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Remediation of Heavy Metals from Electroplating Effluent using Bacterial Strains in Up flow Immobilized Column Reactor

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The contact of effluent containing heavy metals into the soil pose severe environmental and health hazard. Bioremediation of the contaminated soils has been widely attempted to remove heavy metal contaminants. This study was conducted to establish the efficiency of microorganisms present in heavy metal contaminated soil by bioremediation. Six bacterial strains and two exogenous bacterial strains were screened based on their capability to grow in nutrient agar media containing heavy metals. Slurry phase and immobilized column reactor were used to treat contaminated soil. Exogenous bacterial strain *Bacillus sp.* and *pseudomonas sp.* showed enhanced growth, which was chosen for further examination. The results revealed that the combination of *Bacillus sp.* and *Pseudomonas sp.* exhibits excellent efficiency of reducing heavy metals. The soil treatment results showed that, the up flow immobilized column reactor can reduce the heavy metal content appreciably within 24 hours. This evolves as an efficient strategy to replace the less ecofriendly physicochemical approaches and also provides a better insight into the field of bioremediation.

Key words: Bioremediation, *Bacillus sp.*, *Pseudomonas sp.*, Heavy metal, Slurry phase reactor, Immobilized column reactor.

Owing to rapid industrialization and population increase during the past few decades, there has been a significant increase in the utilization and release of chemicals including heavy metals. Industries such as metallurgical, electroplating, metal finishing, tanneries, chemical manufacturing, mine drainage and battery manufacturing are the main source of metals discharge in the environment[1]. Heavy metal pollution has become one of the most severe threats to environment and human health. Most of the heavy metals have toxic effects on living organisms when exceeding a threshold level[2]. The environmental standards and ethics require that wastewater, before being released into the natural water bodies, has to be sufficiently purified[3].

Thus, removal of heavy metals is of great importance for human welfare. The conventional techniques such as chemical precipitation, chemical oxidation and reduction, ion exchange, filtration, reverse osmosis, etc. are used for removing heavy metal ions from dilute solutions. Furthermore, these techniques of removal are often inappropriate or expensive, particularly when unwanted heavy metals are present in very low concentration or in large solution volume[2,4]. In recent years, bioremediation of heavy metals using microorganisms has received a great deal of attention, not only as a scientific novelty but also for its potential[2,5].

Bioremediation is one of the promising technologies that have been expected to play an important role in economical degradation of the toxic contaminants[6]. For survival under metal-stressed conditions, bacteria have evolved several mechanisms to tolerate the uptake of heavy metal

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ions. It mainly involves efflux of metal ions outside the cell, accumulation and complexation of the metal ions inside the cell and oxidation-reduction of the heavy metal ions to a less toxic state[7]. These microbes are widely distributed in the environment and heavy metals, contaminated soil and water are the potential sources of heavy metal-resistant bacteria[5]. Among microorganisms, bacterial biomass (both live and dead) have gained attention of researchers for the removal of metal from aqueous solution during recent years because of their ubiquity and smaller size, which leads to high surface area and fast rates[8,9]. Nevertheless, several functional groups such as carboxyl, amine, and hydroxyl groups are reported to be present as bacterial cell wall component due to which bacteria are capable of sorbing metal ions from aqueous solution.

The members of *Bacillus* genus are easy to culture and have shown high tolerance to heavy metal toxicity. Bacterial species on surfaces of geologic materials can detoxify the metal compounds by reducing them to relatively insoluble and hence significantly less harmful compounds. Biological reduction of heavy metals using indigenous microorganism offers a new cost-effective and environmentally compatible technology[10]. Present study aims at identifying the bioremediation potential in removal of heavy metals contaminated soil. Indigenous and exogenous microbes was used for immobilization technique to degrade heavy metals in soil. In addition, sustaining the treatment efficiency by designing bench-scale bioreactors and optimize the reactor parameters.

MATERIALS AND METHODS

Sampling and isolation of microorganisms:

Effluent sample was collected from an electroplating industry, Madurai district, Tamil Nadu, India for screening heavy metal degrading bacterial isolates. The isolated bacterial strains and bacterial strains obtained from MTCC (*Bacillus sp*., *Pseudomonas sp.*) which were used in this study. The soil collected from the college premises is contaminated with metal effluent obtained. Soil samples were sieved in 2.36 mm sieve and preserved at 4°C to avoid further contamination and chemical characteristics of soil like total organic matter and total nitrogen were determined based on standard protocols for soil analysis. The leachate obtained from soil sample was analyzed for metal concentration by Atomic Absorption Spectrophotometer (AAS).

