Application of Bacteria to Remove Americium from Radioactive Liquid Waste - 11130

Tânia R. de Borba[1], Solange K. Sakata[1], Rafael Vicente de P. Ferreira[1], Maria Helena Bellini[1], Debora Frigi Rodrigues[2], Julio T. Marumo[1]*.

[1]Nuclear and Energy Research Institute, IPEN – CNEN/SP, Brazil
[2]Civil and Environmental Engineering - University of Houston, USA
*Corresponding author (jtmarumo@ipen.br)

ABSTRACT

The awareness and concern about the environment have motivated research for new efficient technologies capable of inexpensively removing metals ions from wastes. The use of biotechnology to remove heavy metals from wastes has great potential in the treatment of radioactive wastes. The aim of this work is to evaluate, for the first time, the ability of bacterial biomasses to remove Am-241 from the aqueous solution as a function of contact time and radionuclide concentrations. For this study *Cupriavidus metallidurans* CH34, *Bacillus subtilis* 102 and *Ochrobactrum* sp. MH1000 were chosen because of their different cell wall structures. *C. metallidurans* CH34 is a well known Gram-negative bacterium for its high capacity of removing metals from different types of waste and for its resistance to solutions with high metals concentrations. *Ochrobactrum* sp. MH1000 is resistant to high Am-241 ion concentrations and is also a Gram-negative bacterium. This bacterium was isolated from leachate samples collected from a lysimeter with Am-241. *B. subtilis* is a Gram-positive bacterium and it is described in many reports as an efficient bacterium to remove metals ions from solutions and soils. The results showed that *Ochrobactrum* sp. MH1000 removed 95% of Am-241 in just 2 minutes of contact time, *Cupriavidus metallidurans* CH34 removed nearly at 100% after 360 minutes of contact time in all studied concentrations and *Bacillus subtilis* 102 was less efficient, the maximum removal percentage was 52% after 720 minutes of contact time in 150 Bq/mL.

INTRODUCTION

The transuranic radionuclide Am-241 can be found in lightning rods, fire alarms and in nuclear industries. The origin of americium liquid waste from the Radioactive Waste Management Laboratory comes from a nuclear research area. The conventional techniques, such as precipitation, ion exchange and electrochemical processes, for removing low americium ion concentrations in large solution volumes are not effective for economic reasons [1].

The search for new efficient technologies to remove radionuclides from liquid wastes has been based on similar techniques employed for removal of heavy metals, such as biosorption. This technique involves the use of biological materials such as microorganisms [2], agricultural wastes [3] and polysaccharide materials [4] that form complexes with metal ions using their ligands or functional groups [5]. On the other hand, in bioaccumulation, the metals ions are incorporated inside the living biomass [6].

Since 1980, many reports have been published about the bacterial capacity to remove heavy metals [2]. Bacterial cell walls can be distinguished base on their structures and compositions. The cell wall of Gram-positive bacteria consists of many layers of peptidoglycan and teichoic acids. Because of the negative charge from its phosphate groups, teichoic acids may bind and regulate the movement of metal ions in and out of the cell [7]. On the other hand, Gram-negative bacteria cell walls contain only a thin layer of peptidoglycan and an outer membrane composed of phospholipids and lipopolysaccharides [8], which confer the anionic character to the cell wall and its metal binding capacity.

Among the other radionuclides, uranium has so far been the most studied radionuclide for biosorption/bioaccumulation studies [9-11]. Moreover there are just a few reports about these technologies for americium removal. Most of the described studies were related to the absorption of americium using yeasts [12] and biopolymers like alginate [13].

The aim of this work is to evaluate, for the first time, the ability of bacterial biomasses to remove Am-241 from aqueous solutions. The experiments were performed using *Cupriavidus metallidurans* CH34, *Bacillus subtilis* and *Ochrobactrum* sp. MH1000 in different experimental conditions, which include different metal concentrations and contact time.

MATERIAL AND METHODS

Bacterial strains and growth conditions

C. metallidurans CH34 and *B. subtilis* 102 were obtained from the Biomedical Sciences Institute at the University of Sao Paulo Culture Collection. *Ochrobactrum* sp. MH1000 was isolated from leachate samples generated in lysimeters which contained organic wastes contaminated with americium [14].

