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Application of immobilized beads of *Ps. aeruginosa4442* and role of various elutants on Cr (VI) recovery from metal containing solution

Kulkarni R. A. and Gupta S. G.

Government college of Arts and Science, Aurangabad**,** Maharashtra431001

Government Institute of Science, Aurangabad, Maharashtra, 431001

Water is the soul of nature; its pollution will perish the whole world. Undesirable changes occurring in water which adversely affects the life activities of human, domesticated animals and causing disturbance to environment is referred as water pollution. The discharge of heavy metals into aquatic systems has become a matter of great concern in India over the last few decades. These pollutants are introduced into the aquatic systems significantly as a result of various industrial operations. Industrialization in India is gaining a momentum with initiation of five years development plan. Since, the industrial revolution, the efforts of removing man-made pollutants from the natural environment have been unable to keep pace with the increasing amount of industrial waste.

Hexavalent chromium compounds are genotoxic carcinogens; chronic inhalation of hexavalent chromium compounds increases the risk of lung cancer. The mechanism of genatoxicity relies on pentavalent and trivalent chromium. The damage is caused by hydroxyl radicals, produced during reoxidation of pentavalent chromium by hydrogen peroxide molecules present in the cells. The sub chronic and chronic oral RFD for hexavalent chromium are 0.02 and 0.005mg/kg/day (U.S.E.P.A. 1990). Based on adequate proof for humans and animals hexavalent chromium is placed in the EPA weight of evidence classification a human carcinogen.

Chromate compounds are known to cause mutations in bacteria and transformation in mammalian cells. The natural intracellular hexavalent chromium reduction occurs by successively accepting one electron, which generates pentavalent chromium. As by product, super active ionization of water results in free radical (OH^-) formation that results in DNA damage (Flessel, 1979). Exposure to hexavalent chromium also produces allergic dermatitis, ulceration of the skin, irritation of the mucosa membranes, nasal septum, renal tubular necrosis, and increase risk of respiratory tract infections. Recent studies have suggested a link between

exposure to hexavalent chromium and several forms of DNA damage. Heavy metals can be accumulated by microbial cells by a variety of processes, both physico-chemical and biological. Metabolism-independent binding or adsorption (biosorption) to living or dead cells, extracellular polysaccharides, capsules and slime layers is frequently rapid. Bacterial cell walls and envelopes and walls of algae, fungi and yeasts are efficient metal biosorbent with binding to chargedgroups frequently being followed by inorganic deposition of increased amount of metal (Burke *et. al.,* 1991). Volesky (1995) has defined utilization of only dead cells as the basis of biosorption and that of living cells as bioaccumulation. In practice there are three categories of biotechnological processes for treating liquid wastes containing toxic metals: biosorption; extracellular precipitation and uptake by purified biopolymers and other processes may be involved (Gadd and White,1993).

Immobilized microbial cells have been reported to be very effective method in recovery of metalions. Toxicity of heavy metal ions and other extreme properties of waste effluents may limit the use of living systems. Freely suspended microbial biomass has disadvantages that include small particle size and low mechanical strength (Katiyer and Katiyer, 1997).Immobilized microbial cells appear to be of a greater potential in controlling particle size, better capability of regeneration, easy separation of biomass from effluent, re-circulation, high biomass loading, minimal clogging etc. Further, it is very convenient to recover toxic metal ions and reuse the immobilized biomass.

Material and Methods

P. aeruginosa 4442, Sodium alginate (4.5%) , Calcium chloride (2%), Sterilized distilled water, Cr (VI) 100ppm with pH 7.0,.Different elutant solutions (1M $NaNO_3$, 1M NaOH, 1M EDTA, HCl 0.1N, HNO_3 0.1N, D. W.)

In order to study the effect of immobilization on Cr (VI) sorption by *P. aeruginosa* 4442 pellet was obtained as described above and it was immobilized in sodium alginate. Cell suspension (5ml) of *P. aeruginosa 4442* was taken andfrom it 3 ml was mixed thoroughly with 6ml of sodium alginate (4.5%).

Final concentration of sodium alginate was 3.0%. Slurry was dispersed drop by drop into 2% calcium chloride solution by a hypodermic syringe and kept for

2hrs at 4^0C. Then beads were washed thoroughly with distilled water and air dried. For storage, beads were dipped in normal saline and stored in air tight bottle. Dry weight of *P.aeruginosa 4442* immobilized was 0.001gm in 1 bead. In order to assess the biosorption of Cr (VI) by immobilized *P. aeruginosa* 4442, 100 ppm Cr (VI) solution with pH 7.0 was inoculated with 20 beads and incubated on a rotary shaker at 100 rpm at 30^0C. After 30 minutes contact, samples were withdrawn and analyzed for residual Cr (VI) using AAS-201, also one sample was analyzed after 24 hours.

Effect of immobilized inoculum on % sorption of Cr (VI) by immobilized *P. aeruginosa* 4442 was studied by inoculating 5 to25 beads in 100ppm Cr (VI) solution with pH 7.0 for 2hrs on a rotary shaker at 100 rpm at 30^0C for the period mentioned in the Table 2.29 .Samples were withdrawn and % Cr (VI) sorption was calculated.

To investigate desorption efficiency of different elutants, Cr (VI) laden immobilized *P. aeruginosa* 4442 cells were filtered and kept on filter paper so as to remove all the metal solution adhered to the immobilized cells, then it was taken into elutent solution (50ml) in 250 ml flask and 10 immobilized beads were added to it. It was allowed to react for 2hrs at 120 rpm at 30^0C. After incubation, it was again filtered and analyzed using AAS 201 for residual Cr (VI) eluted in elutant and its % desorption was calculated.

