects [1] [5] [13].

Conventional remediation methods are costly and inefficient at low concentrations [3][14], whereas microbial bioremediation offers a sustainable alternative [10]. Yeasts, owing to their resilience and functional cell wall groups, remove pollutants via biosorption, bioaccumulation, and enzymatic degradation [9][11]. Laccases play a vital role in dye degradation, while sodium alginate immobilization enhances microbial stability and reusability [4] [5] [8][11][12]. This study evaluates *Trichosporon asahii* for Congo Red degradation and  $Pb^{2+}/Fe^{2+}$  removal, emphasizing immobilization and laccase activity as eco-friendly remediation strategies.

### **MATERIAL AND METHODS**

#### **Collection of Metal-Contaminated Soil**

Soil samples were collected from electroplating and metal-processing industrial areas of Balanagar, Hyderabad (10–15 depth), stored in sterile containers, and transported under controlled conditions for microbial isolation.

#### **Isolation of Yeast**

Yeasts were isolated by serial dilution ( $10^{-3}$  and  $10^{-4}$ ) and spread plating on Sabouraud's agar with streptomycin and Rose Bengal. Plates were incubated at  $28 \pm 2$  °C for 72 h, and distinct colonies were selected for further study."

#### **Morphological and Biochemical Identification**

The isolate was examined with LPCB staining, maintained on YPD agar, and biochemically identified using the VITEK 2.0 system from standardized YPD suspensions."

#### **Metal Tolerance and Dye Degradation Potential of Yeast**

The isolate was tested for tolerance to lead ( $Pb^{2+}$ ), Iron ( $Fe^{2+}$ ) and for Congo Red Dye degradation by culturing in metal-supplemented YPD broth and Congo Red Dye-containing media, with growth and decolorization monitored.[5]

### Immobilization of Yeast

Cells grown in YPD broth ( $OD_{600} \approx 1.0$ ) were harvested by centrifugation (5000 rpm, 10 min), resuspended in 2% (w/v) sodium alginate solution, and dropped into 75 mM Calcium Chloride Solution to form beads. Beads were hardened (30 min), rinsed with 5 mM Calcium Chloride Solution, and stored at 4 °C. Immobilization improved viability, biosorption, and recovery. [1]

### Heavy Metal Biosorption Studies

Biosorption was evaluated in YPD broth containing 0.1% Lead or Iron under three conditions: (i) control (medium + metal, no yeast), (ii) free yeast cells, and (iii) immobilized yeast beads. Cultures were incubated at  $28 \pm 2$  °C with continuous shaking for 5 days. Supernatants were collected post-filtration and analysed for residual Lead and Iron by Inductively Coupled Plasma Mass Spectrometry (ICP-MS). Metal removal efficiency was calculated relative to controls.

### Dye Degradation

Congo Red degradation was tested under three conditions: (i) control (medium + metal, no yeast), (ii) free yeast cells, and (iii) immobilized yeast beads. Cultures were incubated for 5 days at  $28 \pm 2$  °C with continuous shaking. Decolorization was monitored visually and spectrophotometrically. Laccase activity was assessed from cell-free supernatants using guaiacol as substrate [6]. Thin Layer Chromatography (TLC) was performed to identify degradation products [2].

### Laccase Assay

Reaction mixtures contained 3 mL 100 mM acetate buffer, 1 mL 10 mM guaiacol, and 1 mL cell-free supernatant. Absorbance was measured at 470 nm (blank) and 530 nm (test) over 10-minute incubation period to track changes in absorbance. Laccase enzyme activity was expressed in units per millilitre (U/mL), where one unit is defined as the amount of enzyme required to produce one micromole of the coloured oxidation product per minute per millilitre of reaction mixture. [6]

### Thin Layer Chromatography (TLC)

Treated and untreated Congo Red samples were spotted on silica gel TLC plates. Plates were developed in methanol: water (7:3) and visualized in iodine chambers. The appearance of new spots or altered Rf values compared with controls indicated dye degradation [2].

