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Technical Note

Biological removal of zinc from wastewater using *Aspergillus* sp.

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ABSTRACT

The present study was conducted on removal of zinc in a continuous flow system by *Aspergillus* sp. during its growth. The fungal strain was isolated from industrial wastewater. The experiments under continuous culture conditions were performed in two reactors of working volume 650ml and 3 litres respectively. The experiments were conducted using sugar concentrations of 10, 15 and 20g/l and at dilution rates of 0.08, 0.04 and $0.02h^{-1}$. Specific zinc uptake of 44mg and 77mg per gram dry biomass were obtained in 650ml and 3 litre reactors, at 10g/l sugar concentration and $0.02h^{-1}$ dilution rate. No significant increase was observed in the specific zinc uptake with increase in sugar concentration. The specific uptake of zinc increased to 79mg/g of dry biomass at 15g/l sugar concentration and to 84mg/g of dry biomass at the same sugar concentration but with proportional increase in nutrient components . At higher initial concentration (1000mg/l) of zinc, the specific zinc uptake by the fungus was found to be 120mg/g of dry biomass at 10g/l sugar concentration. Further studies using an actual industrial effluent having zinc concentration of 46mg/l resulted in a complete removal of the metal at 10g/l sugar concentration.

Keywords: Zinc; *Aspergillus* sp.; Specific metal uptake; Continuous flow system

1. INTRODUCTION

Removal of heavy metals from industrial wastewaters assumes great importance considering the stringent environmental regulations on heavy metal contamination of water bodies and wastewater treatment sludges meant for land applications. Biosorption has been reported as a potential alternative to the conventional treatment techniques for removal of heavy metals from wastewaters, especially those originating from small and medium scale industries. Microorganisms such as algae, fungi and bacteria have been reported to accumulate the metals and to concentrate them several times (Volesky and Holan, 1995; Volesky, 1993). Fungi belonging to the genera *Rhizopus, Aspergillus, Neurospora, Fusarium, Saccharomyces* and *Penicillium* have already been studied as a potential biomass for wastewater treatment for the removal of heavy metals such as Cd, Cu, Pb, Ni, Zn and As using both growing and non growing biomass (Costa and Leite, 1992; Fourest *et al.*, 1992, Donmez and Aksu, 1999; Niu *et al.*, 1993).The most of the reported studies using actively growing biomass are based on batch systems (Donmez and Aksu, 1999; Costa and Leite, 1992; Omer, 2001). A number of authors have also reported the use of continuous flow systems for the removal of heavy metals like chromium, lead, nickel and copper using both living and non-living algae, fungi and bacteria (Soliso *et al.*, 2000). Valdman *et al.* (2001) have reported the continuous removal of zinc using algal biomass. However, reports on biosorption of zinc using actively growing fungal biomass in continuous flow systems are limited. Such systems can treat larger quantities of wastewaters in

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shorter duration as compared to batch systems. Continuous stirred tank reactors (CSTR) are ideally suited to applications where the microorganism has to be maintained in living or metabolically active state. Studies on CSTR have the advantage that the cell population can adjust to a steady environment and achieve a state of balanced growth. Thus, there is a benefit of obtaining a relatively well defined, reproducible state for the cells. This is more difficult to achieve in batch cultivation.

Batch studies conducted earlier by the present authors using *Aspergillus* sp. isolated from industrial wastewater indicated that the strain was not only tolerant to very high concentrations of zinc but also had potential for metal uptake. A maximum of 833mg of zinc was removed per gram of dry biomass at an initial zinc concentration of 10000mg/l. The organism was also capable of growing in the presence of other heavy metals (chromium, lead, copper, nickel) and thus assumes great importance as a potential candidate for removal of zinc from industrial wastewaters which are often contaminated with more than one heavy metal (Surekha *et al.*, 2002). The objective of the present study was to examine the effects of various parameters such as dilution rate, sugar concentration and nutrient concentration on the removal of zinc by the fungal strain under continuous culture conditions using synthetic metal bearing solutions. The process was then applied to zinc removal from an actual industrial effluent in continuous mode under the optimized conditions.

