Bioremediation of ^{60}Co from simulated spent decontamination solutions of nuclear power reactors by bacteria

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The spent decontamination solutions generated from nuclear power reactors contain radionuclides of cobalt (^{60}Co, ^{56}Co and ^{57}Co) along with a large excess (10^5) of non-radioactive metal ions (Fe, Ni and Cr). Our previous studies demonstrated that bioremediation of ^{60}Co from simulated effluents using fungal biomass can provide an alternative to conventional ion exchangers. In this study, we used several bacteria to further improve the process of bioremediation by decreasing biomass requirement and treatment period. Further, metabolite activation in specific bacterial species resulted in enhanced bioremediation of ^{60}Co from simulated effluent. Optimization of conditions in simulated effluent for the eight bacterial species to accomplish maximum ^{60}Co removal is discussed.

Keywords: Bacteria, bioremediation, cobalt, nuclear power reactor, radionuclides.

THE economic and safety aspects of nuclear power generation call for better optimization of operating conditions to make nuclear energy safe to generate and at the same time economical for industrial and domestic use. In India, a number of nuclear power reactors are being installed and also the existing unit capacities are being upgraded. Many of the reactor vessels are made of stellite, a high cobalt (48% w/w) containing alloy. The high temperatures (~573 K) generated and the aqueous coolant used result in the formation of oxide films containing Fe or Fe + Ni + Cr in different proportions. The corrosion products released to the coolant get transported through the core and get neutron-activated, thereby generating activated corrosion products containing radionuclides such as ^{60}Co, ^{58}Co, ^{54}Mn, ^{51}Cr, etc. Presently, ion exchangers are used to remove radionuclides of cobalt, which results in large amounts of solid waste and poor efficiency due to the presence of a large excess of non-radioactive metals (Fe, Ni and Cr). Microbial bioremediation of radionuclides is increasingly considered as a potential alternative to the conventional organic ion-exchanger-based treatment[1,2]. In our previous studies fungal biomass from various species was used to examine its potential in removing ^{60}Co from simulated decontamination solutions of nuclear power plants[3]. In the present study we have chosen bacteria, which have advantages of rapid growth, large surface-to-volume ratio and efficient metabolism. Further, novel genes and operons are prevalent in bacteria for acquiring cobalt from extremely low concentrations that serves as a cofactor for vitamin B12 and also for other non-corrin cobalt-containing metallo enzymes[4].

Bacterial cultures (*Bacillus megaterium*, *Pseudomonas putida*, *Flavobacterium devorans* (*Fd*), *Salmonella typhimurium* (*St*), *Streptomyces griseus*, *Rhizobium* sp., *Rhodococcus* sp., *Escherischia coli*) were obtained from National Centre for Industrial Microorganisms, Pune, India. *Deinococcus* sp. was obtained from Dr Bandekar's group at the Food Technology Division, Bhabha Atomic Research Centre, Mumbai. Bacteria were cultured in defined minimal medium, MM (w/v) (glucose, 0.5%; Na_2HPO_4, 0.6%; KH_2PO_4, 0.3%; NH_4Cl, 0.1%; NaCl, 0.05%; $MgSO_4$, 0.012%; $CaCl_2$, 0.001%). Nutrient broth was used as the complex medium (CM) for culturing *B. megaterium*, *P. putida*, *F. devorans*, *St* and *E. coli*. MGYP complex medium (w/v) (malt extract, 0.3%; glucose, 1%; yeast extract, 0.3%; peptone, 0.5%) was used for culturing *S. griseus* and *Rhodococcus* sp. TYG medium (w/v) (tryptone, 1%; yeast extract, 0.5%; glucose, 0.1%) was used as CM for cultivating *Deinococcus* sp. Bacteria were cultured at 37°C for 24 h, while *S. griseus*, *Rhodococcus* sp. and *Deinococcus* sp. were incubated at 30°C for 48 h, all under shaking conditions (150 rpm in an environmental shaker incubator). The simulated spent decontamination effluent (SE) used to suspend the pelleted bacterial cultures contained 0.031 μM Co (~1.8 ppb; tagged with ^{60}Co to yield a solution-specific activity of 11.2 nCi/ml) in a mixture of ferric ammonium citrate (10.74 mM), nickel sulphate (0.93 mM) and chromium nitrate (3.0 mM) complexed with 10.33 mM of EDTA adjusted to pH 7.0. In some experiments, SE was supplemented with minimal medium or complex medium or metabolites (DL-homocysteine or urea) as desired. An integral γ-counter coupled to a 2″ × 2″ NaI (T1) well-type detector was used to estimate ^{60}Co removal. A recovery of >95% radioactivity could be accounted for.