## RESULTS AND DISCUSSION

### Isolation and Identification of Yeast

The yeast from metal-contaminated soil in Bala Nagar, Hyderabad, was identified as *Trichosporon asahii* via LPCB staining and colony morphology, confirmed by VITEK 2.0. This species is known for pollutant degradation due to metabolic versatility and resilience [5][9]. [Fig 1 & 2]

### Immobilization of Yeast

Sodium alginate immobilization produced stable beads, enhancing yeast viability, stability, and pollutant contact, consistent with reports of improved microbial biosorption and degradation. [Fig 3]

### Heavy Metal Biosorption

Both free and immobilized yeast reduced lead (Pb) and iron (Fe), with immobilized cells showing higher efficiency due to increased surface area and protection from environmental stress, maintaining stability and activity. [Fig 4&5]

### 4.4 Inductively Coupled Plasma Mass Spectrometry (ICP-MS) Analysis

The efficacy of *Trichosporon asahii* in adsorbing heavy metals specifically lead (Pb) and iron (Fe) was evaluated using Inductively Coupled Plasma Mass Spectrometry (ICP-MS). Removal efficiencies (calculated by mass-balance equation) [15] corroborated the superiority of immobilized yeast.

$$\text{Removal Efficiency(\%)} = \frac{C_i - C_f}{C_i} \times 100$$

Where:

- $C_i$  = Initial metal concentration (ppm)
- $C_f$  = Final metal concentration after treatment (ppm)

The initial lead concentration of 766.80 ppm was significantly reduced to 9.60 ppm by free yeast and 8.97 ppm by immobilized yeast, corresponding to removal efficiencies of 98.75% and 98.83%, respectively. [Graph 1&2]

The initial Iron concentration of 964.86 ppm was significantly reduced to 161.34 ppm by Free Yeast and 58.45 ppm by Immobilized Yeast, corresponding to removal efficiencies of 83.28% and 93.94% respectively. [Graph 3&4]

Immobilized yeast showed higher lead and iron biosorption than free yeast cells, reflecting the benefits of cell entrapment combined with inherent metal-binding capacity. The enhanced performance of

immobilized cells can be attributed to the synergistic effect of the physical entrapment and the yeast's inherent biosorption capabilities.

### Congo Red Dye Degradation Analysis

Controls showed no visible change. Free *T. asahii* caused slight decolorization, while immobilized cells produced marked decolorization, indicating enhanced dye removal. The alginate matrix likely promotes mass transfer and stabilizes extracellular oxidative activity, consistent with earlier reports of improved azo dye degradation by immobilized yeasts. [Fig 6]

### Laccase Enzyme Assay

The calculated laccase activity showed that Immobilized Yeast exhibited higher enzymatic activity (0.00297 U/mL) compared to Free Yeast (0.00237 U/mL), confirming its enhanced potential for effective Congo Red dye degradation. [Fig 7]

Calculation: [6]

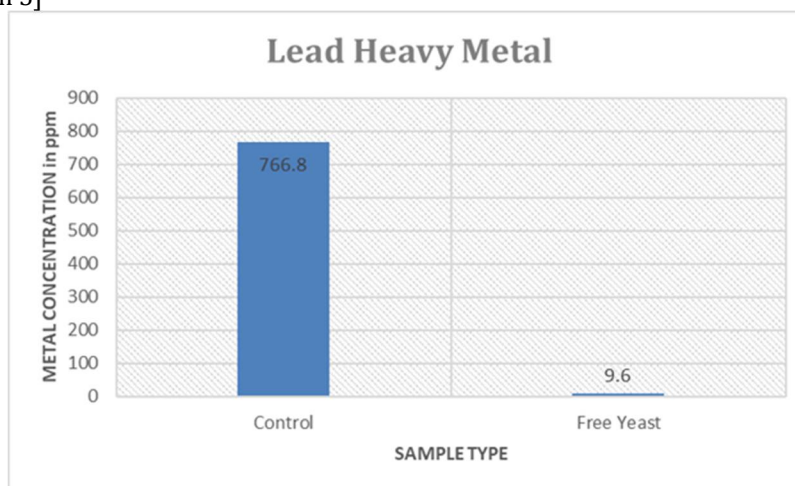
$$\text{Volume Activity (U/ml)} = \frac{\Delta A_{470}/\text{min} \times 4.0 \times V_t \times \text{Dilution Factor}}{\epsilon \times V_s}$$

Where:

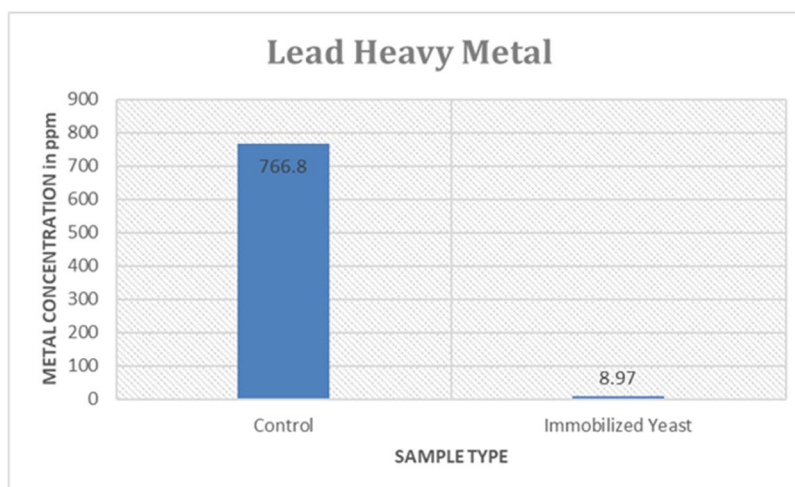
- $V_t$  = Total reaction volume = 5.0ml
- $V_s$  = Sample volume = 1.0ml
- $\epsilon$  = Extinction coefficient of guaiacol ( $6740.0\text{M}^{-1}\text{cm}^{-1}$ )
- 4.0 = Derived constant
- Dilution Factor = 1.0

### Thin Layer Chromatography (TLC) Analysis

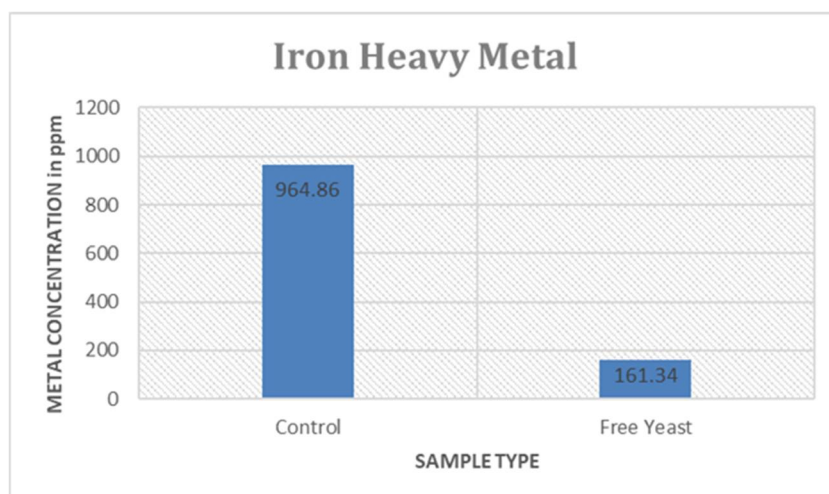
TLC of treated samples showed new spots and shifted Rf values, indicating Congo Red degradation into polar metabolites, supported by elevated laccase activity, suggesting enzymatic oxidation and adsorption [2]. [Fig 8] [Graph 5]



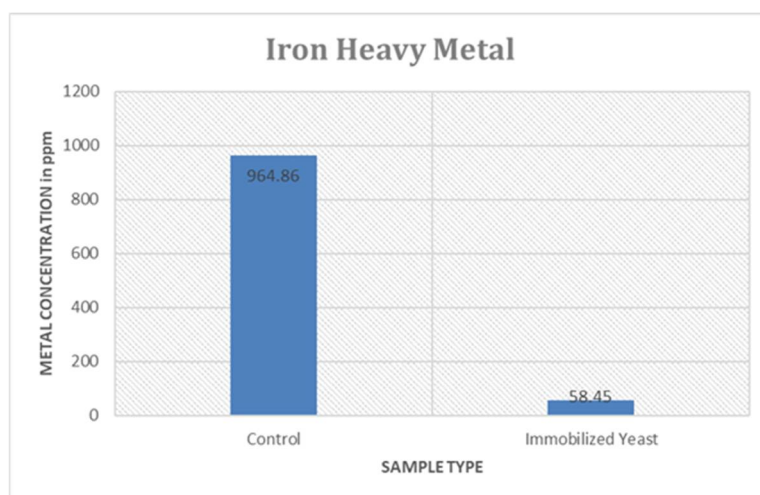
Graph 1: Graph depicting Lead Concentration in Control and Free Yeast-Treated Sample