B. subtilis 102 and *Ochrobactrum* sp. MH1000 were cultured aerobically at 37.0 °C with shaking at a speed of 100 rpm in Brain Heart Infusion at pH 7, and *C. metallidurans* CH34 was cultured in the same conditions, but at pH 5.

Interaction of bacteria with Am-241

After being cultivated for 24 hours, the cells were centrifuged at 2,500 g for 15 min and washed three times with Tris Medium (TSM) [15] at pH 7 for *B. subtilis* 102 and *Ochrobactrum sp.* MH1000, and pH 5 for *C. metallidurans* CH34. The cells were resuspended in TSM and the optical density (O.D.) was adjusted to 2.

The stock solution of Am-241 was prepared by dissolving $AmCl_3$ in TSM Medium to obtain a final concentration of 23.3 kBq/mL. The final concentrations used in the experiments were 150, 300 and 600 Bq/mL, which were obtained by diluting the stock solution as described below.

The Minimum Inhibitory Concentrations for each bacterium was determined by agar dilution method. Briefly, 1 mL of bacterial culture was added to 1 mL of TSM solution containing increasing amounts of Am-241 (20-4000 Bq/mL). These bacterial culture were maintained for 24 hours at 37 ºC and then plated on Plate Count Agar.

The uptake of Am-241 was performed by the batch culture method. Briefly, 1.5 mL of bacterial culture (O.D. 2.0) was mixed to 1.5 mL of TSM solution with Am-241 in a sterile vial to the final concentrations of 75, 150 and 300 Bq/mL. The vials were shaken on an orbital shaker (100 rpm) at room temperature. After the contact time, the solutions were

centrifuged at 2,500g for 15 minutes and 1 mL of the supernatant was removed to assay the residual Am-241 in solution using a scintillator (Tri-carb 2100 TR- Liquid Scintillation Analyzer – Packard-Canberra). All experiments were conducted in duplicate.

RESULTS AND DISCUSSION

The resistance of the bacterial strains to Am -241 was evaluated as shown in Table 1. The *Ochrobactrum* sp. MH1000 strain, when compared to *C.metallidurans* CH34 and *B. subtilis* 102, showed the highest resistance to americium (Table 1). The resistance was between 1.5 to 3 times higher for *Ochrobactrum* sp.

Table 1- Minimum Inhibitory Concentration (M.I.C)

Bacteria	**M.I.C. of Am-241 (Bq/mL)**
Ochrobactrum sp MH1000	1200
Bacillus subtilis 102	700
Cupriavidus metallidurans CH34	400

In order to evaluate the potential of these bacteria to remove Am-241 from radioactive liquid waste, the assays were performed in different americium ion concentrations and contact times for *Ochrobactrum* sp. MH1000, *C. metallidurans* CH34 and *B. subtilis* 102. Figures 1 to 3 show the results obtained. Each point represents the average ± standard error.

