

Treatment of radioactive liquid organic waste using bacteria community

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Abstract Waste management plays an important role in radioactive waste volume reduction as well as lowering disposal costs and minimizing the environment-detrimental impact. The employment of biomass in the removal of heavy metals and radioisotopes has a significant potential in liquid waste treatment. The aim of this study is to evaluate the radioactive waste treatment by using three different bacterial communities (BL, BS, and SS) isolated from impacted areas, removing radioisotopes and organic compounds. The best results were obtained in the BS and BL community, isolated from the soil and a lake of a uranium mine, respectively. BS community was able to remove 92% of the uranium and degraded 80% of tributyl phosphate and 70% of the ethyl acetate in 20 days of experiments. BL community removed 81% of the uranium and degraded nearly 60% of the TBP and 70% of the ethyl

acetate. SS community collected from the sediment of São Sebastião channel removed 76% of the uranium and 80% of the TBP and 70% of the ethyl acetate. Both americium and cesium were removed by all communities. In addition, the BS community showed to be more resistant to radioactive liquid waste than the other communities. These results indicated that the BS community is the most viable for the treatment of large volumes of radioactive liquid organic waste.

Keywords Radioactive waste · Biodegradation · Biosorption

Introduction

The nuclear power industry and research facilities generate hazardous chemicals and radioactive waste, including contaminated liquid organic compounds. Special care must be taken when handling this class of radioactive waste. Hence, most of these compounds are volatile, combustible and toxic, such as lubricants, solvents, decontamination solutions, process fluids and aqueous waste with significant organic content.

The Radioactive Waste Management Laboratory at the Nuclear and Energy Research Institute (IPEN-CNEN/SP), São Paulo, Brazil, is in charge of treating and storing radioactive waste generated by IPEN/CNEN-SP and from radioisotope users in different states in the country. Most of the radioactive contaminated liquid organic waste stored at IPEN is from the organic solvent extraction process.

Radioactive liquid organic waste is conventionally treated with incineration [1, 2], wet oxidation [3], emulsification [4, 5], absorption [4] or distillation [6]. However, these techniques produce secondary waste that needs to be

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treated too [6]. The suitable treatment depends on a number of a technical factors, such as the characteristics and volume of the waste, the availability of technology and costs. However, there is no reliable standardized technology that can be considered a universal reference for the treatment of radioactive liquid organic waste, especially when there is a low concentration of radioisotopes in the water [1].

Recently, microbiological treatment has emerged as a new technology for the treatment of radioactive wastes. This is an environment-friendly process of the degradation of organic compounds in radioactive liquid waste [7, 8]. Moreover, this form of treatment can integrate with non-biological processes to remove radioisotopes from liquid waste through biosorption [9, 10].

In fact, there are just few reports referring to the treatment of real radioactive waste using biosorption. Silva et al. [11], removed U, Ba, Cr and Pb from real radioactive wastewater under dynamic conditions using *Sargassum filipendula* seaweed. The authors demonstrated that the biosorption is selective because Ca, Mg, Fe and Mn ions were not removed from the same radioactive wastewater.

Sacharomyces cerevisiae is so far the most investigated microorganism for radioisotope biosorption purpose, because it is a low cost sorbent, and a by-product of fermentation industry [12]. *Sacharomyces cerevisiae* efficacy in removing uranium, $\text{UO}_2(\text{NO}_3)_2$, from solution of initial concentration between 10 and 1,000 mg L^{-1} and pH of 4.5 was compared to three different types of microorganisms *Debaryomyces hansenii*, *Kluyveromyces marxianus* and *Candida colliculosa* [13]. The results showed that *Sacharomyces cerevisiae* was the most effective sorbent with a maximum sorption capacity of 127.7 mg g^{-1} . This yeast was also studied to remove Am^{241} , Liu and col [14] described that *Sacharomyces cerevisiae* in free form removed 90% of Am^{241} and its sorption capacities were 1.88 mg g^{-1} .

