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Comparative study of Growing/ Immobilized biomass verses resting biomass of *A.lentulus* for the effect of pH on Cu^{2+} metal removal

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ABSTRACT

Biosorption of Cu (II) ions from aqueous solution was studied using *A.lentulus* immobilized by sodium alginate (4.0 g) and gelatin (1.0 g) prepared into a 0.05M $CaCl_2$ solution under constant stirring. Uptake of metal was very fast at 25 min initially and equilibrium was attained within 125 min. Higher Cu(II) uptake was observed by selected biomass (4.0g/l) immobilized in calcium chloride at 35 ^{o}C, 180 rpm when initial Cu^{2+} ions concentration was 100mg/l. The optimum condition of pH, biomass concentration and heavy metal concentration were determine for microbial growth on biosorbents and correlated with heavy metal removal. The observed condition was applied for the biosorption process in immobilized and dead fungal cells. The biosorption of immobilized cells of A. lentulus was 96.6% of Cu whereas the dead cells of A. lentulus were 84%.

Keywords: A lentulus, gelatin, alginate, Cu^{2+} concentration, dead fungal cells, immobilization.

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INTRODUCTION

Heavy metal pollution in wastewater has always been serious environmental problem because heavy metals are not biodegradable and can be accumulated in living tissues. Copper are widely used in various important industrial application. Copper is present in industrial waste primarily in the form of bivalent Cu (II) ion as hydrolysis product. In copper cleaning, copper plating and metal processing, copper ion concentration approach 100-120 mg/l which is very high in relation to water quality standards and permissible [1] Cu (II) concentration (1.0-1.5 mg/l) of wastewater. Copper at extensive concentration is toxic to living organism as humans and other creatures like fish [2]. The removal and recovery of heavy metal from wastewater is important for environmental protection and human health. Conventional methods applied to remove extensive heavy metals from aqueous solutions like evaporation, ion- exchange, and precipitations. However these methods either inefficient or expensive when heavy metals exist at low concentration. These methods may also risk the generation of secondary waste which are difficult to treat [3].It is important to find new technologies and materials for the removal of heavy metals ions from industrial waste water. Biosorption utilizes the ability of certain materials to accumulate heavy metals from aqueous solution by either metabolically mediated or physio- chemical pathways of uptake [4] [5]. The most prominent feature of biosorption is the use of low cost and highly efficient biomass materials to adsorb heavy metals even present at very low concentrations [6]. Various types of biomass including bacteria [7], yeast [8] [9]), fungi [10] [11] have been investigated with the aim of finding more efficient and cost effective metal removal biosorbent. Biological methods, which have better performance and low cost for better remediation, are hiderened by small particle size with low density, poor mechanical strength and rigidity [13]. Immobilization of biomass within a suitable matrix can overcome these problems by offering ideal size, mechanical strength, rigidity and porous characteristic of biological material [14]. Immobilization of biomass, which allows higher biomass concentration, may be well suited for non- destructive recovery [15]. In these studies, adsorption ability of immobilized *A.lentulus* was investigated at different pH for the removal of Cu (II) from waste water.

METHOD AND MATERIALS

Preparation of Biopolymeric Bead

Biopolymeric beads were prepared in two steps. In the first step, a known solution of mixture of sodium alginate (4.0 g) and gelatin (1.0 g) was added drop wise into a 0.05M $CaCl_2$ solution with the help of a syringe and under constant stirring. The beads so produced were of almost identical spherical dimensions and allowed to harden by leaving them in $CaCl_2$ solution for 24 h and thereafter filtered and washed thrice with triple distilled water. The washed beads were further placed in as gluteraldehyde bath overnight and then filtered carefully and washed three water at room temperature and used as such for immobilization.