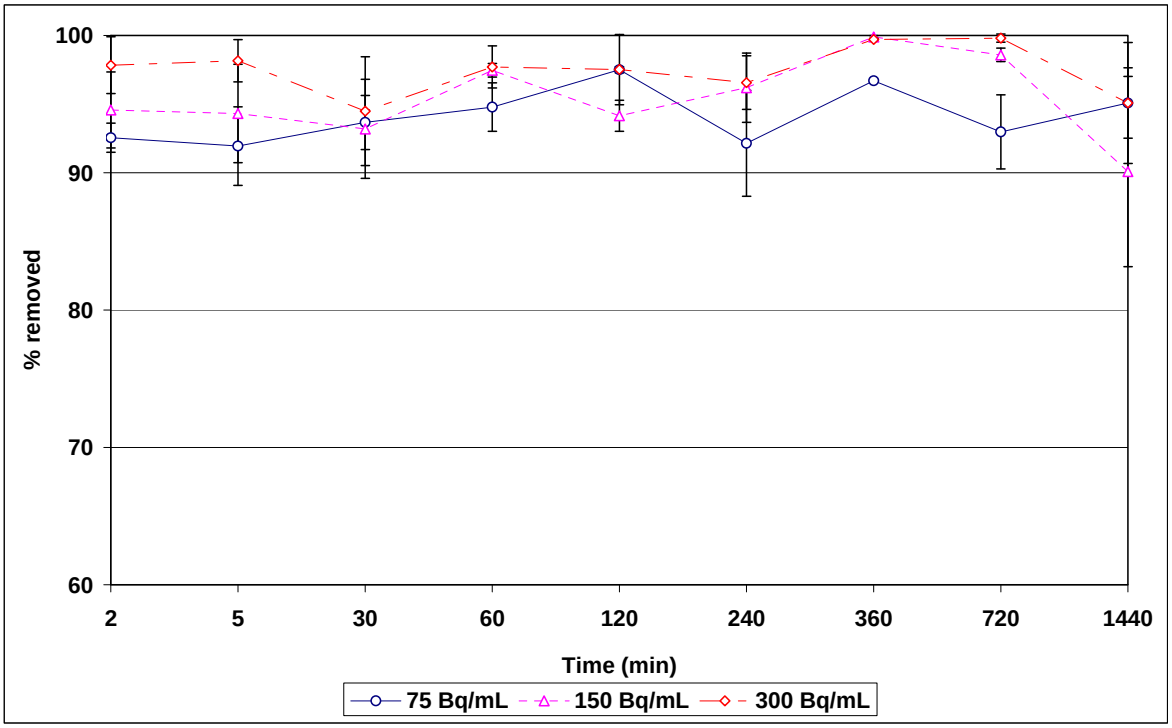

Figure 1 – Bioremoval of Am-241 by *Ochrobactrum sp* MH1000.

Ochrobactrum sp. MH1000 removes on average 95% of Am-241 in just 2 minutes of contact time in all studied concentrations.

C. metallidurans CH34 is a well known model microorganism for heavy metal detoxification. This microorganism has two megaplasmids that encode several genes for heavy metal resistance. The presence of these genes allows it to successfully grow in heavy metal contaminated environments [4].

In this study, we demonstrated for the first time that *Cupriavidus metallidurans* CH34 has the potential to remove Am-241 (Figure 2). The metal concentration decreased rapidly in the first 60 minutes, and remained nearly at 100% after 360 minutes of contact time.

