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Biosorption of Cr and Pb by the Metal Resistant Bacterial Isolates Immobilized in Calcium Alginate Coated with PHBV

Kasthuri, J.[1], Senthil Kumar, D.[2]

[1, 2] Department of Zoology, The Standard Fireworks Rajaratnam College for Women Autonomous, Affiliated to Madurai Kamaraj University, Reaccredited with A Grade by NAAC, College with Potential for Excellence by UGC , Sivakasi – 626 123, Tamil Nadu, India.

Abstract: ***The bacterial isolates of matchworks industrial wastes viz., Bacillus spp. MI1 and Micrococcus spp.MI2 which exhibited resistance to heavy metals, Cr and Pb in terms of Zero Zone size, prepared in suspension and immobilized in calcium alginate matrices were subjected to biosorption studies. The immobilized beads of the bacterial isolates were uniformly 3 mm in size. These smaller sized particles are seemed to play vital role in biosorption because they have higher surface area to bind. The metal uptake experiments carried out at the pH ranging from 2.0 and 4.0 (32 ºC) with the immobilized beads in calcium alginate. Irrespective of the organisms and metals studied , maximal adsorption effiency was reported at pH 3 for Cr and pH 4 for Pb. As for the impact of temperature on metal adsorption, irrespective of the organisms immobilized in Calcium Alginate, the optimal values were reported at 32 ºC for both Cr (pH 3)and Pb(pH 4). The biosorption potential of the immobilized bacterial isolates resistant to metals, coated with PHBV exhibited optimum results for Cr (81.1 mg/L ; 57.4 mg/L) and Pb(76.4 mg/L ; 58.4 mg/L).Further, the desorption studies were carried out with dilute 0.1 N HCl. The results revealed that the beads are seemed to be very effective upto the 3rd cycle. There after desorption studies did not show much promising results .***

Keywords: *Bacillus* spp. MI1, *Micrococcus* spp.MI2, Cr, Pb resistance, calcium alginate immobilization, PHBV coating, biosorption efficiency

1. Introduction

Amelesh Samanta *et al* . [1], opine that the ability of the microorganisms to interact with a wide range of heavy metals such as Cu, Fe, Mg , Au , and Pb etc., is mainly attributed to the difference between the net negative charge of bacteria and the cationic charge of the metals. Actually, the ability of the microorganisms for metal binding is mainly due to the availability of the nucleation sites on their cell surface which would bind the metals of opposite charge. The subsequent binding of heavy metals at high concenteration would result in the precipitation of metals on the cell wall. It has been proved that the metabolically active cells from the exponential growth phase probably contain highly active enzymes, some of which may also be involved in complexing and binding metal ions[3 &4].

However, the disadvantages of the free microbial cells used in laboratory conditions are due to their smaller sized particles, low density, poor mechanical strength and little rigidity. Further, in real application they may also come up with the solid-liquid separation problems, possible biomass swelling, inability to regenerate / reuse and development of high pressure drop in the column mode. High pressures in turn can cause the disintegration of free biomass. Whereas , utilization of dead biomass , on the other hand would offer certain major advantages such as lack of toxicity constraints, non-requirements of nutrients supply and recovery of bound metal species by an appropriate desorption method[5].

Hence the present investigation attempted not only with the live bacterial isolates in suspension but also with the dead bacterial isolates immobilized in calcium alginate matrices to biosorb the heavy metals, Cr and Pb. The biosorption potential was also evaluated with calcium alginate beads coated with PHBV under optimal condition.

2. Materials and Methods

2.1 Bacterial isolates

Pb and Cr resistant bacterial isolates[10] of the Matchworks industrial sites of Sivakasi (Lat.9º 27’ 0” N ; Long.77º 49’ 0” E), *viz*., *Bacillus* spp. MI1, and *Micrococcus* spp.MI2 were chosen for the biosorption studies in the present investigation.

2.2 Preparation of metal (Cr& Pb) stock solutions

For biosorption experiments, Cr and Pb with a concentration of 100 mg / L were prepared using deionized distilled water[15].