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Biomass production and Biosorption method

A.lentulus was grown in 250 ml Erlenmeyer flask containing 100 ml of growth media containing 0.5g/l K_2HPO_4 ; 5g/l Yeast extract; 0.1g/l $MgSO_4$;0.5g/l NH_4NO_3 and 5g/l glucose , pH =6 at 35± ^oC used 180 rpm . Cell suspension (2%) was used as inoculums. After exponential phase of growth, biomass cells were harvested by centrifugation at 4000 rpm and 6ºC for 10 min. Then harvested cells were washed by double distilled water. Then the biomass was used for immobilization.

Immobilization of biomass

A.lentulus was immobilized by mixing sodium alginate, gelatin with above resting biomass in the ratio of 1:1:5 .This mixtures were dropped in the form of beads of sodium alginate, gelatin covering the biomass. These beads were dipped into calcium chloride solution overnight. Finally beads of calcium alginate, gelatin entrapping the fungal biomass were obtained.

Autoclaved biomass

Autoclaved biomass was prepared by autoclaving the pre grown resting biomass at 121ºC and 15 lb for 20 minutes. The autoclaved biomass was then used after filtration.

Copper removal studies using synthetic solution

A weigh amount of treated/ untreated fungal biomass (4 g/l) was added in an Erlenmeyer flask containing 100 ml solution of known concentration of Cu^{2+}. The effect of various parameter like pH (3-8) and contact time on Cu^{2+} removal was studied. Different pH values (3-8) were adjusted by adding 1N HCl and 1N NaOH in media before addition of fungal biomass.

Influence of pH

The pH value of the wastewater has a large influence on the extent of biosorption. 100ml of metal solution at 100 mg/l was tested with 4 g/l of biomass at 180 rpm at varying pH change 3.0-8.0 respectively. The initial and final concentration of solution was measured by AAS. The maximum sorption data was used to find out optimum pH.

RESULT AND DISCUSSION

Growth of A lentulus in medium supplemented with 100 mg/l Cu^{2+} was coupled with significant Cu^{2+} from the solution. Very little Cu^{2+} removal was observed in sterile uninoculated controls indicating that the components of the medium do not reduce Cu^{2+} levels. During the initial 60 h, the increase in fungal biomass was very well correlated with Cu^{2+} removal. Since in first 24 h there was no visible growth, which indicates that the removal process is directly

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dependent on fungal growth. Removal started after the initial 24 h, concentration of total copper in growth medium did not change till 48 h. The colour of medium changed from yellowish orange to greenish brown. (Laxman and More et; al 2002). However after 66 h, the biomass growth stopped while Cu^{2+} removal continued at same rate. Interestingly, during period (66-92 h) both the glucose consumption and Cu^{2+} removal occurred at same rates while no biomass growth was observed. Theses investigations of the changes in Cu^{2+} concentration in the medium during culture growth revealed that these changes are linked to the duration of cultivation. This indicates the active role of a growing culture in the process of Cu^{2+} removal.

At the pH range of 5-8 growing cells showed higher loading capacity compared to non growing cells. At lower metal concentrations and more acidic pH (3-4) non growing cells had higher metal loading capacity than growing cells, obviously because the growth was being inhibited at the extreme lower pH .However showed that throughout the tested pH range (3-8), the metal loading capacity of growing cells was higher than the resting biomass. This would have occurred due to remarkable tolerance of A lentulus to extremes of pH. The use of active cells would be more useful however, if treatment involves mixed waste with potential utilization as energy and carbon source over a longer period. Table 1 gives comparison of the two modes and points that the precultivated biomass may be useful when the effluents are extremely acidic and contain high Cu^{2+} concentration. [16].

