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Remediation Of Metal Contaminated Soil Using A Novel Biotechnological Approach

Corresponding Author

Asha A.Juwarkar
Environmental Biotechnology Division
National Environmental Engineering Research Institute (NEERI)
Nehru Marg, Nagpur – 440020 (INDIA)
Email : aajuwarkar@rediffmail.com

Co-Authors

Prachi Joshi, S.K.Singh, Sadhana Rayalu
Environmental Biotechnology Division
National Environmental Engineering Research Institute (NEERI)
Nehru Marg, Nagpur – 440020 (INDIA)

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ABSTRACT

The technology applied here aims at bioremediation of metal contaminated soil using heavy metal tolerant strains of Azotobacter in combination with zeolite as a carrier. Azotobacter chroococcum strain HM1, being isolated from the soil near mining area contaminated with heavy metals, is not only heavy metal tolerant and withstand stressed conditions but also fixes atmospheric nitrogen nonsymbiotically. Traditionally this nitrogen biofertilizer is mixed with some carrier like lignite and then it is added to soil. But the novelty of this remediation technology is that, a material called zeolite is proposed to be used as carrier material instead of conventional carriers, because of its physicochemical properties. Zeolite is reported to possess properties like high ion exchange capacity, propensity for modification, potential for regeneration and recycling and soil conditioning. Zeolite when added to metal contaminated soil sorb the metal ions from soil and prevent their leaching to groundwater, also they raise pH of the soil to normal which is lowered due to the presence of heavy metal ions and the nitrogen biofertilizer present on zeolite will fix the atmospheric nitrogen, making it available for plants. In this way a dual purpose of metal removal and soil fertilization will be achieved due to zeolite based biofertilizer. Thus this process of bioremediation offers an effective and ecofriendly approach for soil contaminated with heavy metals such as cadmium, arsenic, chromium etc.

KEYWORDS

Bioremediation;
Heavy Metals;
Biofertilizer (Azotobacter chroococcum);
Zeolite.

INTRODUCTION

Metal contamination of soil represents a potential environmental hazard in terms of toxicity to animals (IPCS, 1992) and inhibition of microbial processes (Babich and Stotsky, 1985). Metals being immobile in soil accumulate in the top soil, thus endangering crops and vegetables (Athar and Vohora, 1995). In contaminated sites, heavy metal and oxyanion concentrations may be high enough to inhibit microbial activity. Soil microorganisms are critical to plant growth because they encourage development of stable soil structure by releasing required nutrients in inorganic forms by mineralization and producing growth – regulating substances. Among all nutrients required by plants nitrogen is an important one. Yet not even a single molecule of it can be taken up directly by plants, animals or human beings. Thus in most cases it is the limiting factor as far as growth of the plant is concerned. Apart from chemical fertilizers, certain eubacteria and a few archaebacteria are also attributed with the property of nitrogen fixation. But as mentioned above, under metal contaminated conditions this microflora gets affected. Frequent addition of chemical fertilizers to soil deteriorates soil quality. Hence it will prove to be of great significance to isolate metal tolerant strains of nitrogen fixing bacteria to take up the challenge of remediation of metal contaminated soils.

In spite of the different technologies available, in this study a new and innovative approach is proposed to be applied wherein metal tolerant strain of Azotobacter chroococcum in combination with zeolite as carrier are to be used for remediation of metal contaminated soil with dual purpose viz. remediation & plant growth enhancement.

Zeolites have a rigid, 3-dimensionsal crystalline structure (similar to a honeycomb) consisting of a network of interconnected tunnels and cages. Water moves freely in and out of these pores but the zeolite framework remains rigid. Another special aspect of this structure is that the pore and channel sizes are nearly uniform, allowing the crystal to act as a molecular sieve. The porous zeolite is host to water molecules and ions of potassium and calcium, as well as a variety of other positively charged ions. Now a days zeolite is being widely used for metal contaminated soils such as cadmium, lead etc. where metals are stabilized by immobilization within the zeolite complex structure (Lin and Lo, 1998). Also zeolites by virtue of their chemical & physical properties can be slow nutrient release matrices for chemical fertilizers, which are supplied in plant demand driven fashion.

Hence, in this study zeolite was evaluated as a carrier for free-living, nonsymbiotic, metal resistant strain of nitrogen biofertilizer Azotobacter chroococcum.

MATERIALS AND METHODS

Performance evaluation of zeolite for immobilization of microbes

Azotobacter chroococcum

Azotobacter chroococcum strain HM1 used in this study has been isolated from the heavy metal contaminated site near mining area and tested for metal tolerance.

Evaluation of zeolite-A as a non-toxic material for Azotobacter chroococcum

For this, a five-days old culture of Azotobacter chrococcum, grown in nitrogen free Jensen's medium was centrifuged at 10,000 RPM for 30 minutes so as to get the pure cell biomass. It was then suspended in buffer of pH 7.0. Initial cell count was done using standard plate count method. To this, sterilized zeolite at the rate of 5 g/L was added. A blank sample without zeolite was run along. Both the samples were incubated in incubator cum shaker at 30^0C for 24 hours so as to establish the contact between zeolite and the microbe. Then the experimental sample was centrifuged at a speed sufficient enough to settle the zeolite but not the free microbial cells. The final cell count in the supernatant of experimental sample was determined by standard plate count and was compared with that of control.

