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Eco-Friendly and Effective Ways of Removal of Heavy Metals using Immobilized Cells

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ABSTRACT

Heavy metals in effluents are toxic as well as carcinogenic to living organisms. Chemical and physical methods reviewed for the removal of heavy metal shave several disadvantages. Immobilization of whole cells has gained importance in the area of waste water treatment, as they are simple, economical and effective.
For this, waste water samples from two different places were collected in Thane district and checked for various chemical parameters. In this study, efficacy of bio sorption in removal of heavy metals (Chromium, Nickel and Cadmium) was tested using heavy metal resistant microorganisms immobilized on sand. The bio sorption efficiency was determined by chemical estimation methods.
Immobilization by sand was very efficient in removal of the heavy metals (80-90%) compared to free cells and sand alone. It also improved various parameters of waste water. Thus, it proved that cells immobilised on sand can be an effective and eco-friendly method for removing heavy metals.

Keywords: heavy metals, immobilisation, sand, waste water.

INTRODUCTION

Heavy metal ions, such as cadmium, lead and mercury, are highly toxic to living organisms.[1] Most of the heavy metals discharged into wastewater are found to be toxic and carcinogenic and causes a serious threat to the human health. The release of large quantities of hazardous materials into the natural environment has resulted in a number of environmental problems and due to their non-biodegradability and persistence, can accumulate in the environment elements such as food chain, and thus, may pose a significant danger to human health. To avoid health hazards, it is essential to remove these toxic heavy metals from waste water before its disposal.[2]Environmental pollution particularly from heavy metals and minerals in the wastewater is the most serious problem in India. [3]

A number of efficient methods have been reviewed for the removal of heavy metals such as chemical precipitation, ion exchange, reverse osmosis, electro-dialysis, ultra-filtration, nano-filtration, coagulation, flocculation, floatation, etc. however these methods have several disadvantages such as high reagent requirement, unpredictable metal ion removal, generation of toxic sludge etc. Adsorption process being very simple, economical, effective and versatile has become the most preferred methods for removal of toxic contaminants from wastewater.[4]

A vast array of biological materials, especially bacteria, algae, yeasts and fungi have received increasing attention for heavy metal removal and recovery due to their good performance, low cost and large available quantities.[5]Heavy metal resistant microbes might be present in heavy metal contaminated sites.[6]Recently, immobilised whole cell has been regarded as an alternative method of enzyme immobilization, since it is a tedious and time consuming process.[7]The present study aims at using isolated heavy metal resistant microorganisms in their immobilised form for removal of heavy metals from waste water samples.

MATERIALS AND METHODS

Collection of waste water samples:

Waste water samples were collected from two different locations in Thane District, namely Ambernath and Ulhasnagar. Samples were collected in sterile plastic bottles and the bottles were transported immediately to the laboratory with appropriate care and stored at 4^{o}C till further processing of samples. The samples were then processed as per protocol.

Isolation and preservation of pre-existing isolated heavy metal resistant microorganisms:

Pre-existing heavy metal resistant microorganism, Gram positive rods (with MIC of 500ppm for Cadmium and more than 1000ppm for Nickel and Chromium) from Department of Microbiology, R. K. Talreja College were isolated on St. Nutrient agar, following incubation for 24-72 hours. Further the cultures were maintained on St. Nutrient agar slants and subcultured after every 4 weeks.

Analysis of waste water:

The analysis of waste water was performed before and after treatment with immobilised cells for various parameters like nitrate, nitrite, sulphate, phosphate content, BOD and COD as per standard protocols.[8]

Chemical estimation of individual heavy metals:

The waste water sample was checked for the presence of different heavy metals: chromium, [9]nickel[10] and cadmium[11]qualitatively and quantitatively by diphenyl carbazide method, dimethylgly oxime method and alizarin red S solution method respectively.

Bio sorption efficiency of various heavy metals using microorganisms immobilised on sand:

An inoculum of heavy metal resistant organism, 1ml (O.D. 0.1) was added to defined amount of St. Nutrient broth and incubated for 24 hours under shaker conditions. To this, sterile dried sand (32.5g) was added and further incubated for 24 hrs on shaker. The broth was then decanted and 100ml of stock of heavy metal salt solution was added to it and kept on shaker. Different flasks were maintained for the heavy metals, Cr, Ni and Cd. All the flasks were incubated at R.T. on shaker for 4 days. The samples were withdrawn at regular intervals, diluted and its heavy metal ion concentration was determined by chemical estimation methods. Two controls, free sand and free cells, were also maintained. The stock solutions of heavy metal salts were used at following concentrations: $K_2 Cr_2 O_7$ (700ppm), $NiCl_2$ (700ppm) and $CdCl_2$ (300ppm).

Bio sorption percentage was calculated as-

$$\text{Percentage Bio sorption (\%)} = \frac{\text{initial - final metal concentration}}{\text{initial metal concentration}} \text{x } 100$$

RESULTS AND DISCUSSIONS

Bio sorption efficiency of various heavy metals using microorganisms immobilized on sand:

The bio sorption efficiency for various metals were assessed using immobilised cells on sand and using free sand and free cells as control.