2.3 Immobilization of bacterial isolates with Calcium alginate

Sodium alginate solution was prepared by dissolving sodium alginate with equal volume (50 ml each) of Pb and Cr resistant bacterial inoculum *viz*., *Bacillus* spp. MI1 and *Micrococcus* spp.MI2 which were isolated from matchworks industrial site by Kasthuri and Senthil Kumar[10] and kept in a boiling water bath at 60 ºC. The sodium alginate was then extruded through a 20 mL hypodermic syringe into 3% CaCl solution and left for 2 hrs at 42 ºC for bead formation. The beads were then air dried for 24 hrs. The spherical beads were then rinsed thoroughly with de-ionized water and stored in normal saline (0.85% sodium chloride) solution at 4 ºC until

further use[7].

2.4 Preparation of PHBV Coated Immobilized Beads

With the view to enhance the biosorption efficiency of the freshly prepared calcium alginate beads, they were further coated with PHBV. For which the extracted microbial (*Acinetobacter junii CN1)* PHB (Kasthuri and Poornima[9]) and commercial PHV (Sigma) and Immobilized beads in 1:1:1 ratio were dissolved in 5 ml chloroform and formulated as a PHBV coated bead by casting into clean, dry, glass Petri dishes in a fume hood and then the chloroform was evaporated. The beads were then dried for 24 hrs[9].

2.5 Biosorption studies

Biosorption experiments were carried out not only with live cells / dead cells / the pre-weighed amount of Pb and Cr resistant bacterial isolates immobilized in calcium alginate beads but also with the PHBV coated beads

2.5.1 Biosrption / bioaccumulation of Cr and Pb with both live and dead cells

About 1 % living bacterial biomass were suspended individually in 100 ml solution supplemented with heavy metals. After 12 hrs incubation the cells were harvested and assayed for metal load with Double Beam Atomic Absorption Spectrophotometer (Elico Model 176). The values were then expressed in mg /L[18].

As for dead cells , the dried pellets of 200 mg were added to 1 L distilled water from which about lml cell suspension was added to 100 ml metal solution and evaluated for metal load after 12 hrs of incubation[18].

2.5.2 Biosrption of Cr and Pb with the bacterial isolates immobilized in calcium alginate

One gram bead (about 15 – 17 beads – each weighing approximately 60 mg) was introduced into a 250 ml Erlenmeyer flasks containing 100 mL aqueous metal ion solution. The flask was then provided with the constant agitation of 150 rpm speed using a rotary shaker for 6 hrs. The differences between the initial and final metal concentration in the solution were determined using Double Beam Atomic Absorption Spectrophotometer (Elico Model 176)[7] .

2.6 Evaluation of the Efficiency of PHBV-Coated Immobilized Beads

Calcium alginate beads of Pb and Cr resistant bacterial isolates coated with PHBV were evaluated for their absorption potential at different temperatures 30, 32 and 35º C as in the previous case[7]. The process was also evaluated at different pH values such as 2.5, 3 and 3.5 for Cr and pH 3, 4 and 5 for Pb resistant bacterial isolates.

2.7 Evaluation of bead Capability for Metal Biosorption

Five cycles of adsorption and desorption experiments were conducted to examine the capability of the calcium alginate beads coated with PHBV to retain metal removal capability.

2.8 Desorption studies

Calcium alginate beads of Pb and Cr resistant bacterial isolates coated with PHBV were subjected to metal ion recovery experiments using 0.1 M HCl solutions. After 6 hrs of adsorption, the bound metal ions were eluted into 20 mL elutant for 60 min. After elution, the mixture was filtered and filtrate was measured for metal ion concentration. After elution, calcium alginate beads were washed with deionized water till the pH of wash solution reached the range of 2.0 – 2.5. Beads regenerated were air dried and then again suspended in metal containing solutions for the next adsorption run[14].

3. Results and Discussion

It is noteworthy that in biosorption technology, the uptake of various metal ions is largely depend upon the ionic strength of metals, the nature and distribution of active groups on the biosorbent and the mode of interaction between the metal ions and the biosorbent[11]. Further, the mechanism of interaction of heavy metals with biopolymers is of extracellular in principle. This can be achieved only *via* surface binding by involving the specific chemical sites or functional groups available on the surface of the adsorbent. However, the electrosatic attraction seems to be the main mechanism responsible for the biosorption of the negatively charged anions to the positively charged cell surface[12].