Isolation and Enrichment Technique:

1mL of the metal effluent was added to 99mL of sterile distilled water (10^{-1}). For serial dilution, 4.5 mL distilled water was taken in series of tubes and 0.5mL of sample was transferred from 10^{-1} dilution to next tube upto 10^{-8} dilution. The serially diluted samples (10^{-5} and 10^{-6}) were plated on nutrient agar plates and the plates were incubated at 37ºC for 24 hours. The incubated plates were observed for the predominant types of organisms. Six strains with different colony morphology were isolated by streaking on Nutrient agar plates and incubated at 37° C. All the isolated cultures were studied by inoculating them in an enriched nutrient medium. The inoculated medium was incubated at 37° C for 24 hrs under shaking in an orbital shaker at 140 rpm. Two exogenous strains *Bacillus sp*., *Pseudomonas sp.*, were also checked for its metal tolerance by plating technique.

Slurry phase reactor (SPR):

For slurry biotreatment experiment, soil to be treated was mixed with distilled water in solid concentration of 30% by weight (30g soil and 70mL water) and taken in a 250 mL Erlenmeyer flask. Aeration is provided by air spargers through diffuser along with microbial strains which constitute the SPR. Treatment trails were performed by adding 3 mL of the various strains and its combination (1.5+1.5 mL). The SPR was kept at 37° C for 24 hrs under shaking in an orbital shaker at 200 rpm. pH of 7-8 was maintained throughout the phase. The treatment is used as assessment trial to identify efficient organisms to treat the contaminated soil.

Metal removal analysis:

Bioremediation ability of each strain was performed in SPR containing different metal concentration. About 3 mL inoculum of each isolate was used separately. Uninoculated SPR functions as control. Inoculated SPR and control were incubated at 37°C for 24 hrs under shake culture condition. From SPR, about 2 mL sample was taken at 2 hrs regular interval and centrifuged at 10,000 rpm for 10 minutes. Supernatant collected was used for biosorption analysis using UV-Vis spectrophotometer at 590 nm and growth curve was generated. Bioaccumulation study was carried out after 24hrs by comparing the outlet sample with inlet sample of SPR. The metal concentration of metal effluent, contaminated soil and treated samples were determined using AAS.

Immobilization:

Bacteria showing maximum metal removal efficiency was identified as *Bacillus sp.* (MTCC 10439), *Pseudomonas glumae* (MTCC 10462) and its combination. The identified bacteria were used in Up Flow Immobilized Column Reactor (UFICR). Kitchen scrubber cut into pieces of size 2 cm X 3 cm was used as immobilized media. For immobilization, scrubber is inoculated with identified organism in nutrient broth (15 pieces per 250 mL of nutrient broth). After 24 hrs of incubation, immobilized media is prepared and used for further reactor studies.

Up flow immobilized column reactor (UFICR):

In UFICR, for treatment the water from eluent tank helps to elude the heavy metals present in the soil as leachate. The reactor having the dimensions 36inch X 3inch X 3inch (split into 3 layers) was fabricated using acrylic material for the experiment. The leachate to be treated flows upwards through a column containing immobilized media. The immobilized bacterial column absorbs and adsorbs the heavy metals as the leachate moves upwards. The collection flask helps to collect the treated effluent. Immobilized media helps in preventing the escape of bacteria along with the treated sample and thus extends the bed life and reduces the cost of recycling biomass. Figure 1 shows the schematic diagram of UFICR.

RESULTS

The heavy metal content in contaminated soil analyzed using AAS and chemical characteristics of the soil are mentioned in (Table 1). The increase in total nitrogen and heavy metal content in the soil is due to contamination by metal effluent. The slight increase in total nitrogen content does not have any influence in contamination. The heavy metal content should be reduced for the survival of plants and any other beneficial activity in the soil.

No inhibition of growth of microorganism was observed during increase in concentration of metal content in effluent. Based on this concept, six strains from the metal effluent and two exogenous strains were identified. Trails were performed using all these strains in SPR in different combinations. After 24 hours of reaction, the soil and the leachate were collected and analyzed for heavy metal content. The results revealed that *Bacillus sp., Pseudomonas sp,* and combination of both shows better metal removal efficiency. The metal removal efficiency of efficient organism is shown in figure 2 and 3.