Evaluation of zeolite- A as a carrier material for Azotobacter chroococcum

Same procedure mentioned above was performed and % adsorption of bacteria to zeolite was deter-

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mined using the following formula% Adsorption =(Initial cell count- Final cell count)/Initial cell count x100

Normal zeolite A carrying positively charged replaceable ion i.e. cation could not adsorb of Azotobacter chroococcum cells and hence the zeolite was surface modified with the surfactant (Haggerty, 1994) so as to introduce hydrophobicity as well as to alter the surface charges.

Evaluation of surface modified zeolite- A (SMZ-A) as a carrier material for Azotobacter chroo coccum

Same procedure as given for the evaluation of zeolite A as carrier was performed for SMZ-A-450 and % adsorption was determined.

Optimization of different parameters

Type of zeolite and pH

Here three different types of zeolites with different surfactant treatments that is SMZ-A-100, SMZ-A-200, SMZ-A-were used for adsorption process at pH values 6.0, 7.0 and 8.0.

Effect of dose of SMZ-A-200

Studies were conducted for SMZ-A-200, at varying doses ranging from 0.1-5 g/L of broth culture of at pH 7.0.

Effect of contact time

Studies were conducted at varying contact times from 4-24 hours for the adsorption process of Azotobacter chroococcum on SMZ-A-200 at pH 7.0.

Determination of nitrogen fixing capacity of Azotobacter chroococcum mixed with surface modified zeolite

The nitrogen fixing capacity of the biofertilizer mixed with SMZ-A-200 was demonstrated in nitrogen free Jensen's medium using standard Kjeldahl method (Yadav and Mowade, 2005).

RESULTS AND DISCUSSIONS

Evaluation of zeolite-A as a non toxic material for Azotobacter chroococcum

Cell count of Azotobacter chroococcum in the initial as well as in the final sample was found to remain unaffected even in presence of zeolite, which proves its nontoxicity towards the given biofertilizer.

Evaluation of zeolite A and SMZ-A-450 as carrier material for Azotobacter chroococcum

Results presented in TABLE 1 show comparative account of adsorption of Azotobacter chroococcum on zeolite-A with that of SMZ. It is clear that surface modification of normal zeolite enhances the adsorption process. It is favorable to use SMZ instead of normal zeolite since adsorption prevents the leaching of the biofertilizer away from root zone of plant.

Effect of type of zeolite at different pH values

Results presented in figure 1, 2 and 3 indicate that SMZ-A-200 and SMZ-A-450 show 100% adsorption of Azotobacter chroococcum irrespective of the pH of buffer used. Thus it can be concluded that pH plays no significant role in adsorption process and hence for further studies pH 7.0, which is the most favourable pH for growth and survival of Azotobacter chroococcum, was selected. Whereas SMZ-A-100 could not show complete adsorption at

TABLE 1: Percentage adsorption of Azotobacter chroococcum on zeolite A and SMZ-A-450

Sr. No.	Type of zeolite	Initial cell count (cfu/ml)	pH of the broth before zeolite addition	Final cell count (cfu/ml)	pH of the system after zeolite addition	% Adsorption
1.	Zeolite A	$26x10^6$	7.0	$26x10^6$	7.81	0%
2.	SMZ-A-450	$36 x10^6$	7.0	-	7.81	100%