In the initial studies, optimal conditions for cobalt removal by bacteria with respect to time and biomass were established. Incubation period of 6 h and 50 OD units of bacterial biomass (in 20 ml) were optimal for cobalt removal from simulated effluent. Hence in all experiments the above conditions were used. Subsequently, optimal growth media for cobalt removal from SE were evaluated. Bacteria were cultured in MM/CM and suspended in SE, SE + MM and SE + CM. Cobalt-removal capacity of various bacteria was then assessed in these media. The results of the above experiments showed that the bacterial cells cultured in MM were least effective in ^{60}Co removal upon subsequent introduction into SE alone. However, enhanced cobalt-removal capacity (above 25%) was ob-

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Table 1. Effect of supplements (DL-homocysteine[a] or urea[b]) on ^{60}Co removal

Supplement		Cobalt removal (%)		
		Bacillus megaterium	*Deinococcus* sp.	*Rhodococcus* sp.
DL-homocysteine	–	8.4	7.2	–
	+	16.7	7.0	–
Urea	–	–	–	4.9
	+	–	–	20.9

[a]Effect of DL-homocysteine was checked in *B. megaterium* and *Deinococcus* sp.
[b]Effect of urea was checked only in *Rhodococcus* sp.

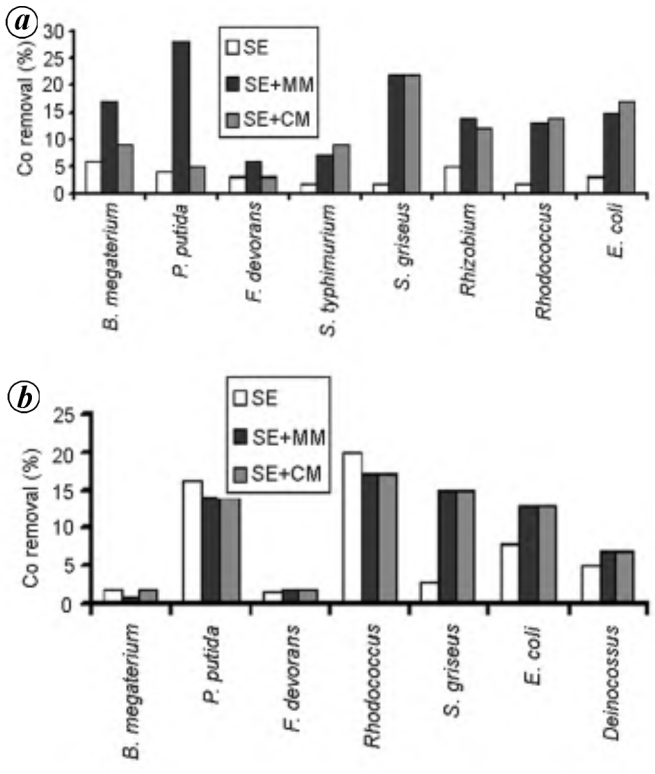

Figure 1. ***a***, Bacteria cultured in MM suspended (20 OD units) in 20 ml of SE alone or supplemented with MM/CM for 24 h. ***b***, Bacteria cultured in CM suspended (20 OD units) in 20 ml of SE alone or supplemented with MM/CM for 24 h.

served for *P. putida* (Figure 1 *a*) when supplemented with MM (SE + MM), but not with CM (SE + CM). In case of *S. griseus*, culturing in MM increased ^{60}Co removal significantly both in SE + MM and SE + CM. A marginal increase in both the supplemented media (SE + MM and SE + CM) was also observed for *B. megaterium*, *Rhodococcus* sp., *Rhizobium* sp. and *E. coli*. *Fd* and *St* were least effective in cobalt removal among the bacterial cultures tested. On the contrary, culturing in CM had a pronounced effect on ^{60}Co removal in SE alone without any supplementation, in case of *Rhodococcus* sp. and *P. putida* (Figure 1 *b*). When SE was supplemented with MM/CM in this experiment, significant increase in ^{60}Co removal was observed for *Rhodococcus* sp. followed by *S. griseus*, *P. putida*, *E. coli* and *Deinococcus* sp.; *Fd* and *B. megaterium* did not show any increase in ^{60}Co cobalt removal using this method.