In the present investigation , when compared to the live cells the dead cells were seemed to be more efficient in metal adsorption (Table 1). In contrast, Kapoor *et al.*[8] have reported with relatively higher Ni biosorption capacity in live *Aspergillus niger* cells when compared to the dead ones

Table 1: Biosorption of Cr and Pb with both live and dead bacterial isolates

Organisms	Metals Studied			
	Cr mg /L		**Pb mg /L**	
	Live Cells	Dead Cells	Live Cells	Dead Cells
Bacillus spp. MI-1	58.5	70.4	54.2	64.6
Micrococcus spp. MI-2	38.6	48.9	34.5	46.4

It is worth mentioning that the immobilized beads of the bacterial isolates were uniformly 3 mm in size. Supportingly, Vijayaraghavan and Yun[19], opine that the smaller sized particles play vital role in biosorption because they have higher surface area to bind.Further, it is imperative to determine the factors that impact the sorption and elution characteristics of the beads for the industrial application . Thus the optimal conditions of pH and temperature required for Cr and Pb binding to the metal adapted bacterial isolates entrapped in calcium alginate beads were determined with the laboratory experiments.

Among all the factors, the effect of pH (Table 2 & 3), has been identified as the most important variable, governing metal adsorption in the biosorbent[13]. The removal of metal ions from the solution by adsorption is highly dependent on the pH of the solution, which affects the surface charge of the adsorbent and the degree of ionization as well. It was

therefore important to study the effect of pH on the adsorption of Cr(VI) and Pb(II).

Table 2: Biosorption efficiency of the immobilized bacterial isolates for Cr (mg /L) with varied pH at 32 ºC

	Organisms	
pH	***Bacillus* spp. MI-1**	***Micrococcus* spp. MI-2**
2.5	72.3	49.8
3	79.2	55.4
3.5	75.6	54

Table 3: Biosorption efficiency of the immobilized bacterial isolates for Pb (mg /L) with varied pH at 32 ºC

pH	Organisms	
	Bacillus spp. MI-1	*Micrococcus* spp. MI-2
3	68.3	48.5
3.5	70.5	51.9
4	74.2	56.3

The results of the present investigation reveal that the biosorption of calcium alginate beads depends on solution pH. Further, irrespective of the organisms studied, the maximum absorption was reported at pH 3 for Cr and 4 for the Pb. While evaluating the biosorption efficiency of calcium alginate beads at varied pH, metal uptake by calcium alginate beads decreases as the pH increases16. The maximum removal efficiencies were found to be 57% and 64%, for Pb(II) and Cu(II) respectively. This reduction in limitations in the binding and movement of metal ions or either affinity of sites for metal or binding sites on relevant biopolymer.

Tsezos and Volesky17, have also reported that above pH 2.0, biosorption of metal ions by calcium alginate beads was found to be relatively constant for Pb(II) and Cu(II) ions. Where as Cr (VI) biosorption seemed to be increasing upto pH 3.0 having 86% removal efficiency. Thus, different metals have different pH optima, due to the different solution chemistry of the metals.

Followed by pH, effect of temperature do equally play key role in biosorption of metals. As in the present investigation(Table 4 & 5), Brierley and Brierley2 and Gadd5, had also observed the extent of sorption of metal ions by the sorbent is seemed to be increased with increase in temperature upto 35ºC. Further increase in the temperature of the reaction mixture showed reduction in biosorption.

However, the trend was seemed to be increasing when the temperature was increased from 4 to 25 ºC but decrease in uptake on further increase in temperature to 50 ºC. It has been suggested that increase in metal uptake at increased temperature is due to either higher affinity of sites for metal or an increase in binding sites on relevant biosorbent2&5. Interestingly , in the present investigation, the optimal metal aborption was reported at 32 ºC for both Cr and Pb.