The identified efficient strains were used for immobilization. Since metal removal efficiency is high when mobility of the microbes is arrested, kitchen scrubber is used as an immobilization media for this technique. The identified efficient organisms and their combinations were immobilized and introduced in SPR. Comparatively, the combination shows excellent removal metal efficiency in SPR. The combination trials were carried out in leachate for metal removal efficiency studies and the results obtained is shown in figure 4.

UFICR Studies:

The optimum reactor parameters like Bed life, HRT (Hydraulic Retention Time) and saturation in leaching were determined by considering chromium alone since it is the predominant heavy metal contaminant in the soil is shown in figure 5.

From figure 5, it is clear that the efficiency in metal reduction increases with increase in HRT. The degree of increase between 6 and 8 HRT is very low. Therefore, In case of large scale operations 6 hours can be maintained as the economically efficient HRT.

Saturation in leaching:

The saturation in leaching time represents the time to change the treated soil with untreated soil. From figure 6, it is clear that the saturation time is 24 hours.

Bed life of Bacterial column

The bed life of the bacterial column was estimated at every shift (8 hours) by considering the chromium reduction. The efficiency is appreciable until the sixth shift, which implies that, after 48 hours the bacterial column has to be removed, washed and suspended in nutrient media for re-bloom so that it can be reused. The graphical representation of bed life is shown in figure 7.

DISCUSSION

Bacterial strains tested in this study revealed unique capability of biosorption of heavy metals due to the tolerance to heavy metals by the bacteria was in agreement with the results obtained by Niu[11], who isolated microbes such as *Bacillus sp., Pseudomonas sp., Alcaligenes sp.,* and *Flavobacterium sp.,* from the Zhangshi irrigation area and identified the distinct biosorption of heavy metals by bacteria might rely on physiology, categories of heavy metals and environmental factors.

Marcus[12] published that the physiology of microbes play a major role in tolerance to heavy metals which could affect the zinc accumulation by bacteria, fungi, and yeasts isolated from a coal mining area in Santa Catarina.

Distinct microbes show dissimilar mechanism of heavy metal biosorption. The entrapment of heavy metal ions by bacterial cell

Table 1. Heavy metal and nitrogen content in soil

	Uncontaminated soilmg/Kg	Contaminated soilmg/Kg
Total nitrogen	2.8	3.5
Copper	1.46	24.08
Magnesium	0	11.24
Zinc	3.47	12.52
Lead	0	8.67
Chromium	0	43.34
Cadmium	0	21.63