The aim of our study is to evaluate ability of the microbial communities from impacted areas to remove radioisotopes and degrade of organic compounds from real radioactive liquid organic waste stored at the Radioactive Waste Management Laboratory (IPEN-CNEN/SP).

Materials and methods

Characterization of radioactive liquid organic waste

The radioactive liquid waste was analyzed by gamma spectrometry (HPGe detector with beryllium window 0.5 mm in thickness) that identified cesium-137 and americium-241. The specific activities of the Cs^{137} and Am^{241} were calculated based on the photopeak areas at 661.66 and 59.54 keV, respectively. Total uranium concentration was determined using the Arsenazo III,

according to the method described by Silva et al. [15]. The determination was performed by spectrophotometry at a wavelength of 650 nm. A factor of 24.4 was used in the conversion of the concentration of uranium (in mg L^{-1}) into U specific activity (in Bq L^{-1}).

The organic compounds were extracted from the radioactive liquid-organic waste with 5 mL of dichloromethane (1:1 v/v) and analyzed by gas chromatography (CG) (Agilent model 6890 N) using standard solutions. The separation was carried out in a DB-XLB (J & E Scientific) (30 m, 0.25 mm i.d.; film thickness: 0.25 μm). The GC temperature program was 30 °C for 5 min, up to 180 °C (10 °C min^{-1}), maintained for 1 min, increased to 230 °C (10 °C min^{-1}) and maintained at this temperature for 3 min to ensure the removal of all compounds. The analysis were performed in a split/splitless injector (split ratio 1:14) at 250 °C. The carrier gas was helium at a rate of 1.3 mL min^{-1} and detector temperatures was 280 °C.

Volatile organic compounds were analyzed by a direct measurement of the radioactive waste samples using a Shimadzu Headspace System composed of a GCMS-QP5050 and HSS-4A. The radioactive waste samples were introduced into a split/splitless injector (split ratio 1:14) at 250 °C, using helium as the carrier gas at a constant flow rate of 1.3 mL min^{-1} . Separation was carried out with a DB-5 J&W column (30 m \times 0.25 mm i.d.; film thickness: 0.25 μm). The GC temperature program was 40 °C for 2 min, increasing 3 °C min^{-1} up to 65 °C, which was maintained for 1 min, then raised to 15 °C min^{-1} until 220 °C and maintained at this temperature for 3 min. The interface temperature for MS was 230 °C. The mass spectrometer was operated in scan mode (electron impact at 70 eV, 1,000 V).

Bacterial assay in radioactive waste

The presence of bacteria in the radioactive-liquid-organic waste was determined based on the Standard Methods for the Examination of Water and Wastewater [16]. A 10 mL aliquot was aseptically removed from the liquid waste and transferred to sterile tubes containing 90 mL of buffer solution (0.25 M KH_2PO_4 /0.4 M MgCl_2 , pH 7.2). After a serial dilution, ranging from 10^{-1} to 10^{-7} , the cultures were inoculated on plate count agar in Petri dishes for aerobic and anaerobic incubation. For acidophilus microorganisms, the pH of the buffer solution and plate count agar was adjusted to pH 3.0. Anaerobic atmosphere was achieved with anaerobic jars and CO_2 generators. After incubation at 37 °C for 48 h, the colony count was determined.

Bacterial enrichments

The bacterial communities were obtained separately by enrichments of soil (BS) and water from Bia Lake (BL)

sites at Caldas uranium mine and sediments from São Sebastião channel (SS).

Both Caldas uranium mine and São Sebastião channel are environmentally impacted areas that could have bacteria which could be used to treat radioactive waste. Caldas uranium mine was the first facility to produce uranium concentrate in Brazil and it operated from 1982 to 1995. The waste generated during mining activities are currently a source of acid drainage, which leads to the solubilization of uranium, thorium, radium and stable elements, such as manganese, iron, zinc and fluorine [17, 18]. São Sebastião channel, in the north coast of the state of São Paulo, is where the most important oil terminal in Brazil is located. A total of 305 oil spills occurred from 1974 to 1997, exposing coastal ecosystems to crude oil [19].