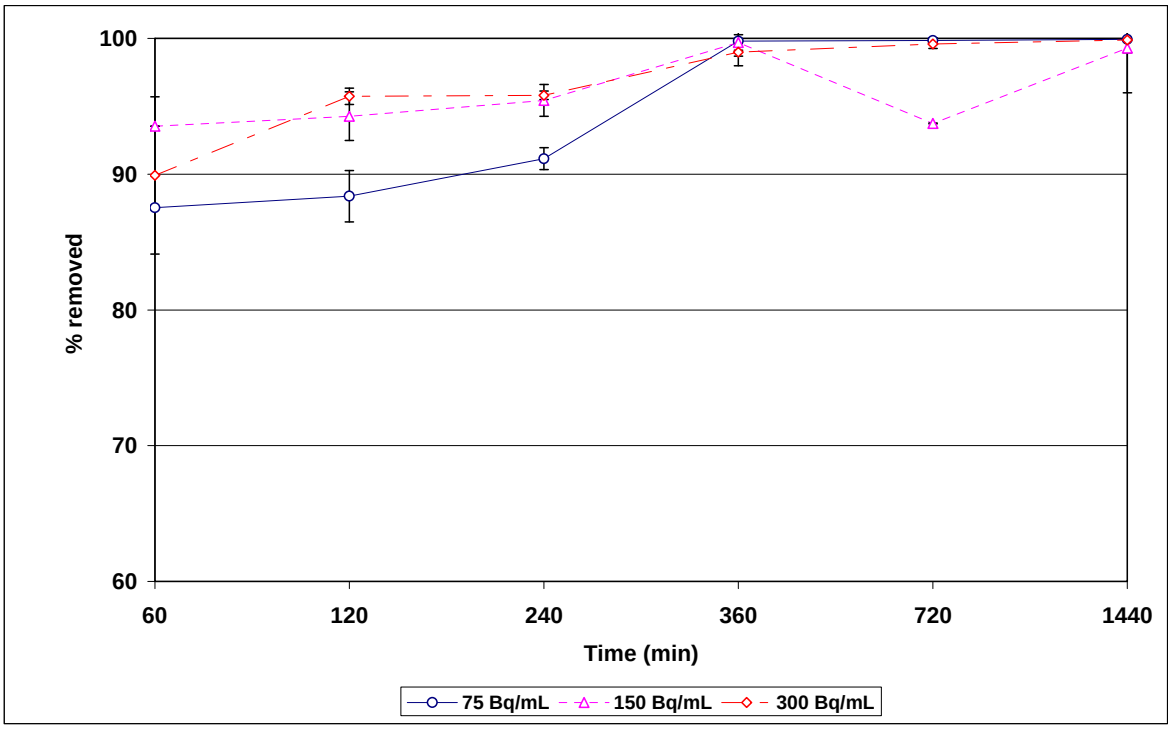

Figure 2 - Bioremoval of Am-241 by *Cupriavidus metallidurans* CH34.

The ability of a Gram-positive bacterium to remove Am-241 was performed by *B. subtilis* 102 (Figure 3).

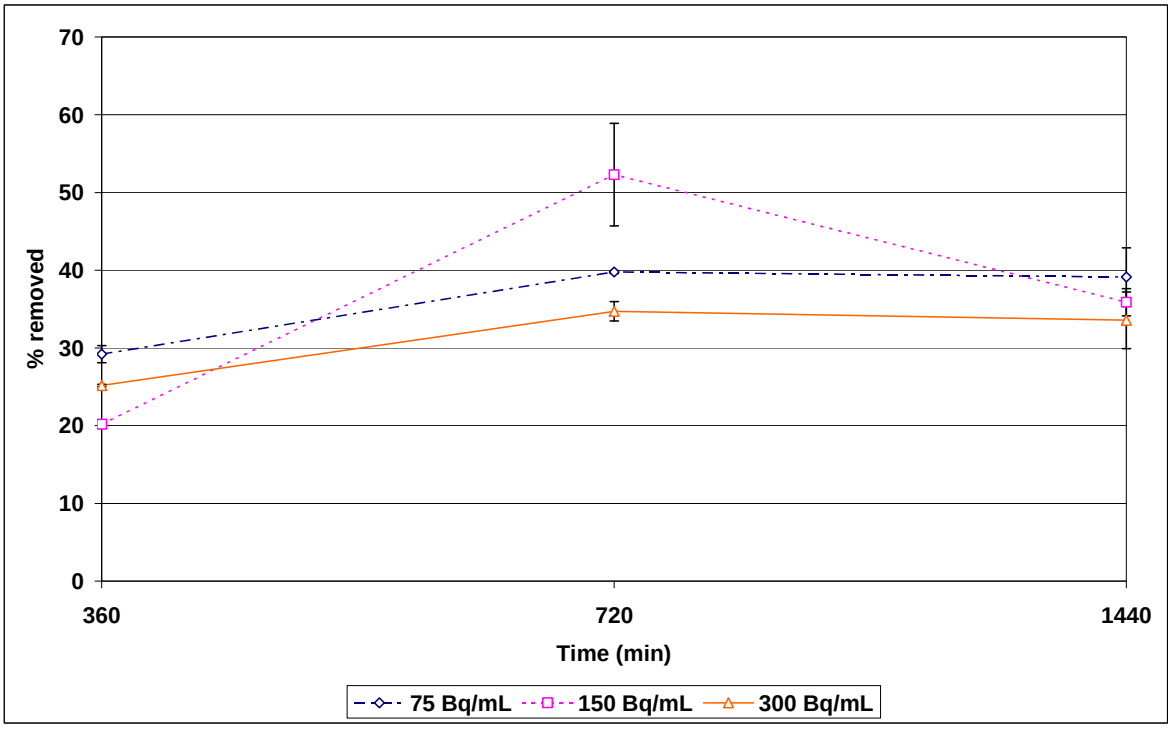

Figure 3 - Bioremoval of Am-241 by *Bacillus subtilis*102.

