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Isolation and screening of bacterial isolates for bioremediation of lead, cadmium, chromium and nickel from wastewater: An experimental approach

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ABSTRACT

Removal of heavy metals such as Cadmium, Chromium, Lead, and Nickel from wastewater was carried out through microorganism. For this purpose, heavy metals tolerant bacterial isolates were isolated from samples collected from Karnal, Panipat and Sonepat districts of Haryana using enrichment culture technique. The bacterial isolates tolerant up to 400 ppm concentration of heavy metals (Pb, Cd, Cr and Ni) were tested for removal of heavy metal from liquid media containing 50 ppm concentration of Pb, Cd, Cr and Ni each. The Maximum uptake capacity of Pb, Cd, Cr, Ni tolerant bacterial isolates (BPb11, BCd4, BCr24 and BNi12) were 55.88 mg/g, 2.42 mg/g, 4.29 mg/g and 5.39 mg/g respectively.

Key words: Wastewater; Cadmium; Chromium; Nickel; Lead.

INTRODUCTION

Several electroplating industries including agricultural sector industries, sewage treatment and mining operations all combine to generate hazardous waste water containing enormous amounts of toxic heavy metal[1,2,3,4]. The accumulation of these toxic heavy metals in human beings, animal through food chain. Many methods for treatment of heavy metal contaminated wastewater have been used in the world. Among them, chemical precipitation[7], ion exchange[8], reduction[9], adsorption[10], electrochemical precipitation[11], solvent extraction[12], membrane separation[13], Cementation, evaporation, reverse osmosis, foam separation and freeze separation[14,15]. Despite effective treatment, these methods are expensive with the high cost of equipment, chemical and manpower. So, the use of microbial method that minimizes metal contaminants has been considerably interested now. In this study bioremediation investigator solved heavy metal problem in wastewater through microorganism. Efficiency for removal of heavy metal from wastewater using microorganisms has been varied from one to another microorganism. The present work describes the isolation and screening of the heavy metal resistant microorganisms from wastewater, sewage, sludge, industrial effluents and testing of efficient bacterial isolates for removal of heavy metals (Pb , Cd, Cr and Ni).

MATERIAL AND METHODS

Collection of samples

Samples of sewage, sludge and industrial effluents were collected in sterilized containers from sewage treatment plants at Karnal, Panipat and electroplating industry at Sonepat. These samples were brought to laboratory and kept in refrigerator at 4 °C.

Isolation of Bacteria

Bacterial isolates were isolated from samples of sewage, sludge and industrial effluents by serial dilution method using nutrient agar medium containing 25 ppm of Pb, Ni, Cr, and Cd individually. The 1000 ppm stock solutions of Pb, Ni, Cr and Cd were made in double distilled water using $Pb(NO_3)_2$, $NiCl_2$ .6 H_2O, $CdCl_2$, and $K_2Cr_2O_7$. The 25 ppm solution of these heavy metals was prepared from

1000 ppm stock solution by dilution with double distilled water. The stock solution of heavy metals were sterilized separately through bacteriological filters and added to sterilize nutrient medium (Hi Media Pvt. Ltd, Mumbai) to make its concentration 25 ppm. A serial dilution of each sample was made up to 10^{-6} and one ml of dilution 10^{-4} and 10^{-6} was added in sterilized Petri plates. 20 ml Nutrient agar medium containing 25 ppm of one of these heavy metals individually was poured and incubated at 37°C for 48 hours. The colonies of predominant genera of bacteria were picked and purified by serial dilution method[4].

Screening of bacterial isolates for tolerance to heavy metals

The isolated heavy metal tolerant (25 ppm) bacterial isolates were further screened for tolerance to Pb, Ni, Cr and Cd at 50, 100 and 400 ppm of heavy metals individually on nutrient agar medium. All the bacterial isolates were streaked on nutrient agar medium containing 50, 100 and 400 ppm of each of the four heavy metals separately. Streaking of bacterial isolates on normal nutrient medium served as control (normal growth) for comparison of growth of bacterial isolates on nutrient medium containing different concentration of heavy metals. Observations on growth of bacterial isolate were made after 48 hours of incubation of inoculated Petri plates.

Uptake of heavy metals by bacterial isolates from liquid media

The highly tolerant bacterial isolates to different heavy metals were evaluated for uptake in nutrient broth medium containing 50 ppm concentration of different heavy metals Pb, Ni, Cr and Cd individually. Nutrient broth containing 50 ppm of one of the heavy metals was dispensed in100 ml lots to 250 ml conical flask and sterilized at 15lbs/psi for 15 min. These flasks were inoculated with 1 ml of bacterial cell suspension (10^6 to10^7 cells/ml) of each bacterial isolate and put on shaker at 150 rpm at 37°C for 48 hours. Uninoculated flasks containing nutrient broth of 50 ppm concentration of different heavy metals served as control. Bacterial growth was centrifuged at 5000 rpm for 10 minutes after 48 hours and washed with phosphate buffer saline (PBS).The harvested bacterial biomass was rinsed with double distilled water and dried in hot air oven at 80 ^{0}C for 18 hours. The dried bacterial biomass was weighed and heavy metal concentration in it was estimated by digestion with nitric acid and perchloric acid (3:1 ratio)[16]. The digestion mixture was filtered through Whatman filter No. 42. and made the volume of filtrate to 50ml in volumetric flask. The heavy metals concentration in filtrate was estimated by Atomic Absorption Spectrophotometer (AAS)[16].