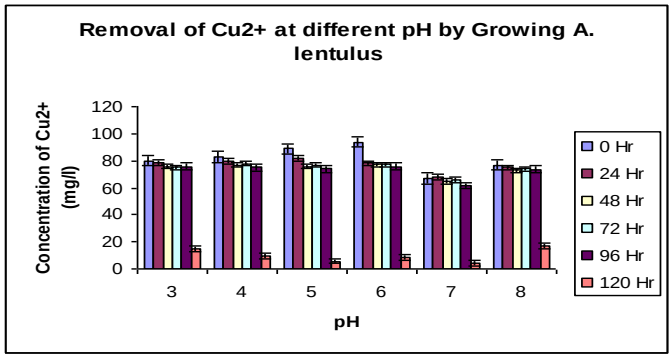

Fig.1 Removal of Cu (II) at different pH by growing *A.lentulus*

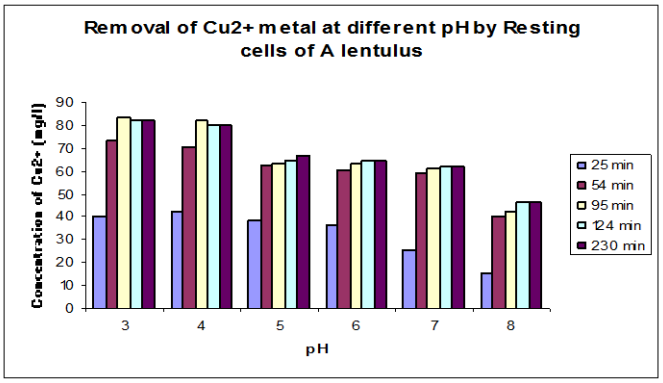

Fig.2 Removal of Cu (II) metal at different pH by resting cells of *A.lentulus*.

Removal of Cu (II) employing growing biomass

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Growth kinetics of *A.lentulus*

Fig.3 and 4 shows the change in glucose concentration and biomass production during the growth of *A.lentulus* in absence and in presence of 100 mg/l Cu^{2+} using the optimized composite media. No lag phase was observed in absence of Cu^{2+} as compared to 12 h of lag phase in its presence. In absence of metal, very fast growth and glucose consumption was observed during the first 18 hr which was coupled with decrease in pH (initial pH-6) and it was dropped to 5.0 at 24 hr. Subsequently, pH values displayed a rise in pH-6.5 in next 48 h. In presence of Cu (II), the fall pH initiated at 12 h and maximum, (5.0) was recorded pH at 48 h, beyond which it started rising. This could be attributed to the delayed and slow rate of glucose consumption in presence of Cu (II), which was led to substantial reduction in the rate of fungal growth. A entulus displayed toxicity response and its growth was delayed in presence of Cu (II), however the organism could eventually acclimatize to produce almost equal quantity of biomass (in absence of Cu (II)) after 120 h [17].

Table.1 Comparison of growing and non growing biomass for metal removal.

Parameter	Growing (G)	Non-growing (NG)	Comment
Process time	Long treatment time (5 days)	Short (120 min)	If cultivation and biomass preparation time included then= 2 days in non growing
pH sensitiveness	Often yes but in case of *A.lentulus* not significantly affected in broad pH range (3- 11)	Yes, Removal significantly reduced in neutral range	
Pollutant Toxicity	Yes , above 550 mg/l $Cu2^{+}$	No	At higher pollutant load non-growing may be preferred.
Recovery of metals and recycling of biomass	Generally not attempted	Possible	
Interference from other contaminants	Yes	No	Choice of robust organism for growing