Despite the fact that lipoteichoic acids in Gram-positive bacteria give an overall negative charge to the microorganisms' cell wall and therefore should have a better affinity to metal ions, the Gram-positive *B. subtilis* 102 was less efficient in removing americium than the other studied bacteria. B. subtilis 102 had a maximal percentage removal of only 52% after 720 minutes, as shown in Figure 3.

CONCLUSION

It is noteworthy that *Ochrobactrum* sp. MH1000 showed great tolerance to higher concentrations of americium compared to the other microorganisms. Furthermore, *Ochrobactrum* sp. MH1000 and *Cupriavidus metallidurans* CH34 were more effective in removing different concentrations of americium in aqueous solutions than *Bacillus subtilis* 102. Moreover, *Ochrobactrum sp.* showed faster removal than *Cupriavidus metallidurans* CH34. Hence, *Ochrobactrum sp.* has a great potential for americium removal from radioactive liquid wastes.

REFERENCES

[1] B. Volesky, "Detoxification of metal-bearing effluents: biosorption for the next century", *Hydrometallurgy*, 59, 203–216, (2001).
[2] K. Vijayaraghavan, Y.S. Yun, "Bacterial biosorbents and biosorption", *Biotechnol.Adv.*, 26, 266–291, (2008).

[3] M. Minamisawa, H.Minamisawa, S. Yoshida,N.Takai, "Adsorption Behavior of Heavy Metals on Biomaterials", *J. Agric. Food Chem.,* 52, 5606-5611, (2004) .
[4] D. Feng, C. Aldrich, "Adsorption of heavy metals by biomaterials derived from the marine alga *Ecklonia maxima", Hydrometallurgy,* 73, 1-10, (2004).
[5] J. Wang, C. Chen, "Biosorbents for heavy metals removal and their future", *Biotechnol Adv.* , 27, 195–226, (2009).
[6] A.Y. Dursun, G. Uslu, O. Tepe, Y. Cuci, H.I. Ekiz, "A comparative investigation on the bioaccumulation of heavy metal ions by growing *Rhizopus arrhizus* and *Aspergillus niger*". *Biochem. Eng. J*., 15, 87–92, (2003) .
[7] G.J. Tortora, B.R. Funke, C.L.Case, "Microbiology, an introduction", 7th ed., Benjamin-Cummings Pub Co, (2001).
[8] Sherbert GV. "The biophysical characterization of the cell surface". London: Academic press; (1978).
[9] K.C. Bhainsa, S.F. D'Souza, "Biosorption of uranium(VI) by *Aspergillus fumigates*". *Biotechno. Techniques* ,13, 695–699, (1999).
[10] C.Gok, S. Aytas, "Biosorption of uranium(VI) from aqueous solution using calcium alginate beads", *J. Hazard. Mater*., 168, 369–375, (2009).
[11] J. Yang , B. Volesky, "Biosorption of uranium on *Sargassum* biomass", *Water Res*., 33, 3357-3363, (1999).
[12] Liu, N.; Lou, S.; Yang, Y.; Zhang, Y.; Jin, J.; Liao, J. "Biosorption of americium-241 by *Saccharomyces cerevisiae*", *J. Radional. and Nuc. Chem.,* 252,187-191, (2002).
[13] Mimura, H.; Ohta, H.; Akiba, K.; Onodera, Y. "Uptake behavior of americium on alginic acid and alginate polymer gels", *J. Radional. and Nuc. Chem*, 247, 33-38, (2001).
Ferreira, R. V. P.; Sakata, S. K.; Isiki, V. L. K.; Miyamoto, H.; Bellini, M. H.; Lima, L. F.
[14]C. P.; Marumo, J. T. "Influence of americium-241 on the microbial population and biodegradation of organic waste". *Environ.Chem. Letters***, (**2009).
[15] Mergeay, M.; Mies, D.; Schlegel, H.G.; Gerits, J.; Charles, P.; Gijgesem, Van. "Alcaligenes eutrophus CH34 is a facultative chemolithotroph with plasmid-bound resistance to heavy metals". *Journal of Bacteriology*, 162, 338-324, (1985).