2. MATERIALS AND METHODS

2.1 Microorganism and growth conditions

The fungal strain was grown in 250ml Erlenmeyer flasks containing 100ml of optimized growth media of the following composition (g/l): glucose, 10; K_2HPO_4, 0.5; NaCl, 1.0; $MgCl_2$, 0.5; NH_4NO_3, 0.5; yeast extract, 5.0. The initial pH of the media was 5.6. The flasks were incubated on a rotary shaker at 180rpm at a temperature of 30°C.

2.2. Reactor assembly

Figure 1 shows the schematic diagram of the reactor assembly. The continuous stirred tank reactor consisted of a cylindrical glass vessel, 45cm high with 12cm inner diameter. There were three overflow ports at different heights of the reactor corresponding to different working volumes. The reactor was heated externally with a heating tape connected to a temperature control module. Aeration was provided at the bottom of the reactor using a multipoint injection system. Efficient mixing was ensured by a mechanical stirrer. The media was fed to the reactor through a peristaltic pump.

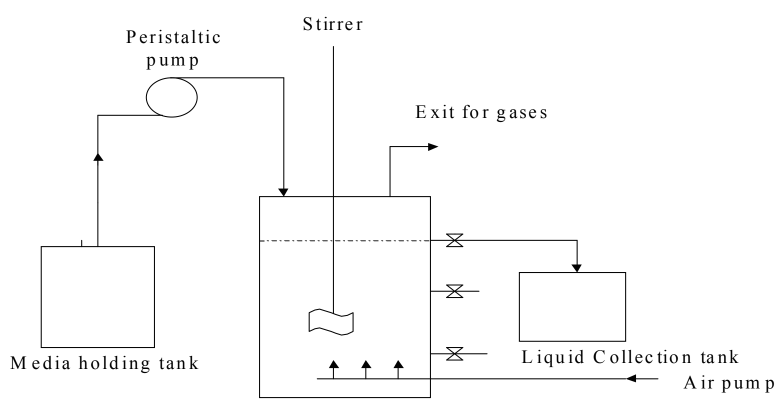

Figure 1. Schematic diagram of the reactor assembly

2.3. Reactor run

Initially the reactor was operated in the batch mode using 500mg/l zinc concentration at a temperature of 30°C in order to develop sufficient cell concentration. Thereafter, it was operated in the continuous mode. A peristaltic pump was used to feed the media into the reactor at the desired dilution rate. The process was monitored for 10-12 days. The treated effluent was collected in the liquid collection vessel through the overflow port. Samples were drawn twice a day and were analysed for residual zinc concentration and sugar concentration. The biomass concentration inside the reactor was determined by separating the biomass from the liquid by centrifugation and then drying it at 80°C. Since the biomass was in the form of granules or beads, the effluent collected over a 24 hour period was used for this purpose so as to minimize estimation errors. The effect of the following parameters on the zinc uptake by the organism was studied:

Dilution rate	: 0.08, 0.04, 0.02h^{-1}
Sugar concentration	: 10, 15, 20g/l
Sugar concentration with proportional increase in nutrients	: 15g/l
Zinc concentration	: 500, 1000mg/l
Working volume	: 650, 3000ml

Similar methodology was followed to study the specific zinc uptake by the organism from an actual industrial effluent. This effluent had an initial zinc concentration of 46mg/l.

2.4 Analytical procedure

Zinc concentration was estimated by analysing the samples using atomic absorption spectrophotometer (model AAS 4129, ECIL, India). The sugar concentration was estimated by DNS method (Miller G.L., 1959). The weight of biomass was determined after drying it at 80°C until constant weight was achieved.

