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Bioremediation of Phenol, Ammonia, Nickel, Hexavalent Chromium and Iron from Steel Plant Effluent of Visakhapatnam City by Live, Killed and Immobilized Bacteria

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Bioremediation of phenol, ammonia, nickel, hexavalent chromium and iron from untreated steel plant effluent of Visakhapatnam city was carried out using different bacteria. Live *Bacillus* species could remove 8% phenol, 100% ammonia, 92.5% nickel, 88% hexavalent chromium and 73.1% iron(II), from industrial effluent. Biosorption technique showed 100% and 97% of chromium removal by *Staphylococcus aureus* and *Bacillus* species (BS2) respectively. There was 90.7 and 47.7 removal of ammonia by *Bacillus* species and *Staphylococcus aureus* respectively by immobilization technique. Chi-square test was employed to test whether the results obtained were significant or not at the level of $P < 0.05$.

Key Words: Bioremediation, Pollutants, Steel plant, Effluent, Bacteria, Biosorption, Immobilization

INTRODUCTION

Direct and indirect anthropogenic activities such as dumping of municipal wastes, legal and illegal discharge of industrial, agricultural wastes, into various water bodies, are the major sources of pollution of the environment. These wastes emit different toxic pollutants such as phenol, ammonia, heavy metals like nickel, hexavalent chromium, copper, mercury, zinc, cadmium, arsenic, lead and other radionuclides into the biosphere which by entering the food chain are hazardous to the health of entire life on this globe[1–5]. Several conventional chemical techniques, use of different adsorbents to remove the heavy metals and radionuclides from the environment have been in practice for the last several years[6–10]. But the chemical methods generate secondary pollutants and are ineffective at low concentration of metals. Environmental biotechnology is the technology of application of micro organism to improve the environment. Bioremediation is a recent, ecofriendly technique of application of living organisms to remove the hazardous wastes from the environment. There are several reports on removal of toxic pollutants, particularly the heavy metals, by chemical methods as mentioned above. But bioremediation of these toxic chemicals using bacteria, by biosorption and immobilization techniques are scanty[11, 12]. Therefore the aim of this investigation was to remove phenol, ammonia, nickel, hexavalent chromium and iron from untreated steel plant effluent using various species of *Bacillus* and *Staphylococcus aureus*, the gram positive bacteria.

EXPERIMENTAL

Bacterial growth condition: *Bacillus subtilis* was procured from MTCC[12], Chandigarh, India. *Staphylococcus aureus* was isolated in our laboratory from the water and identified as *S. aureus* by Gram staining, mannitol fermentation by growing on mannitol salt agar and novobiocin sensitivity assay. Two other gram +ve bacilli are isolated as laboratory contaminants and identified by several biochemical tests as *Bacillus* species. This was labelled as BS1, another bacterium identified as *Bacillus* species and temporarily named as BS2. These four types of bacteria were maintained in the laboratory on nutrient agar slants and plates. They were stored in refrigerator and periodically maintained in fresh media.

Bioremediation of pollutants by live bacteria: Sterile steel plant effluent was inoculated with *S. aureus* (loopful/mL effluent) and incubated at room temperature in an orbital shaker for 24 h. Later the culture was spun at 5000 rpm for 15 min and the supernatant was collected to estimate various pollutants such as phenol, ammonia, nickel, chromium(VI) and iron. Different pollutant concentrations were obtained from the standard curves of pollutants. The phenol was estimated spectrophotometrically by direct photometric method using 4-aminoantipyrine[13]. Standard curve was plotted by taking various concentrations of phenol on x-axis and OD : 540 nm on y-axis. Ammonia was estimated spectrophotometrically by Nessler's method[13]. Nickel[13], hexavalent chromium[13] and iron[14] were estimated by dimethyl glyoxime, diphenyl carbazide and 1 : 10 phenanthroline methods respectively using spectrophotometer. A control sterile steel plant effluent was also simultaneously incubated with experimental flask but without addition of *S. aureus*. The pollutant content in the treated effluent with *S. aureus* was compared with those of control and percentage removal of pollutant was calculated. Similarly other bacteria were also incubated separately and the contents of pollutants were estimated. To assess whether all the results obtained in this investigation were significant ($P < 0.05$) or not, Chi-square test was employed.

Bioremediation of pollutants by biosorption technique: It was done as described previously[12]. All four types of bacteria were cultivated on nutrient agar plates separately at 37°C for 18–24 h. Later the cells were harvested, washed twice in sterile distilled water and the pellet was dried in a hot air oven at 100°C for 1–3 h. Dried pellet was pulverized to fine powder. This powder is the sorbent. Then the sterile steel plant effluent was taken in a conical flask and inoculated with sorbent (20 mg/L effluent). The flask was incubated at room temperature (29°C) for 24 h, spun at 5000 rpm and the supernatant was collected to estimate different pollutants as mentioned above.

Bioremediation of pollutants by immobilization: Each bacterial culture was cultured on nutrient agar plates. The sodium alginate solution 3% (w/v) was sterilized and heated to make a slurry. Ten loopfuls of each culture were added in 5 mL of alginate slurry separately and mixed very well. This slurry was dropped into beads with the help of a syringe into 50 mL of sterile calcium chloride solution (0.1 M). After 1 h the beads were collected aseptically and the bead diameter was calculated to be 46.2 mm. The sterile steel plant effluent was inoculated with beads (1 g of beads/2 mL effluent) and incubated at room temperature in an orbital shaker

for 24 h. Later the solution was collected and the contents of pollutants were estimated. Control beads were prepared only with sodium alginate slurry without addition of bacterial culture. Control beads were also incubated simultaneously with experimentals and pollutant concentration was estimated.