Enrichment cultures were prepared by adding 1.0 g (or 1.0 mL) of sample in mineral salt medium with 0.5% *n*-dodecane, tributyl phosphate (TBP) and ethyl acetate as the carbon source. The mineral medium contained 1.0 g (NH₄)₂SO₄, 0.2 g KH₂PO₄, 1.6 g K₂HPO₄, 0.2 g MgSO₄·7H₂O, 0.1 g NaCl, 0.01 g FeSO₄·7H₂O, 0.02 g CaCl₂·2H₂O and 1,000 mL deionized water [20]. The cultures were incubated in a rotary shaker at 28 °C and 160 rpm for approximately 48 h. Following three serial transfers in the enrichment medium, the cultures were streaked on nutrient agar plates.

Minimum inhibitory concentration assays

The lowest amount of waste that inhibits microorganism growth from each community was determined through minimum inhibitory concentration (MIC) assays. The MIC was determined based on the method recommended by the US National Committee for Clinical Laboratories Standards [21], the waste concentration ranging from 1 to 64% v/v. MIC was evaluated after 24, 48 and 72 h of incubation at 30 °C by comparisons with control tubes and inoculation on nutrient agar plates.

Biosorption and biodegradation experiments

Experiments were performed for each bacterial community (BS, BL and SS). The inoculum was incubated using 1.0 mL of culture in mineral medium with 1,000 ppm of TBP, ethyl acetate and dodecane as the carbon source at 30 °C in a rotary shaker (150 rpm) until OD₆₀₀ = 2. The cultures were centrifuged at 3,000 rpm for 20 min. The pellet was washed up twice with saline sterile solution (NaCl 0.85%) and re-suspended in the mineral medium for a final OD₆₀₀ of 1.

Both biosorption and biodegradation experiments were performed in 50 mL glass vials, containing 1 mL of culture medium with an OD₆₀₀ of 1 and mineral medium and

amount of radioactive liquid waste nearest to the MIC for a total volume of 10 mL. The cultures were incubated at 30 °C in a rotary shaker (150 rpm) for 1, 2, 4, 10 and 20 days.

To evaluate biosorption after each incubation time, the microbial cells were separated by centrifugation at 3,500 rpm for 15 min and the supernatants were analyzed by gamma spectrometry and spectrophotometry, as described above. The results were statistically analyzed and the amount of radioisotope remaining in the solution was calculated as follows:

$$q = \left(\frac{C_i - C_f}{m} \right) V$$

in which

q = metal uptake (mg metal per g of biosorbent),

V = liquid sample volume (mL),

C_i = initial concentration of metal in the solution (mg L⁻¹),

C_f = final concentration of metal in the solution (mg L⁻¹) and

m = amount of added biosorbent on a dry basis (mg).

For the biodegradation assay following each incubation time, the cultures were heated to 100 °C for 30 min to stop any further degradation and 1,000 ppm of octane were added to each vial as the internal standard, followed by 5 mL of dichloromethane for liquid–liquid extraction of organic compounds. A 0.2 μL aliquot was analyzed by gas chromatography, as described above.

As a control, 1 mL of each heated culture at 100 °C for 30 min was also inoculated in a glass vial to check the recovery of each pollutant and radioisotope. All experiments were performed in duplicate.

Results and Discussion

One of the requirements of the radioactive waste management is the radioisotopic and chemical characterization in order to determine the best method for treating it. For the radioactive liquid organic waste, the amount of Am²⁴¹, Cs¹³⁷, U (Total) stored at the Radioactive Waste Management Laboratory (IPEN-CNEN/SP) is shown in Table 1.