3. RESULTS AND DISCUSSION

Figure 2 shows the batch growth kinetics of the fungus in a 3 litre bioreactor at initial zinc concentrations of 500mg/l and 1000mg/l along with the control run (without zinc). This was studied prior to the starting of the process in continuous mode. The specific growth rate of the organism was calculated during the exponential phase of growth using equation (1):

$$\ln X/X_i = \mu . t \quad (1)$$

where, X and X_i are the biomass concentrations at the start of the exponential phase and after a time t, respectively, while μ is the specific growth rate of the organism (h^{-1}).

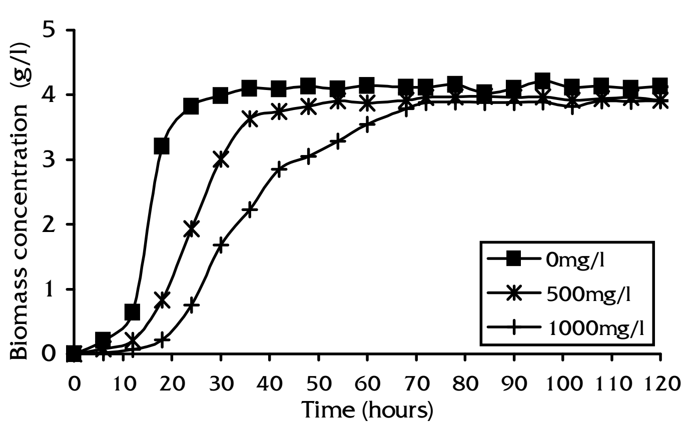

Figure 2. Change of biomass concentration with time

The value of μ was calculated to be $0.147h^{-1}$ at 500mg/l and $0.128h^{-1}$ at 1000mg/l zinc concentration. In the absence of any zinc in the media, the specific growth rate was found to be $0.16h^{-1}$. These values indicate that the growth is affected by the presence of the metal. Literature information available on growth kinetics of different fungal strains indicate that growth rate of fungi is adversely affected by the presence of heavy metal ions in the medium. Donmez and Aksu (1999) studied the effect of copper ions on the growth of the fungus *Saccharomyces cerevisiae, Kluveromyces marxians, Schizosaccharomyces pombe* and *Candida* sp. In the case of *Candida* sp. the specific growth rate decreased from $0.276h^{-1}$ to as low as $0.076h^{-1}$ when the concentration of copper was increased from 25.9mg/l to 708mg/l.

In the continuous mode of operation for different reactor volumes the dilution rates were maintained below 50% of the specific growth rate in order to avoid washout conditions.

Table 1
Maximum zinc removal, biomass concentration and specific zinc uptake at different dilution rates using different reactor volumes under steady state conditions

Reactor volume	Dilution rate h^{-1}	Maximum zinc removal mg/l	Biomass concentration g/l	Specific zinc uptake mg/g
	0.02	250	3.26	77
3 litres	0.04	180	3.01	59.8
	0.08	75	2.41	31.1
	0.02	114	2.6	44
650ml	0.04	77	1.98	39
	0.08	45	1.5	30

Table 1 shows the maximum zinc removal, biomass concentration and specific zinc uptake by the organism at different dilution rates in different reactor volumes using 10g/l sugar concentration. For a particular reactor volume, increase in maximum zinc removal, biomass concentration and specific zinc uptake was observed with decrease in the dilution rate. Continuous flow system studies conducted by Soliso *et al.* (2000) on Cr (III) biosorption by *Sphaerotilus natans* also report similar results. In the present study, at all the dilution rates, lower values were obtained for both biomass concentration as well as specific zinc uptake in the 650ml reactor as compared to the values obtained in the 3 litre reactor. While any experimental evidence is not available at this moment to explain this observation, this can possibly be attributed to the higher shear rates in the smaller reactor, thereby leading to reduced growth of the filamentous organism.