RESULTS AND DISCUSSION

There was 76 and 75% removal of chromium and nickel respectively by live *S. aureus* in 24 h of incubation indicating that there was very high percentage removal of chromium and nickel by this organism (Table-1). Live *B. subtilis* could remove 100% chromium indicating that this organism has a strong potential for chromium removal. The live *Bacillus* species (BS1) showed 100% removal of ammonia, 92.5% of nickel, 88.5% of chromium and 73.1% of iron indicating that this organism has the highest potential for bioremediation. So far *Nitrosomonas* and *Nitrococcus* species are recognized as the potential removers of ammonia which takes a very long time to culture these organisms as they are very slow growing organisms. But this *Bacillus* species which was isolated from our laboratory is a fast growing bacterium with highest removing capacity of ammonia, not only ammonia but also nickel, chromium and iron. The live *Bacillus* species (BS2) could remove 86.6 and 75% of iron and chromium respectively. These results are similar to those of Elizabeth[15], where there was 96.4% of ammonia removal by *Bacillus* species (BS1) when cultured for 72 h in basal salt medium at pH 7, when used at 100 mg/L concentration. Most of the results were statistically significant and summation of calculated chi-square values is higher than tabulated values, that is, 3.8 and $P < 0.05$. Insignificant values are denoted by asterisk (*). Bioremediation of these pollutants by biosorption technique (Table-2) shows that there was 53.8 to 100% removal of phenol by these organisms, *B. subtilis* with highest percentage removal. Highest percentage removal of ammonia is obtained by *Bacillus* species (BS2) *i.e.*, 94.5%; highest percentage removal of nickel by *Bacillus* species (BS1) *i.e.*, 83.4%. There was 86 to 100% removal of chromium by all four bacteria used, *S. aureus* with 100% removal. The percentage removal of iron ranged from 58.8 to 90.0 by sorption technique, *Bacillus* species (BS2) with highest percentage removal and *S. aureus* with least percentage removal. These results correlate well with our unreported results[15], where there was 86% removal of nickel and 86.5% removal of chromium by BS1. The removal of positively charged heavy metals and other pollutants may be due to the adsorption to the negatively charged bacterial components such as teichoic acid, phospholipids which are present in the cell walls of these gram +ve bacteria. The percentage removal of phenol by immobilization technique (Table-3) showed a range of 37 to 68, *Bacillus* species BS2 with highest removal and BS1 with least. Percentage removal of ammonia by this technique ranged front 47.7 to 90.7%, BS1 with highest and *S. aureus* with lowest percentage removal. There was 11.7 to 40% removal of nickel by this technique. Highest percentage removal of chromium was exhibited by *Bacillus* species (BS1). *Bacillus* species (BS2) could remove 66.4% of iron by immobilization technique. These results are similar to those of Churchill *et al.*[11], who observed higher percentage removal of chromium(VI) by biosorption technique using bacteria.

TABLE-1
BIOREMEDIATION OF POLLUTANTS BY LIVE BACTERIA

S.No.	Live organism	Percentage removal of				
		Phenol	Ammonia	Nickel	Chromium	Iron
1	*S. aureus*	0.0*	17.9	75.00	76.0	64.5
2	*B. subtilis*	20.0	40.0	69.20	100.0	42.4
3	*Bacillus* species (BS1)	8.0*	100.0	92.50	88.5	73.1
4	*Bacillus* species (BS2)	5.0*	37.1	29.16	75.0	86.6
5	Control	0.0	0.0	0.00	0.0	0.0
6	Pollutant concentration in sterile steel plant effluent after incubation (control) (mg/L)	600	800	12.50	95	330

* insignificant; rest of the chi-square values are > the tabulated value, *i.e.*, 3.8 and $P < 0.05$.

TABLE-2
BIOREMEDIATION OF POLLUTANTS BY BIOSORPTION TECHNIQUE

S. No.	Killed bacteria Biosorption	Percentage removal of				
		Phenol	Ammonia	Nickel	Chromium	Iron
1	*S. aureus*	58.3	91.5	35.0	100.0	58.8
2	*B. subtilis*	100.0	43.5	37.9	87.8	64.5
3	*Bacillus* species (BS1)	71.0	64.7	83.4	86.0	70.2
4	*Bacillus* species (BS2)	86.3	94.5	72.8	97.0	90.0
5	Control	0.0	0.0	0.0	0.0	0.0
6	Pollutant concentration in sterile steel plant effluent (mg/L)	600	800	12.5	95	330

TABLE-3
BIOREMEDIATION OF POLLUTANTS BY IMMOBILIZATION TECHNIQUE

S. No.	Immobilized bacteria	Percentage removal of				
		Phenol	Ammonia	Nickel	Chromium	Iron
1	*S. aureus*	65.0	47.7	13.7*	37.9	64.4
2	*B. subtilis*	62.0	35.0	40.0	51.5	52.0
3	*Bacillus* species (BS1)	37.0	90.7	15.6*	58.0	51.0
4	*Bacillus* species (BS2)	68.0	56.0	11.7*	48.5	66.4
5	Control bead	17.0	13.0	15.0	20.0	10.0
6	Pollutant concentration in sterile steel plant effluent (mg/L)	600	800	12.50	95	330

* indicates that those values are not significant and rest of the values are significant as the calculated chi-square value exceeds tabulated value of 3.8. So, $p < 0.05$.

Conclusion

These results clearly indicate that *Bacillus* species BS1 is a very potential microbe which can remove phenol, ammonia, nickel, chromium and iron in live, killed and immobilized forms. Of all the techniques used, biosorption technique proved to be the most efficient in the removal of most of the pollutants of Visakhapatnam steel plant effluent.

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