Bio sorption of Chromium, Nickel and Cadmium by immobilized cells using sand:

The percentage removal of heavy metals, by cells immobilized on sand, increased with an increase in incubation period from 0 hour to 96 hours as shown in Table 1. The bio sorption efficiency of Cr, Ni and Cd was 84.8 %, 93.26% and 95.5% by the end of 96 hour. With increase in the incubation period no increase in bio sorption was seen in both the controls, free cells and free sand.

Bio sorption of Nickel and Cadmium was much faster compared to Chromium, as around 85-86% of Ni and Cd were removed within the first 24 hours. By the end of 96 hours, efficiency of removal of Cadmium by immobilised cells was the highest (36.7%) followed by Nickel (31.73%) and Chromium (15.2%) compared to control.

Table 1: Bio sorption of Chromium, Nickel and Cadmium by immobilized cells on sand

Time (hours)	Percentage of heavy metals removed								
	Chromium			Nickel			Cadmium		
	A	B	C	A	B	C	A	B	C
0	0	0	0	0	0	0	0	0	0
24	69.6	69.6	69.6	86.53	49.61	49.61	85.3	58.8	71.2
48	78.7	69.6	69.6	86.53	49.61	49.61	85.3	58.8	71.2
72	84.8	69.6	69.6	93.26	61.53	61.53	90.6	58.8	71.2
96	84.8	69.6	69.6	93.26	61.53	61.53	95.5	58.8	71.2

A-Immobilised cells, B- Free cells and C- Free sand

Gram-positive bacteria accumulate much higher concentrations of heavy metals on their cell walls than that of metals Gram-negative bacteria **(Nanda M.et al., 2019)**. Studies have reported that bacteria which are multi-heavy metal resistant have greater MIC values as compared to bacteria showing resistance to a single heavy metal**(Goyal Pet al., 2020).**

Elahi and Rahman, 2018 have reported that B. aerius S1 and B. iodinum S2 were capable of removing upto 99% Cr^{6+} from tannery effluent after 6 days of incubation.

Ilunga Kamika and Maggy NB Momba (2013) reported that living Pseudomonas putida had the highest removal rates of heavy metals Ni-51% in 5 days. **Hany Hussein and et al (2004)** also reported that maximum removal of Ni(II) was in the range between 35 to 88% by Pseudomonas species in 20 mins.

K. Mathivaan et al, 2014 study showed that strain TT-10 belonging to Pseudomonas species showed 99% biosorption of cadmium in 10 days. **Hany Hussein et al (2004)** also reported that maximum removal of Cd (II) was in the range between 35 to 88% by Pseudomonas species in 20 mins.

Analysis of waste water:

The chemical analysis of waste water was performed before and after treatment with immobilised cells.[8] The analysis was done for various parameters like nitrate, nitrite, sulphate, phosphate content, BOD and COD as per standard protocols.The results were as shown in Table No. 2.

Table 2: Percentage removal of Nitrate, Nitrite, sulphate, phosphate, BOD&COD from Ambernath & Ulhasnagar wastewater effluent

ParameterTests	Ambernath	Ulhasnagar
	% Reduction	% Reduction
Nitrate(ppm)	35.71	58.8
Nitrite(ppm)	41.66	71.4
Sulphate(ppm)	46.8	50
Phosphate(ppm)	35.6	18.36
BOD (mg/L)	50	50
COD(mg/L)	7.69	7.69
Crcontent(ppm)	88.5	87.8
Nicontent(ppm)	91.75	80.8

Cdcontent(ppm)	95.3	94.3

On treatment with immobilized cells, the nitrate, nitrite and sulphate content were reduced effectively upto the permissible limits from both the waste waters. As compared to others, the phosphate reduction was less; it was around 18-35%. Also, BOD of both the waste waters were reduced by 50%, while COD reduction wasn't much significant. The heavy metals Cr, Ni and Cd were bio sorped from both the waste waters by 80-95%.

Adhoni S A et al. (2018) reported similar results as immobilized algal cells biosorped most nitrates such that the nitrate content was almost negligible after 30 days. According to J. Abarnadevi, M. Anu, M. Bharani (2013) the level of BOD was recorded maximum as 340 mg/l initially. It was reduced nearly 65% in the effluent treated with Aspergillus when compared to control.

CONCLUSIONS

Heavy metal pollution is one the most important environmental problems in marine, terrestrial, and freshwater areas. In the present study, bio sorption efficiency of heavy metals resistant isolate was determined. Bio sorption of heavy metals like Chromium, Nickel & Cadmium was performed by immobilizing heavy metal resistant organisms on sand. Rate of removal of heavy metals was very high in first 24 hours. As contact time increased, bio sorption capacity of immobilized cells also increased. Treatment with immobilised cells also improved the chemical parameters of waste water along with its stabilization. The maximum heavy metals were bio sorped after 96 hours. Thus, use of heavy metal resistant organisms immobilised on sand proved to be highly effective & eco-friendly method for bio sorption of heavy metals and stabilization of waste water.

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