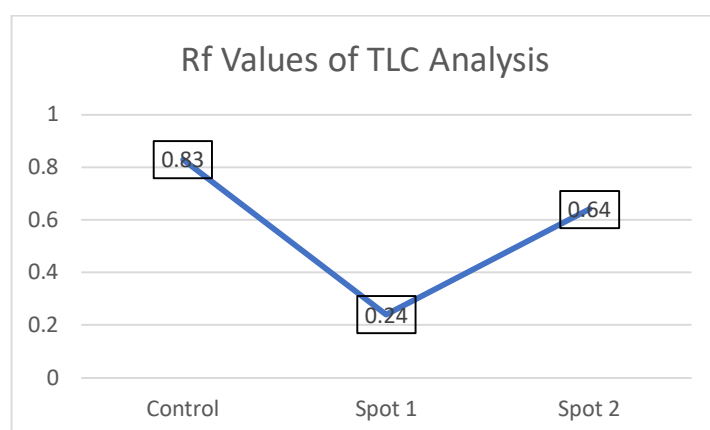
Graph 2: Graph depicting Lead Concentration in Control and Immobilized Yeast-Treated Sample



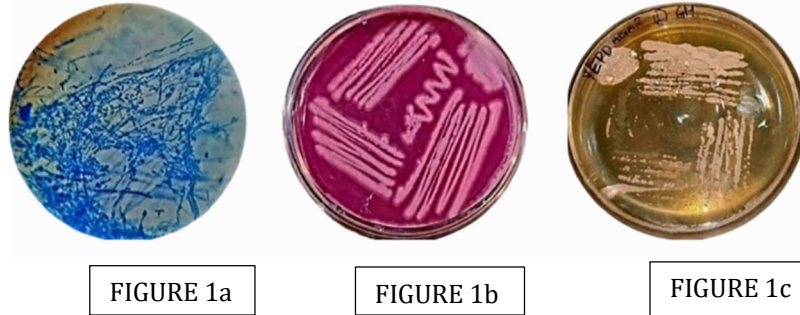
Graph 3: Graph depicting Iron Concentration in Control and Free Yeast-Treated Sample



Graph 4: Graph depicting Iron Concentration in Control and Immobilized Yeast-Treated Sample



Graph 5: Line Graph Representing Rf Values Obtained from TLC Analysis of Congo Red Degradation in Control and Treated Samples.



**Fig 1.** Microscopic view of Lactophenol Blue Wet Mount of *Trichosporon asahii* **(a)**, Colony Morphology of *T. asahii* plated on Sabouraud's Agar **(b)**, Isolated Yeast on Yeast Potato Dextrose Agar **(c)**.

**FIGURE 2:** VITEK Report of *Trichospron asahii*



**FIGURE 3:** Immobilized Sodium Alginate Beads of *Trichosporon asahii*





FIGURE 4a

FIGURE 4b

FIGURE 4c

**Fig 4.** Flask containing Free Yeast with Lead Heavy Metal **(a)**, Flask containing Immobilized Yeast and Lead Heavy Metal **(b)**, Flask containing only Lead Heavy Metal (control) **(c)**.



FIGURE 5a

FIGURE 5b

FIGURE 5c

**Fig 5.** Flask containing Free Yeast with Iron Heavy Metal **(a)**, Flask containing Immobilized Yeast and Iron Heavy Metal **(b)**, Flask containing only Iron Heavy Metal (control) **(c)**.



FIGURE 6a

FIGURE 6b

FIGURE 6c

**Fig 6.** Flask containing Free Yeast with Congo Red Dye **(a)**, Flask containing only Congo Red Dye (Control) **(b)**, Flask containing Immobilized Yeast with Congo Red Dye; depicting a colour change **(c)**.



1,

2

**Fig 7** Tube-1 indicating the Control sample showing no colour change and Tube-2 indicates the development of colour due to oxidation of Guaiacol by Laccase.



**Fig.8** Spot 1 depicts the control sample indicating single spot and Spot 2 depicts Treated sample, shows different spots indicating breakdown of compounds.

## CONCLUSION

*Trichosporon asahii* is a promising candidate for heavy metal and dye bioremediation. Sodium alginate immobilization enhanced stability and removal efficiency, with combined biosorption and enzymatic degradation offering a cost-effective, sustainable approach for treating industrial effluents.

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## CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest related to this work. The study was carried out purely for academic and research purposes, without any financial or commercial interests influencing the results.

## AUTHOR'S CONTRIBUTION

S.V.S.R. Lakshmi Lavanya (First Author): Executed the experimental work, performed data collection, analysis, and interpretation, and prepared the manuscript.

Dr. V. GAYATHRI (Corresponding Author): Provided supervision, conceptual guidance, resources, critical review, and final approval of the manuscript.

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## ETHICS STATEMENT

This study did not involve experiments on humans or animals; hence, ethical approval was not required.

## INFORMED CONSENT

Not applicable, as the study did not involve human participants.

## DATA AVAILABILITY

All data generated or analysed during this study are included in the manuscript. No external datasets were used.

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