RESULTS AND DISCUSSION

Bacterial growth and uptake of heavy metals by bacterial isolates

Total 60, 16, 24, 41 isolates selected for tolerance towards the Pb, Cd, Cr and Ni respectively (data not shown). Out of these isolates only 14, 4, 4 and 10 isolates tolerant 400 ppm Pb , Cd , Cr and Ni respectively (Table 1).

The maximum dry weight (0.361 g) was observed by bacterial isolate BPb23 followed by BPb24 (0.335 g) in nutrient broth containing 50 ppm of Pb[17]. The minimum dry weight (0.024g) was observed by bacterial isolate BPb10, 15. The maximum uptake (55.88 mg/g) of Pb was observed in bacterial isolate BPb11. Minimum uptake Pb (0.52 mg/g) found in bacterial isolate BPb19 (Table 2)[18].

Table 1: Screening of different bacterial isolates on nutrient agar media containing different concentration of heavy metals

Heavy metal	25 ppm	50 ppm	100 ppm	400 ppm
Pb	43	31	27	14
Cd	14	11	10	4
Cr	10	5	4	4
Ni	28	24	16	10

The maximum dry weight (0.155g) was observed by bacterial isolate BCd9 in Nutrient broth containing 50 ppm of Cd. The minimum dry weight (0.040g) was observed in BCd3. The maximum uptake (2.42 mg/g) of Cd was observed in BCd4. Minimum uptake of Cd (0.34mg/g) was observed in BCd9 (Table 3)[19,22,23,24].

Table 2: Uptake of Pb by different bacterial strains from liquid media containing 50 ppm Pb

Strain No.	Dry weight (g)	Uptake (mg/g)
BPb 6	0.048	19.38
BPb 7	0.030	27.39
BPb 8	0.046	18.2
BPb 9	0.082	8.94
BPb 10	0.024	7.56
BPb 11	0.032	55.88
BPb 12	0.031	6.57
BPb 13	0.031	25.8
BPb 14	0.037	53.8
BPb 15	0.024	15.31
BPb 16	0.067	5.27
BPb 17	0.030	1.83
BPb 18	0.132	6.5
BPb 19	0.178	0.52
BPb 20	0.025	23.29
BPb 21	0.050	5.56
BPb 22	0.036	6.72
BPb 23	0.361	1.19
BPb 24	0.335	0.69

Table 3: Uptake of Cd by different bacterial strains from liquid media containing 50 ppm Cd

Strain No.	Dry weight (g)	Uptake (mg/g)
BCd 1	0.076	0.38
BCd 2	0.073	1.37
BCd 3	0.040	1.48
BCd 4	0.062	2.42
BCd 5	0.095	1.33
BCd 6	0.075	2.28
BCd 7	0.076	1.70
BCd 8	0.129	0.76
BCd 9	0.155	0.34
BCd 10	0.104	1.90

Table 4: Uptake of of Cr by different bacterial strains from liquid media containing 50 ppm Cr

Strain No.	Dry weight (g)	Uptake (mg/g)
BCr 1	0.079	0.37
BCr 2	0.049	0.60
BCr 3	0.045	0.34
BCr 5	0.048	0.03
BCr 7	0.054	0.03
BCr 8	0.065	0.24
BCr 9	0.046	0.03
BCr 10	0.023	0.06
BCr 11	0.065	0.02
BCr 12	0.098	1.43
BCr 13	0.065	0.02
BCr 15	0.079	0.19
BCr 16	0.068	0.02
BCr 17	0.046	0.03
BCr 18	0.059	0.02
BCr 23	0.068	2.06
BCr 24	0.065	4.29

Table 5: Uptake of Ni by different bacterial strains from liquid media containing 50 ppm Ni

Strain No.	Dry weight (g)	Uptake (mg/g)
BNi 2	0.066	0.34
BNi 3	0.025	1.19
BNi 5	0.095	0.94
BNi 6	0.079	0.19
BNi 7	0.054	1.38
BNi 9	0.049	1.83
BNi 10	0.064	1.52
BNi 11	0.046	0.16
BNi 12	0.018	5.39
BNi 16	0.036	2.07
BNi 17	0.064	0.47
BNi 18	0.034	1.76
BNi 21	0.079	0.57
BNi 22	0.064	1.05
BNi 23	0.058	1.42
BNi 24	0.038	0.79
BNi 25	0.065	1.26

The maximum dry weight (0.098g) was observed in BCr12. The minimum dry weight (0.023g) was found in bacterial isolate BCr10. The maximum uptake (4.29 mg/g) and minimum uptake of Cr (0.02) was observed in bacterial isolate BCr24 and BCr11,16,18 in Nutrient broth containing 50 ppm of Cr respectively (Table 4)[24,25,27,28].

The maximum dry weight (0.95g) was observed in BNi15 in Nutrient broth containing 50 ppm of Ni. The minimum dry weight (0.025g) was found in bacterial isolate BNi3. The maximum uptake (5.39 mg/g) and minimum uptake of Ni (0.16 mg/g) from Nutrient broth containing 50 ppm of Ni respectively (Table 5)[4,26,28].

The above data of dry weight of bacterial isolate and uptake of heavy metal by bacterial isolate indicate, in general, that where there is high dry weight of bacterial isolate, there is less uptake of heavy metal and vice versa. Similar results have been reported by earlier workers[4]. The bacterial isolates were tested in liquid media containing 50 ppm of Pb, Cd, Cr and Ni. Those bacterial isolates which are showing maximum uptake capacity for heavy metals (Pb, Cd, Cr, Ni) are useful for removal of heavy metals from wastewater and industrial effluents.

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