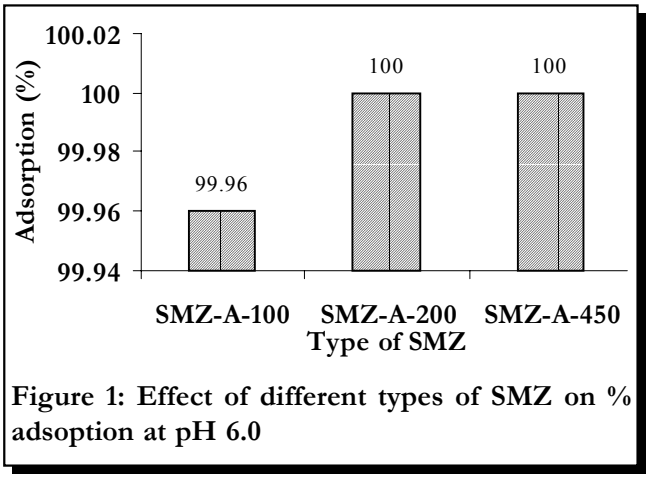

Figure 1: Effect of different types of SMZ on % adsoption at pH 6.0

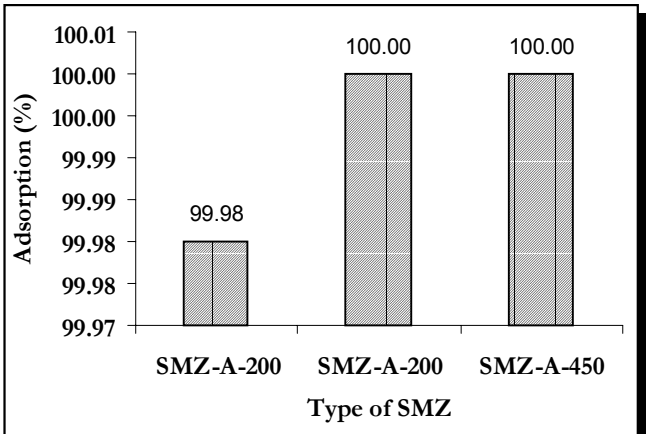

Figure 2: Effect of different types of SMZ on % adsorption at pH 7.0

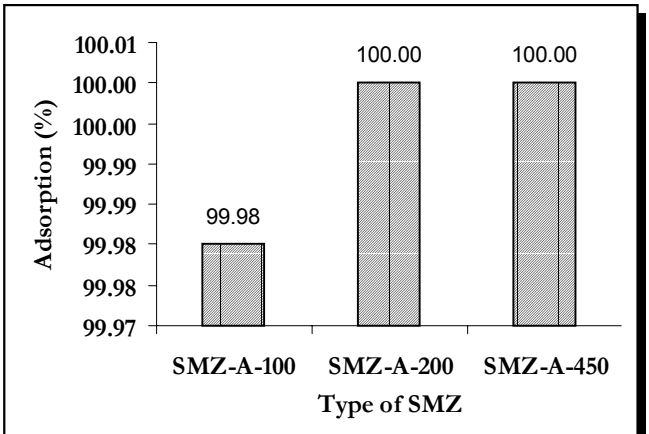

Figure 3: Effect of different types of SMZ on % adsorption at pH 8

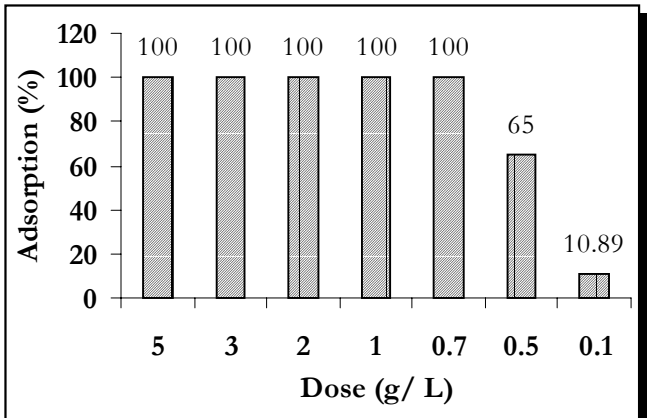

Figure 4: Effect of dose of SMZ-A-200 on % adsorption of Azotobacter chroococcum

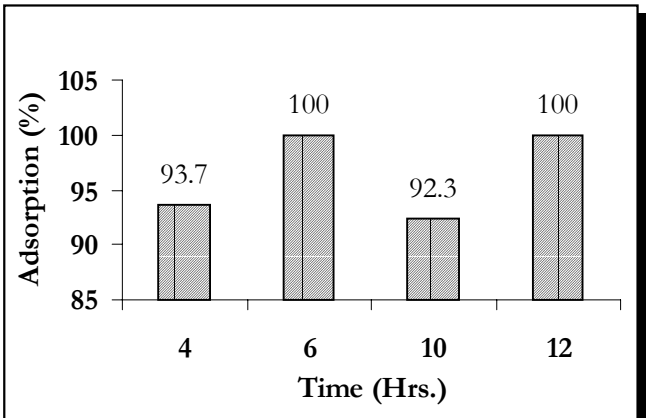

Figure 5: Effect of contact time of SMZ-A-200 on % adsorption of Azotobacter chroococcum

any of the pH values and hence cannot be used. SMZ-A-200 is selected as an economical and efficient carrier material since it requires less surfactant treatment and also shows 100 % adsorption.

Effect of dose of SMZ-A-200

Figure 4 indicates that dose of SMZ-A-200 at the rate of 0.7g and above per liter of broth culture with the cell number of 10^7cfu/ml shows 100% adsorption and hence minimum dose of 0.7g/L was optimized for further studies.

Effect of contact time

Minimum contact time required to achieve complete adsorption of the bacterial cells using SMZ-A-200 (0.7g/L) was 12 hours. Results are presented in figure 5

Demonstration of nitrogen fixing capacity of Azotobacter chroococcum mixed with surface modified zeolite

Nitrogen fixing capacity of Azotobacter chroococcum cells mixed with the surface modified zeolite was demonstrated. It was observed that Azotobacter chroococcum cells adsorbed on surface modified zeolite show better nitrogen fixation as compared to the free living Azotobacter cells, which proves that the nitrogen fixing potential of the adsorbed microorganisms remains unaffected.

CONCLUSION

Thus application of metal tolerant strain of biofertilizer can be used for remediation of metal contaminated soils with following added advantages-

(1) Restores back the fertility of soil

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(2) Minimizes the use of chemical fertilizers
(3) Being used in combination with Zeolite, which is now well known as a versatile option for targeting metal contamination, the process of decontamination of metal affected soil is expected to become faster and more effective.

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