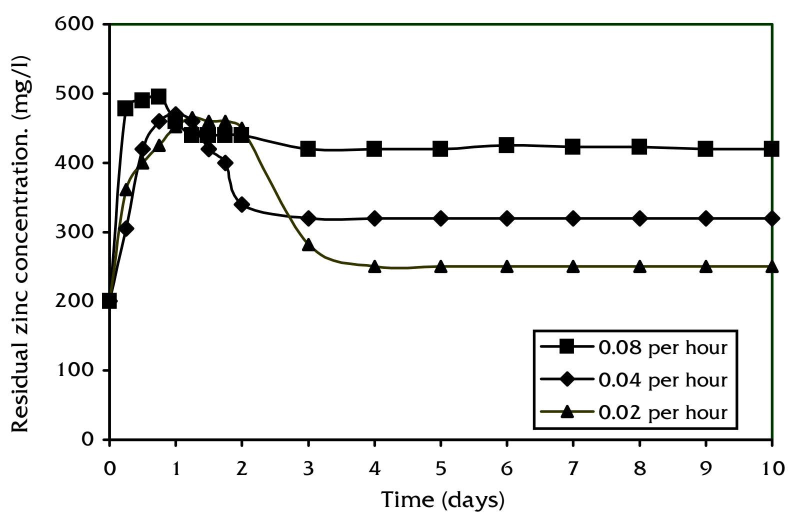

Figure 3. Change in residual zinc concentration with time at different dilution rates

Figure 3 shows the change in residual zinc concentration with time in continuous mode of operation at different dilution rates for a reactor working volume of 3 litres using 10g/l sugar concentration. During transient state operation of the reactor, immediately following the commencement of continuous feed, the residual zinc concentration initially increased. This continued until the reactor stabilized and achieved a steady state operation. The onset of steady state operation was indicated by the constant value of residual zinc in the effluent. The residual zinc concentration decreased to 250, 320 and 425mg/l at dilution rates of 0.02, 0.04 and $0.08h^{-1}$ respectively, from an initial zinc concentration of 500mg/l. The time required to attain steady state operation increased with decrease in dilution rate. This is expected since lower dilution rates would mean lesser volume treated per unit time. CSTRs typically require 3 to 5 reactor volumes to reach steady states.

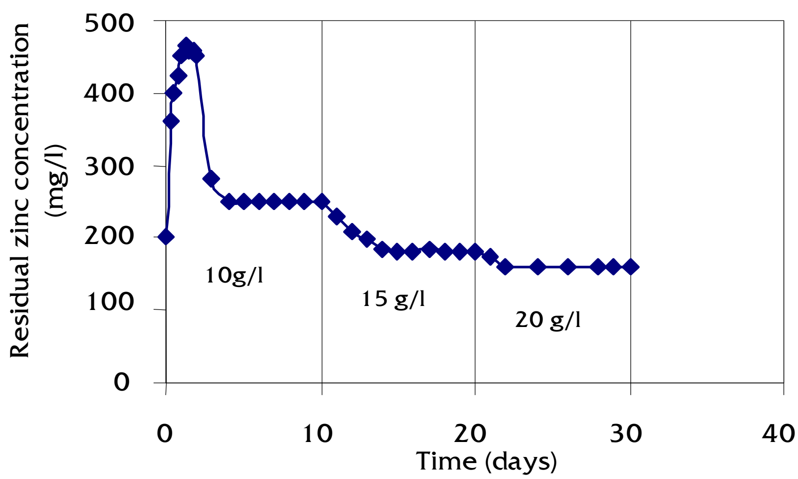

Figure 4. Change of residual zinc concentration with time at different sugar concentrations

Figure 4 shows the change in residual zinc concentration with time at different sugar concentrations. The residual zinc concentration decreased from 250mg/l to 180mg/l by increasing sugar concentration from 10g/l to 15g/l. However, further increase in sugar concentration (20g/l) did not result in appreciable improvement in zinc uptake by the organism, as is indicated by the residual zinc concentration of 160mg/l.