Table 4: Biosorption efficiency of the immobilized bacterial isolates for Cr (mg /L) at varied temperatures (pH 3)

	Organisms	
Temperature	***Bacillus* spp. MI-1**	***Micrococcus* spp. MI-2**
30 ºC	76.8	54.6
32 ºC	79.2	55.4
35 ºC	75.1	53.7

Table 5: Biosorption efficiency of the immobilized bacterial isolates for Pb (mg /L) at varied temperatures (pH 4)

	Organisms	
Temperature	***Bacillus* spp. MI-1**	***Micrococcus* spp. MI-2**
30 ºC	73.4	53.9
32 ºC	74.2	56.3
35 ºC	71.1	52.5

Yet another fascinating factor related to biosorption efficiency of beads is also being governed by the percentage of sorbent loading. Accordingly the optimum sorbent loading was found to be 3% for Cr(VI), 3% for Pb(II) and 1% for Cu(II) respectively(Spiniti *et al* ., 1995).. With this view, the sorbent loading percentage of the present investigation is fixed at 3 %.

However, increase in sorbent loading beyond a certain point resulted in a decrease in metal adsorption. This can be attributed to the difference in porosity of the beads when a higher quantity of sorbent was loaded. The increase in dose of sorbent in relation to amount reduced the surface area of the bead[16]. As the sorbent dose was increased, the beads became less porous and the free transport of metal ions to the interior adsorption sites was affected. Because of the reduced porous nature, the total surface area of entrapped biosorbent particle, interacting with metal ions is reduced.

Table 6: Biosorption of Cr and Pb with PHBV coated beads at 32 ºC

Organisms	**Metals Studied**	
	Cr (pH 3) mg /L	**Pb (pH 4) mg /L**
Bacillus spp. MI-1	81.1	76.4
Micrococcus spp. MI-2	57.4	58.9

In the present investigation (Table 6) the pores were further reduced by coating the immobilized beads with PHBV. Which comparatively showed very promising results for Cr (81.1 mg /L ; 57.4 mg /L) and Pb (76.4mg /L ; 58.9 mg /L) when compared to the uncoated ones.

It is worth mentioning that till now the biosorption potential for different metal ions and capacity of reusability in multiple adsorptions – desorption cycles has not been properly investigated. Fascinatingly, for commercial application, Iqbal and Edyvean[8] ,have determined the capacity of the calcium alginate beads to adsorb metal ions by repeating the adsorption and desorption experiments in three consecutive cycles. As in the same way , in the present investigation , 5 cycles of desorption experiments were conducted at 32 ºC with the pH value 2 for Cr and 2.5 for Pb .

Fascinatingly , the desorption studies were carried out with dilute 0.1 N HCl . In the present investigation, irrespective of the isolates and metals , results (Table 7) revealed that the beads are seemed to be very effective upto 3 cycles . On 4th

cycle, the metal sorption efficiency remained as the same. There after desorption studies did not show much promising results Thus the present experiment confirms the reusability of beads .upto 3 cycles

It is expected that soil receiving long-term application of wastewater / industrial effluents containing toxic metals may result in the development of selection pressure on the organisms / bacterial isolates. In the present investigation, both the bacterial isolates studied revealed high resistance to Pb which in turn indicates the contamination of the industrial sites with Pb. This might cause increased level of metal tolerance as well as metal adsorption capacity in the bacterial isolates.

Table 7: Desorption Cycles at pH 2.0 for Cr and pH 2.5 for Pb (32ºC)

Cycles	Metals Studied			
	Bacillus spp. MI-1		*Micrococcus* spp. MI-2	
	Cr mg /L	Pb mg/L	Cr mg /L	Pb mg /L
1	32.44	20.56	22.96	23.56
2	24.21	17.42	11.75	12.10
3	11.50	15.75	08.02	09.52
4	09.33	10.54	06.95	08.65
5	08.10	07.12	05.23	07.33

Further, the metal absorption efficiency shall be improved with immobilization in calcium alginate and coating the same with other polymeric systems such as PHBV. Such systems were also monitored for better results under optimal pH and Temperature. Furthermore, the reusability of the beads also confirms its potential application in the bioremediation of metallic pollutants. To conclude the Authors suggest that all these data obtained with the simple experiments can be of great interest in scale-up process to optimize the biosorption of heavy metals.

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