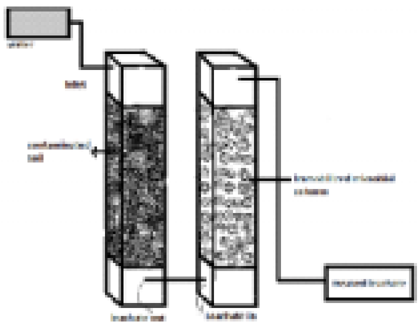

Fig. 1. Schematic diagram of Up flow Immobilized Column Reactor

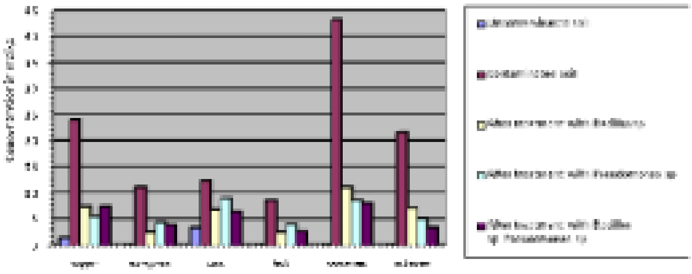

Fig. 2. Concentration of various trials in metal reduction

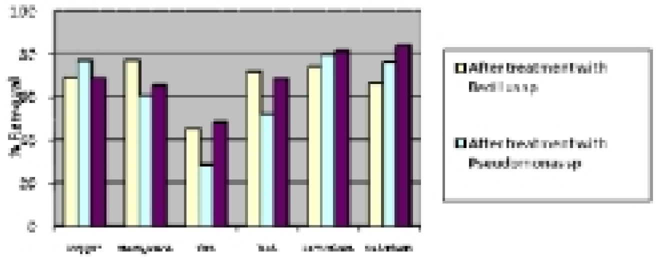

Fig. 3. Efficiency of various trials in metal reduction

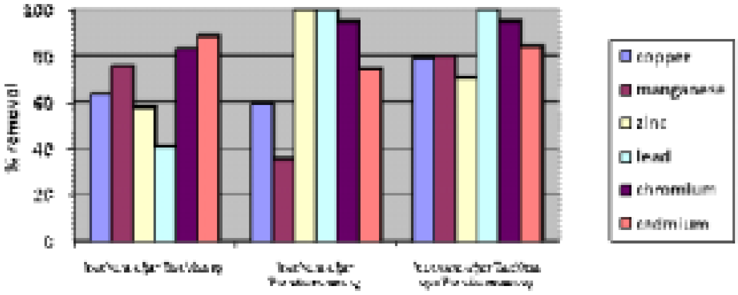

Fig. 4. Efficiency of various trials in metal reduction (leachate)

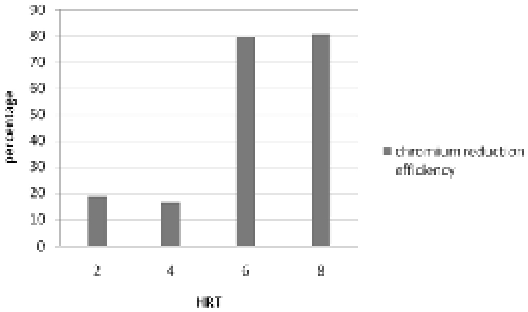

Fig. 5. Optimum HRT of Bacterial column

wall also involves extra cellular sediment, complexation, accumulation and combination[13]. Gram-negative bacteria such as *Pseudomonas sp.*, *Alcaligenes sp.*, and *Flavobacterium sp.* which has carboxylate groups lying on the cellwall might be the key aspirant for binding protons and metal cations[14,15]. The Gram-positive *bacillus* confirmed bioaccumulation of Cd^{2+}, Pb^{2+}, and Cu^{2+} [16,17,18]. The cell wall of *bacillus sp.* was the initial locality for heavy metal biosorption[19]. Metal uptake by

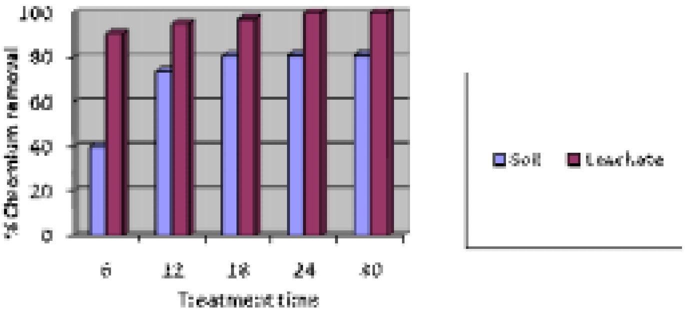

Fig. 6. Saturation time

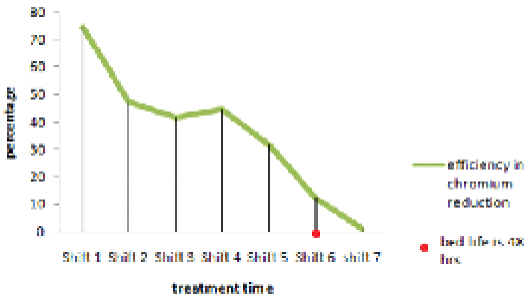

Fig. 7. Optimum bed life of Bacterial column

bacillus was an outcome of intracellular and/or extracellular deposition with OH, NH, CO, CO NH, and PO_4^{3-} groups involved[20]. Interface among metal ions and biopolymers from the cell wall (polysaccharides, proteins, and some hydrolysis) of *bacillus* develop micro sediment[21].

CONCLUSION

The metal contaminated soil support the growth of *Bacillus sp.* and *Pseudomonas sp.* obtained from MTCC. *Bacillus sp.* and *Pseudomonas sp.* combination had better ability of adsorbing and accumulating metal ions than using them individually. The biosorption and accumulation in this research might be influenced by the physiology of bacteria, toxicity, and bioavailability of heavy metals and environmental factors. After the treatment of metal contaminated soil, the soil can be used for land filling, construction etc since the leachate produced during the practice of treated soil doesn't possess any hazard. Thus the harmful characteristics of the contaminated soil were reduced economically and efficiently by bioremediation process.

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