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## Fungal Biomass as Biosorbent for Removal of Heavy Metal from Industrial Wastewater Effluent

<sup>1</sup>Shipra Jha, <sup>1</sup>Ritu Chauhan and <sup>2</sup>S.N. Dikshit

<sup>1</sup>Amity Institute of Biotechnology, Amity University, Noida, Ghaziabad, (Uttar Pradesh), India

<sup>2</sup>Shrimant Madhavrao Scindia Government Science College, Jiwaji University, Gwalior, India

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**Abstract:** In this study, selective biosorption of Cu ions by micro-organism from industrial wastewater were investigated. Micro-organism was isolated and in the study a micro-organism which was identified as *A. lentulus* was used. In this study, the effects of dilution and nutrient supplementation for efficient Cu(II) removal from effluents and initial concentration of metal ion on the biosorption capacity were investigated. Under supplementations, comparatively faster Cu(II) removal by *Aspergillus lentulus* was observed resulting in 97-99.8% removal in 120 h.

**Key words:** Industrial wastewater, heavy metal, *Aspergillus lentulus*, biosorption

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### INTRODUCTION

Pollution caused by heavy metals is increasing with the increased usage of chemicals in industry and agriculture (Velaippan *et al.*, 2002; Akbar and Gupta, 1985; Anonymous, 1950; Akbal and Camci, 2011). The environmental contamination by trace and heavy metals through industrial wastes is one of the main problems in industrial countries. Metal contaminants can easily enter to food chain if contaminated water, soil and plants are used for food production. The industrial effluents generally consist of organic compounds, inorganic complexes and other non-bridgeable substances (Ferre-Huguet *et al.*, 2009; Deng *et al.*, 2007; Elzein *et al.*, 2001; Evans and Rotar, 1987; Goplan *et al.*, 1988). The untreated industrial and domestic waste disposal into environment affects quality of soil and ground water and considered as undesirable soil use (Qazilbash *et al.*, 2006; Jain and Gupta, 1985). These pollutants not only alter the quality of soil and ground water but also pose serious problems (Karthikeyan *et al.*, 2010; Jackson *et al.*, 1996; Kapoor *et al.*, 1989; Gadd *et al.*, 2001). There is rising sense of global urgency concerning the environmental pollution by chemicals arrangement used in various activities (Palaniappan *et al.*, 2009; Misra and Siddiqi, 2004; Paraszkiwicz *et al.*, 2007; Rawat and Hazra, 1999; Salpekar and Khan, 1997; Sayer and Gadd, 1997).

Wastewater generated from industrial treatment plant contains considerable metal contaminants. Their concentration must be reduced to levels before being released into the environment. Rapid industrialization has

led to increase disposal of heavy metal into the environment. These heavy metals entered into the water bodies through waste water from metal plating industries and industries of mining, alloys etc. Therefore, effective recovery of heavy metals is as important as their removal from waste streams (Freeman, 1988).

The removal of metals from industrial effluents can be achieved by ion exchange, chemical oxidation, chemical precipitation etc. (Aksu *et al.*, 1992). As an alternative to these methods, recently the method of the removal of heavy metal contaminants by means of bacteria has been focused. Biological removal of heavy metal contaminants from aquatic effluents offers great potential when metals are present in trace amount (Fourest and Roux, 1992; Panchanadikar and Das, 1993). Many microbial species such as bacteria, fungi, yeast and algae are known to be capable of adsorbing heavy metals on their surface and accumulating within their structure (Campbell and Martin, 1990; Luef *et al.*, 1991; Mitani and Mistic, 1991; Panchanadikar and Das, 1993). It is possible that micro-organisms can be used in the removal of toxic metal ions from the water and even in the recovery of them by using these adsorption properties of the micro-organisms. Accumulation occurs in living cells is slow, related to metabolic activity (Gadd, 1993; Nourbakhsh *et al.*, 2002). Although there are a number of studies on removal of heavy metals, the knowledge of the application of biosorbents in the environment and industry has not been clear yet.

In this study, some important parameters that should be considered in the removal of heavy metals from the industrial waste waters were investigated and an

approach concerning with application to the industrial waste water containing heavy metals have been presented.

## MATERIALS AND METHODS

**Removal of Cu(II) from industrial effluents:** Industrial effluents were collected from two sources: Electroplating effluent from industry located in Malanpur and Banmore area, Gwalior and combined effluent treatment plant (India). Cu(II) removal from effluents was studied using growing biomass.

**Under growing conditions:** Experiments with electroplating effluent were conducted by following two approaches: Dilution and supplementation. To dilute the effluent, composite media was used in the ratio of 1:1 and 1:5 (Effluent:Media). For supplementation, two main components of composite media i.e., glucose and yeast extract were used in different ratios (5 g L<sup>-1</sup> yeast extract +10 g L<sup>-1</sup> glucose; 5 g L<sup>-1</sup> yeast extract+ 0.5 g L<sup>-1</sup> glucose). All the flasks were incubated at 180 rpm and 30°C for 120 h. The crucial media components responsible for maximum Cu(II) removal were identified; a series of effluent supplementations (Yeast extract alone, glucose alone, Yeast extract+Glucose) was tested.