Increase in sugar concentrations in the media did not result in appreciable zinc removal in this experiment since the concentration of other media components were not increased along with increase in sugar concentrations. Further experiments were conducted to see the effect of proportional increase of other media components on growth and zinc removal. The change in residual zinc concentration with time at different sugar concentrations both with and without proportional increase in other media components is shown in Figure 5. The residual zinc concentration decreased to 110mg/l from an initial zinc concentration of 500mg/l at 15g/l sugar concentration with proportional increase in other media components. The biomass concentration also increased from 4.08g/l obtained at 15g/l sugar concentration to 4.68g/l with the same sugar concentration but with proportional increase of nutrients. This is attributed to the availability of required nutrients for the growth of the organism at higher sugar concentration leading to higher biomass production and therefore, metal uptake.

The change in residual zinc concentration with time at 1000mg/l initial zinc concentration, with 10g/l and 15g/l sugar concentrations in the media, is shown in Figure 6 (The growth curve for the fungus at 1000mg/l zinc concentration is shown in Figure 2). The residual zinc concentration was lowered to 600 mg/l from the initial value of 1000mg/l when 10g/l sugar was present in the media at a dilution rate of $0.02h^{-1}$. Further increase in sugar concentration to 15g/l decreased the residual zinc concentration to 400mg/l. The decrease in residual zinc concentration with further increase in sugar concentration is due to the higher biomass concentration (3.52g/l) achieved at 15g/l sugar concentration as compared to the value

(2.81g/l) at 10g/l sugar concentration. The specific zinc uptake values were found to be 141mg/g and 170mg/g at 10g/l and 15g/l sugar concentrations respectively. The specific uptake increased at 1000mg/l initial zinc concentration as compared to the specific zinc uptake at 500mg/l initial zinc concentration (Table 1).

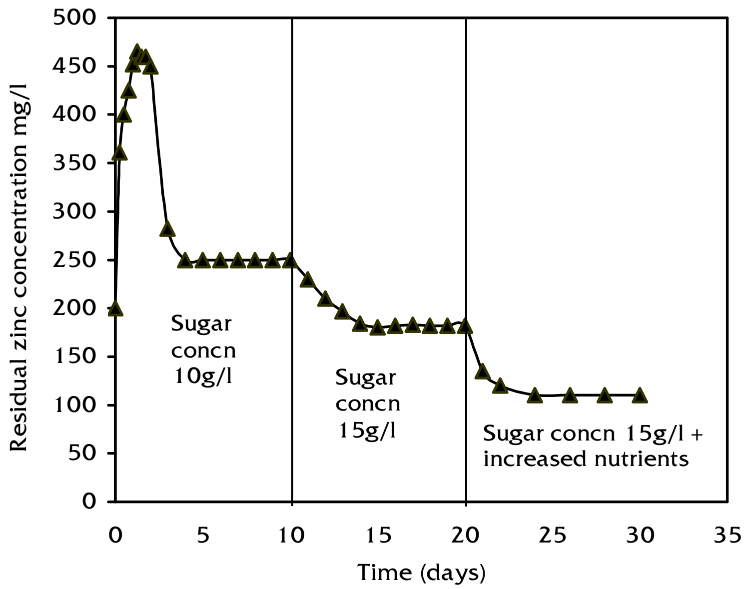

Figure 5. Change in residual zinc concentration with time at different sugar concentrations with proportional increase of nutrients