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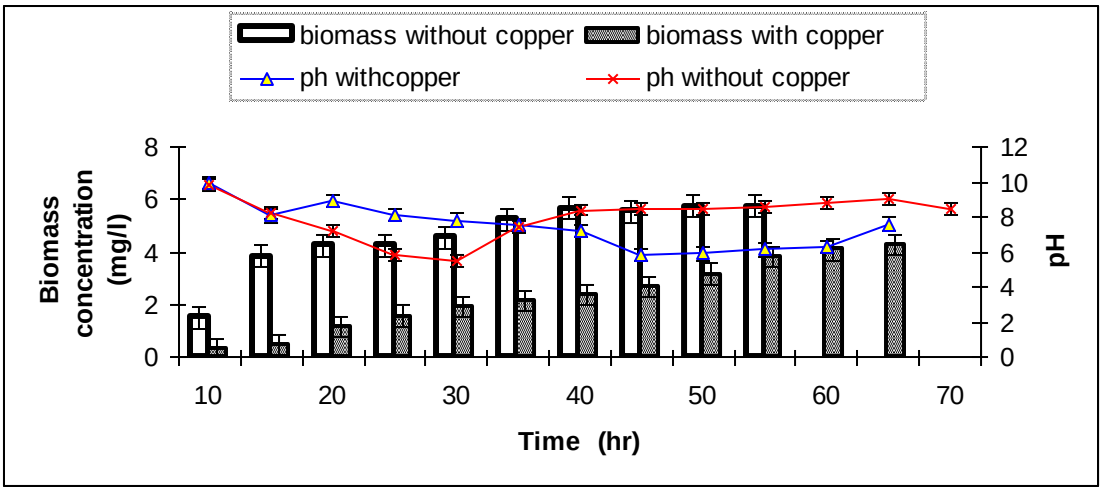

Fig.3 Variation in pH, biomass during growth of *A.lentulus* in presence (100 mg/l) and absence of Cu (II)

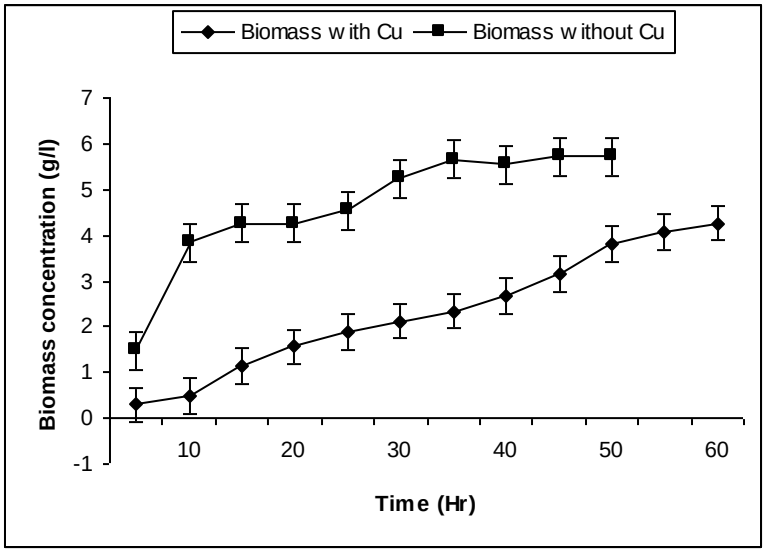

Fig.4 Variation in biomass during growth of *A.lentulus* in presence (100mg/l) and absence of Cu (II).

CONCLUSIONS

The kinetics of growth and Cu (II) removal by *A.lentulus* was studied using the optimized media. Presence of Cu (II) resulted in delayed growth and decreased growth rate of fungal strain. The results revealed that growing *A.lentulus* employs several mechanisms such as sorption, bioaccumulation within cells as well as extra cellular reduction to achieve Cu removal from the industrial effluent. This enables the outstanding performance of *A.lentulus* as more tolerant and efficient bio- treatment agent. *A.lentulus* displayed the ability to extreme range of pH (3 to 8) and even its Cu (II) removal ability was only slightly reduced at pH 3 or pH 8 as compared to the maximum removal at pH 6. Marginal difference in the growth and metal removal at extreme pH indicates the suitability of this strain for treating acidic effluents (electroplating) or an alkaline effluents (e.g. textile) at their ambient pH eliminating the need for neutralization. Further, the strain also showed the tolerance against high temperature and

faster growth was observed at 35 ^{o}C. Till 35 ^{o}C almost 96.6 % of Cu (II) was removed within 96 h. Further, the specific Cu removal and uptake capacity in case of non- growing was much lower than the growing mass implicating higher sludge production.

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