**Statistical analysis:** Data was analysed by one way analysis of variance (ANOVA).

## RESULTS AND DISCUSSION

### Using growing biomass

**Neat effluent:** The effluent was inoculated with fungal isolate and incubated at 180 rpm and 30°C. No fungal growth and Cu removal was observed in the effluent. The concentration of Cu(II) in the effluent was 47 mg L<sup>-1</sup> while that of the total Cu was 93 mg L<sup>-1</sup>. Hence, the reason for

absence of fungal growth in the effluent could be either due to the lack of nutrients necessary to support organism's growth or toxicity due to other pollutants such as Zn, Mn etc (Hatfield *et al.*, 1996).

Fischer revealed the biosorption capacity of both fungal species, significantly increased as the initial concentration of metal ion was raised in the medium. According to them, electroplating effluents have impurities in form of other co-ions in electroplating effluents may compete for binding sites on the fungal cell wall and low pH than acidity may increase the H<sup>+</sup> ions decreasing binding of metal ion on biomass surface (Zhao and Duncan, 1998; Sousa *et al.*, 2009). Hence, it was decided that the effluent must be supplemented with nutrients and diluted to reduce the effective concentration of the co-contaminants (Vidyarthi *et al.*, 2006; Yezza *et al.*, 2006).

**Dilution and nutrient supplementation of effluents:** The diluted effluent (1:1 and 1:5; effluent: composite media (glucose and yeast extract) was inoculated with spore suspension (5% v/v) and incubated at 180 rpm and 30°C for 120 h. Cu(II) concentration was monitored at regular intervals. As shown in Fig. 1, the initial concentration of Cu(II) was effectively reduced due to the dilution.

In 1:1 dilution, the removal took approximately 120 h while in 1:5 dilutions the same was completely in 72 h only. This could be attributed to lower concentration of nutrients in the diluted effluents as compared to the neat growth media. Therefore, further experiments targeted towards optimization of the nutrient supplementation for efficient Cu(II) removal from the effluent.

**Supplementation only:** This series of experiment was set up without any dilution of effluent and series of supplementations was investigated. The effluent was investigated with glucose (carbon source) and/or yeast extract (nitrogen source) (Fig. 2). Glucose

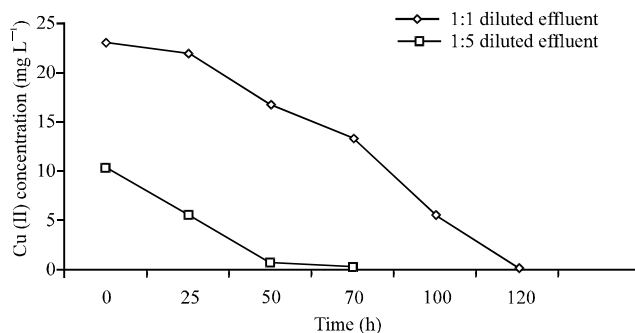


Fig. 1: Removal of Cu(II) from diluted (Effluent:Composite media) and nutrient supplemented EFT effluent using *Aspergillus lentulus*

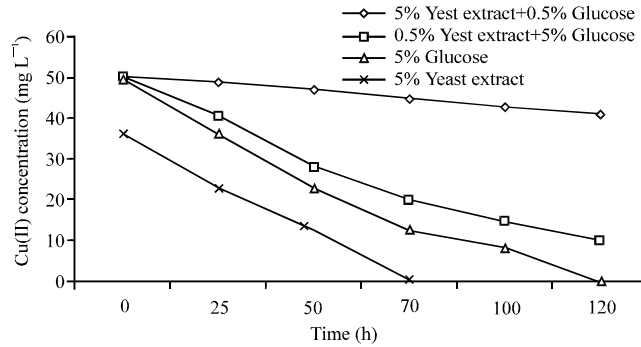


Fig. 2: Removal of Cu(II) from undiluted EFT effluent with different nutrient supplementations using *Aspergillus lentulus*