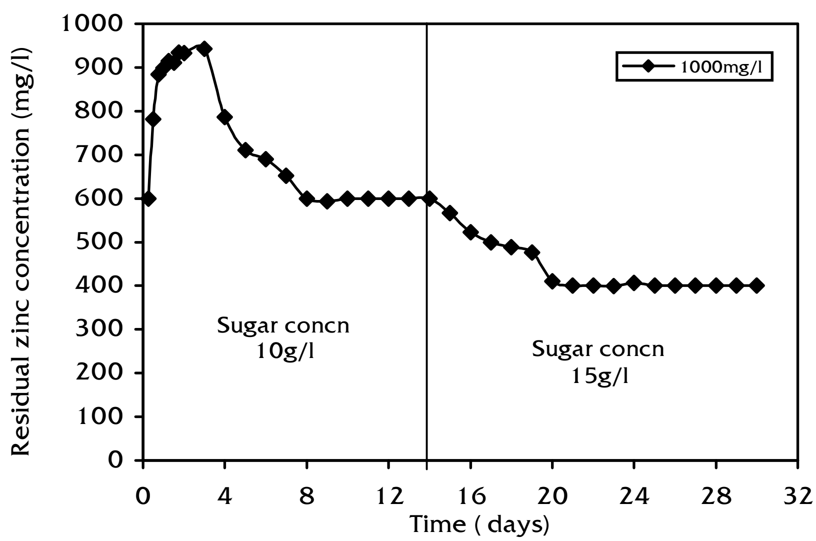

Figure 6. Change in residual zinc concentration with time at 1000mg/l of zinc concentration

Figure 7 shows the performance of the reactor when an actual industrial effluent was used. This industrial effluent had a zinc concentration of 46mg/l. Almost complete removal of zinc was obtained within 2 days in a 3 litre bioreactor at a dilution rate of $0.02h^{-1}$. A biomass concentration of 1.01g/l and specific zinc uptake of 45.5mg/g were obtained under these conditions. The time required to reach the steady state condition was reduced at lower initial concentration .of zinc.

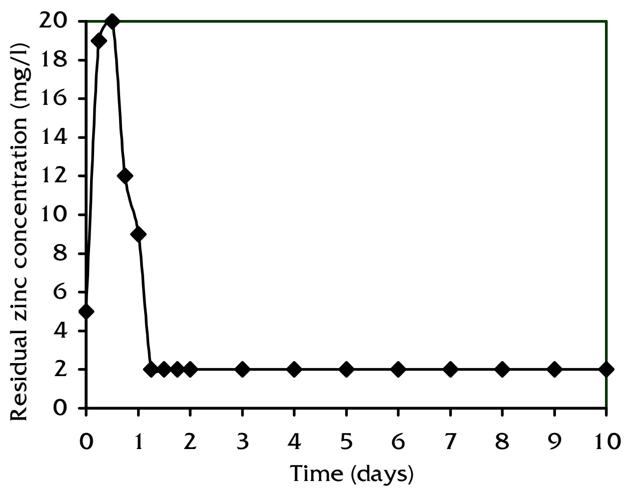

Figure 7. Change in residual zinc concentration with time in effluent

4. CONCLUSIONS

The organism was able to achieve very good removal of zinc continuously from the medium when the treatment was carried out in a continuous, well-mixed reactor. The extent of zinc removal was 80% when the influent to the continuous reactor contained 500mg/l zinc at 15g/l sugar concentration. This decreased to 60% when the influent zinc concentration was increased to 1000mg/l. Further improvement in zinc removal was observed with proportional increase of nutrients at the same sugar concentration for 500mg/l initial zinc concentration. The results indicate that limitation in zinc removal was primarily due to the biomass concentration in the reactor and the upper limit for the specific metal uptake by the organism. This also indicates that higher concentrations of zinc could be removed either by enhancing the biomass concentration in the reactor or by using two or more reactors in a staged manner. Since the biomass concentration that could be achieved in a CSTR is dependent on the dilution rate, a staged operation consisting of two or more CSTRs seems to be more attractive.

Almost complete removal of zinc was obtained using an industrial effluent containing 46mg/l of zinc in addition to other metal ions. This shows the possibility of developing a biological process for removal of zinc from industrial effluents. It is evident from the above study that increase in sugar and nutrient concentration increases the growth leading to enhanced metal uptake. Therefore, supplementing the media with cheaper sources of carbon and nutrients will make the process economically viable for the treatment of zinc-containing effluents. Further studies are needed in this direction.

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