The requirement for cobalt in trace amounts in some bacteria is primarily due to vitamin B_{12} metabolism, which acts as a cofactor for the enzyme homocysteine methyltransferases[5]. It was found that cobalt (5 ng/ml) could be substituted for methionine to permit the growth of *Sinorhizobium meliloti* strains in MM[6]. This enzyme is involved in the synthesis of methionine from homocysteine. In order to see whether cobalt removal efficiency changes in the presence of DL-homocysteine, *B. megaterium* and *Deinococcus* sp. were suspended in simulated effluent containing DL-homocysteine (100 µg/ml). *B. megaterium* removed twofold excess of ^{60}Co from simulated effluent containing DL-homocysteine (Table 1), which accounts for 16.7% (0.24 ng/mg dry wt) when compared to 8.4% in control SE alone (0.12 ng/mg dry wt). The effect of DL-homocysteine was not observed in *Deinococcus* sp. (Table 1) and also in other bacterial species tested (data not shown).

Cobalt is an essential component of specific bacterial nitrile hydratase, which catalyses the hydration of nitriles to the corresponding amides. These enzymes are selectively induced by urea and cyclohexanecarboxamide in the presence of cobalt ions. A functionally characterized cobalt transporter belonging to the NiCoT family was reported[7] in *Rhodococcus rhodochrous* J1, with a physiological significance for nitrile hydratase. Based on the above reports we studied the effect of urea on cobalt-removal efficiency in *Rhodococcus* sp. A fourfold increase in ^{60}Co removal (20.9%) was observed when cells were cultured in MM and subsequently introduced into SE + urea than in SE alone (4.9%).

The results of the present work with bacteria showed distinct advantages over the previous reported work using fungal biomass[3]. Maximum cobalt-removal efficiency of 1 µg of ^{60}Co/g dry wt of bacterial mass could be achieved within 6 h compared to 8–500 ng/g attained after 24–48 h for fungal mycelial biomass. This twofold decrease in biomass requirement along with shorter time-span for maximum efficiency reiterates the potential of bacterial bioremediation.

In order to further improve ^{60}Co removal, we are focusing on prokaryotic genome analysis. Based on the previous

study of cobalamine synthesis gene clusters (cob/cbi), where a multitude of potential cobalt transporters belonging to various families (ABC family, NiCoT family, etc.) have been identified[8], we performed comparative genome analysis of eight different bacterial species employed in this study. This *in silico* approach allowed us to identify high-affinity cobalt transporters in the bacterial species used in this study. Genome-wide analysis of radiation-resistant bacterium, *Deinococcus radiodurans*[9] revealed the presence of three putative cobalt transporter genes (unpublished data). Genetic engineering strategies to overexpress these transporters in *D. radiodurans*[10] would be the next ideal step to increase the overall cobalt removal efficiency from a margin of 40–90%, and such a recombinant strain could be more promising for bioremediation.

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Assessment and monitoring of mangroves of Bhitarkanika Wildlife Sanctuary, Orissa, India using remote sensing and GIS

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The present study deals with periodic assessment and monitoring of the mangroves of Bhitarkanika Wildlife Sanctuary, Orissa, India using remote sensing and Geographic Information System techniques. Satellite data of Landsat MSS for 1973, IRS-1A LISS II for 1988 and IRS-P6 LISS III for 2004 along with other spatial and non-spatial data were used to find out the changes that occurred in mangrove and other land-cover categories during the last 30 years. It was found that the sanctuary is occupied by agriculture (51.76%), followed by dense mangrove (21.77%), water bodies (20.19%) and open mangrove (2.73%). A loss of 1534 ha mangrove area and an increase of 2436 ha agriculture area clearly depict anthropogenic activities by local villagers. A significant increase of 270 ha plantations illustrates plantation activities taken up by the Orissa Forest Department to protect the coastal shoreline.

Keywords: Bhitarkanika Wildlife Sanctuary, Geographic Information System, mangrove, multi-spectral data, remote sensing.

MANGROVE forest is a vegetation community formed by a variety of salt-tolerant species growing in the inter-tidal areas and estuary mouths between the land and the sea. Mangrove forests are one of the most productive wetlands on earth. They provide critical habitat for diverse marine and terrestrial flora and fauna. Yet, these unique coastal, tropical forests are among the most threatened habitats in the world. Traditionally, local communities in mangrove ecosystems collected fuelwood, harvested fish and other natural resources[1,2]. However, in recent decades, many coastal areas have come under intense pressure from rapid urban and industrial development, compounded by a lack of governance or power among environmental institutions. Mangroves have been overexploited or converted to various other forms of land use, including agriculture, aquaculture, salt ponds, terrestrial forestry, urban and industrial development and for the construction of roads and embankments[3,4]. Mangroves are affected by several different activities simultaneously, or over time as land-use patterns change[5].

India has a total area of 4461 sq. km under mangroves, which is 0.14% of the country's total geographic area. It

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