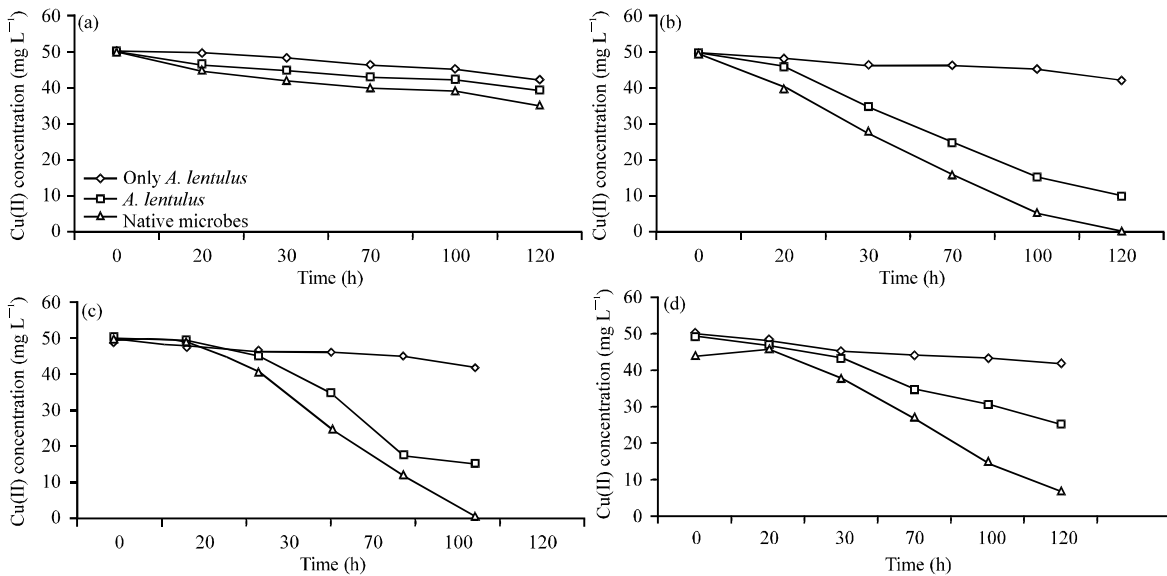


Fig. 3(a-d): Comparison of the role of native microbes, *Aspergillus lentulus* and native microbes+*Aspergillus lentulus* under different supplementations (a) 5 g L<sup>-1</sup> glucose, (b) 5 g L<sup>-1</sup> glucose+0.5 g L<sup>-1</sup> yeast extract, (c) 5 g L<sup>-1</sup> yeast extract and (d) 5 g L<sup>-1</sup> yeast extract+0.5 g L<sup>-1</sup> glucose

supplementation alone did not show good performance and could only remove less copper.

In remaining three flasks where either yeasts extract alone or combination of yeast extract and glucose were supplemented, 92% Cu(II) removal was observed. The combination of 5% yeast extract +0.5% glucose performed best and caused 95% Cu(II) removal within 96 h. Addition of 0.5% yeast extract +5% glucose that caused 89% Cu(II) removal would offer the most economical supplementation option. These results indicate that the performance of *A. lentulus* with nutrient supplemented industrial effluents is comparable to that observed with the Cu(II) amended media.

**Role of native microbes (present in actual effluent) in Cu(II) removal:** For each supplementation series,

Table 1: Role of native microbes and *Aspergillus lentulus* in Cu(II) removal under different nutrient supplementations

Supplementation	Removal (%)		
	Only <i>A. lentulus</i>	<i>A. lentulus</i> + native microbes	Only native microbes
5 Y+0.5 G	98.8	90.2	21
5 G+0.5 Y	87.2	74.3	18
5 G	28.0	40.0	10
5 Y	95.8	88.2	12

unsterilized and non-inoculated effluent counterpart was run to evaluate native flora’s contribution to Cu removal. In almost all the supplementations, Cu(II) removal by native flora alone was insignificant as compared to that by *A. lentulus* (Table 1).

Least Cu(II) removal (10%) was observed in unsterilized, non-inoculated flask (representing native

microbes) and also in sterilized inoculated flask (representing *A. lentulus*) both with only glucose supplementation indicating that neither native flora nor *A. lentulus* were able to perform well with only glucose substrate (Fig. 3a). Interestingly, this was the only supplementation in which the Cu(II) removal in unsterilized inoculated flask (representing *A. lentulus*+ native microbes) was higher than that recorded with only *A. lentulus* indicating a synergistic contribution by native microbes and fungal isolate together.

It is clearly observed that the rate of Cu(II) removal in all the three cases (only native microbes, only *A. lentulus* and native microbes+*A. lentulus*) was slow till initial 50 h in 5 g L<sup>-1</sup> glucose supplementation (Fig. 3a) as well as in 5 g L<sup>-1</sup> glucose+0.5 g L<sup>-1</sup> yeast extract combination (Fig. 3b). Hence, during the initial growth phase, lack of proper nitrogen source have delayed the microbial growth and biomass generation. In other two supplementations, where yeast extract was the dominant component (Fig. 3c, d) the Cu(II) removal by native microbes was comparatively enhanced to 18% (5 g L<sup>-1</sup> yeast extract) and 21% (5 g L<sup>-1</sup> yeast extract +0.5 g L<sup>-1</sup> glucose). Under these supplementations, comparatively faster Cu(II) removal by *A. lentulus* was observed resulting in 97-99.8% removal in 120 h. In the unsterilized inoculated series representing *A. lentulus*+native microbes, comparable Cu(II) removal was observed indicating that the native micro flora did not suppress the performance of *A. lentulus*. If the treatment time is extended beyond 120 h, more complete removal of Cu(II) could have been obtained in these cases also, as the rate of Cu(II) removal between 100-120 h was quite high (Fig. 3c, d). Infact, it is very important observation which demonstrates that *A. lentulus* shows compatibility with the native microbial flora of the effluent. Visual observations of the growth forms indicated presence of turbidity due to growth of native microbes but distinct fungal pellets still dominated the liquid phase.

### CONCLUSION

The present study, findings suggest that sterilization of the particular EFT effluents shall not be required prior to biological treatment with *A. lentulus* making the process simple and cost effective.

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