The % metal desorption efficiency (D) is calculated using following formula

% desorption efficiency- Ce x V/QxWX100

Where,

C al concentration in the elutant. V =

e volume of the elutant.

Q = metal uptake

=

W = weight of biomass

m

To investigate the reusability of the immobilized matrix containing Cr (VI), the 10

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immobilized beads were used for sorption-desorption cycles. The beads were allowed to sorb Cr

(VI) from the 100 ppm solution for 1hr and then allowed to desorption using EDTA as elutant for 5 cycles.

Results and Discussion

Cell immobilization studies showed that in 100 ppm Cr (VI) solution maximum 68% of sorption was observed after 150min of incubation at 30^0C(Table 1) which remained constant even after 24hrs of incubation.

Table 1 Impact of immobilized *P. aeruginosa* 4442 on adsorption of Cr (VI) using(20beads)

Contact time (minutes)	% sorption of Cr (VI)
30	40
60	49
90	64
120	67
150	**68**
after24hrs	**69**

Table 2. Effect of *P. aeruginosa* 4442 immobilized beads and contact time on Cr (VI) uptake

Number of immobilized beads	% sorption of Cr (VI)	
	2hrs	**24hrs**
5	33	35
10	46	48
15	54	56
20	**67**	68
25	**67**	**70**

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Table 2 showed the effect of increase in number of beads on % sorption of Cr (VI). It can be concluded from the results that 20 beads showed optimum % sorption (67%) further increase in number of beads failed to show further increase in % sorption at 2hrs of incubation. It was also noticed that Cr (VI) sorption was a fast process and completed in about 2hrs. Further incubation upto 24hrs had not resulted in much increase in *%* sorption of Cr (VI).

Table 3. Desorption of Cr (VI) from immobilized*Ps.aeruginosa 4442* (%of Cr (VI) was 65 %)

Elutant	% desorption of Cr (VI)
$NaNO_3$ (0.1M)	73
NaOH (0.1M)	74
EDTA (0.1M)	**79**
HCl (0.1N)	65
HNO_3 (0.1N)	77
Distilled water	20

Various eleutants applied for the Cr (VI) recovery from adsorbed material is presented in Table 3 Beads (10) immobilized with *P. aeruginosa* cells adsorbed 65% of 100 ppm of the Cr (VI) solution. These 10 Cr (VI) loaded beads were used for the desorption studies. All the eleutants were found to be responsible to recover Cr (VI). The recovery of Cr (VI) from the beads was maximum (79%) by using EDTA followed by HNO_3 (77%), NaOH and $NaNO_3$ (74% and 73%) respectively. Distilled water showed very poor (20%) elution of Cr (VI).

The free cells generally have low mechanical strength and small particle size and therefore excessive hydrostatic pressures are required to generate suitable flow rate in commercial biosorbent systems, such high pressures can cause disintegration of microbial cells and also they are not suitable for continuous sorption desorption systems and such problems can be solved by trapping them on

some suitable matrix. Immobilized beads (10) used in the first cycle of desorption studies. These beads were contained 65% of Cr (VI) immobilized on it. For desorption studies, EDTA (1M) solution was used.

Table 4. Effect of recycling of beads on sorption and desorption

(S/D) of Cr (VI)

S/Ds cycles	Percent of Cr(VI)	
	Sorption	Desorption
1	65	52
2	54	50
3	45	44
4	41	30
5	30	26

Results presented in Table 4 indicated that Na-alginate beads immobilized with *P. aeruginosa* cells can be reutilized for at least 5 cycles. Results also confirmed that reuse of beads results in a minimum decrease in adsorption-desorption percent from 52-26% Cr (VI) in 5 cycles (Table 4). The results thus indicated the reusability of alginate beads by repeated sorption-desorption cycles. Nearly 100% of Cr (VI) removal was observed by immobilized *P. aeruginosa 4442* that remedied Cr at a rate of 2.33mg/l / hour and compared to live free cells immobilized *Pseudomonas* beads were more efficient (Benazir *et. al*., 2010).

The form of the biomass to be used is an important consideration whether living or dead cells are used in a metal recovery system, while cells in free suspension can provide valuable information in laboratory experimentation. For more rigorous industrial applications microbial biomass has a number of disadvantages. It is generally of small particle size, low mechanical strength, and low reactor systems and make biomass/effluent separation difficult (Tsezos, 1986). For use in packed-bed or fluidized-bed

reactors immobilized or pelleted biomass appears to be of greater potential. The use of immobilized cell systems has many advantages over the use of freely suspended cells. These include better capability of re-using the biomass, easy separation of cells from the reaction mixture clogging in continuous flow systems. Whole cell immobilization within a polyacrylamide gel provides a useful laboratory scalesystem.

Both living and dead biomass can be used in immobilized systems. If living cells are used, there are possibilities for the removal of other undesirable substances, as well as heavy metals and radionuclide from waste waters, although toxicity is an important consideration. Living cells of *Pseudomonas aeruginosa* have been immobilized on particles of PolyVinyl Chloride (PVC) and melt blown polypropylene webs and used in batch and column systems for simultaneous denitrification and heavy metal removal from contaminated waste water (Hollo *et.al.,* 1979).

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