Table 1 Radioisotopes present in radioactive waste and their respective concentrations

Radioisotopes	Activity (Bq/L)	Concentration (mg/L)
Am ²⁴¹	2.0 × 10 ⁵ ± 1.5 × 10 ³	1.6 × 10 ⁻³ ± 1.2 × 10 ⁻⁵
Cs ¹³⁷	2.1 × 10 ⁴ ± 1.8 × 10 ³	6.6 × 10 ⁻⁶ ± 5.6 × 10 ⁻⁷
U (Total)	2.3 × 10 ³ ± 2.1 × 10 ²	1.8 × 10 ² ± 1.7 × 10 ¹

The volatile compounds revealed in the chromatograms were predominantly acetone (30 ppm), followed by ethanol and hexane. The non-volatile organic compounds quantified were ethyl acetate (196 ppm) and TBP (227 ppm).

Removing TBP from the waste via the traditional distillation technique was considered impracticable due to the high boiling point (289 °C) of this compound. Therefore, biodegradation was considered for the treatment of this liquid waste. Bacterial communities were selected from two different sites: (a) the BL and BS communities were isolated from water and soil samples, respectively, taken from Caldas uranium mine (state of Minas Gerais, Brazil); and (b) the SS community was isolated from sediments taken from São Sebastião channel (state of São Paulo, Brazil). We determined the absence of bacteria in the radioactive waste studied before carrying out experiments.

The MIC values revealed that the BS community was more resistant than the other communities. The amount of radioactive-liquid-organic waste capable of inhibiting bacterial growth ranged from 32 to 50% for the BS community and 16 to 32% for the BL and SS cultures. Thus, the biodegradation experiments were performed using the radioactive liquid organic waste at a concentration of 32% for the BS community and 16% for the BL and SS communities over a 20 day period.

The degradation of TBP and ethyl acetate were analyzed on days 4, 10 and 20 days of experiments. The degree of biodegradation caused by both the BS and SS communities was nearly 80% of the TBP and 70% of the ethyl acetate by day 20 (Figs. 1 and 2).

Although the BL community was somewhat less effective, it degraded nearly 60% of the TBP and 70% of the

Fig. 1 Degradation rate of TBP present in raw, observed in the test of biodegradation using different bacterial community after 96, 240 and 480 h

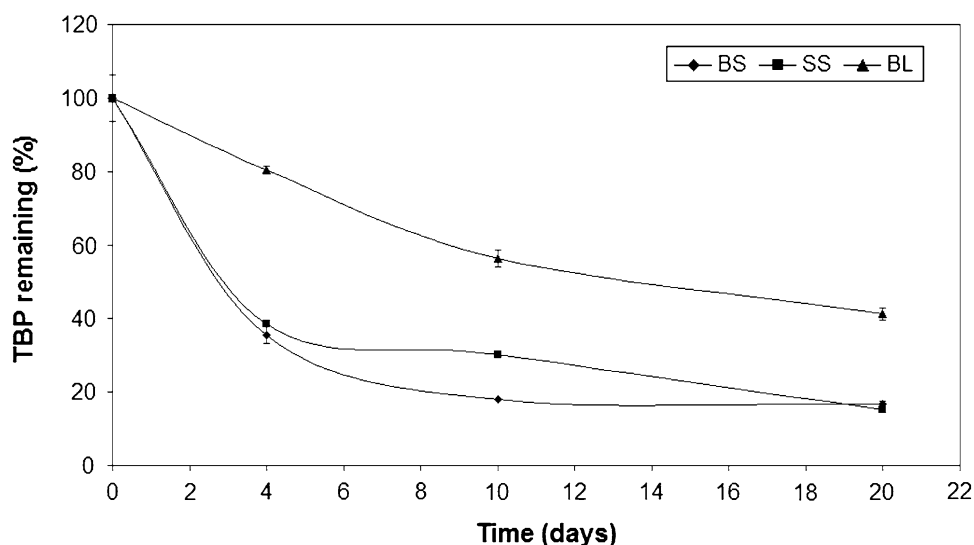
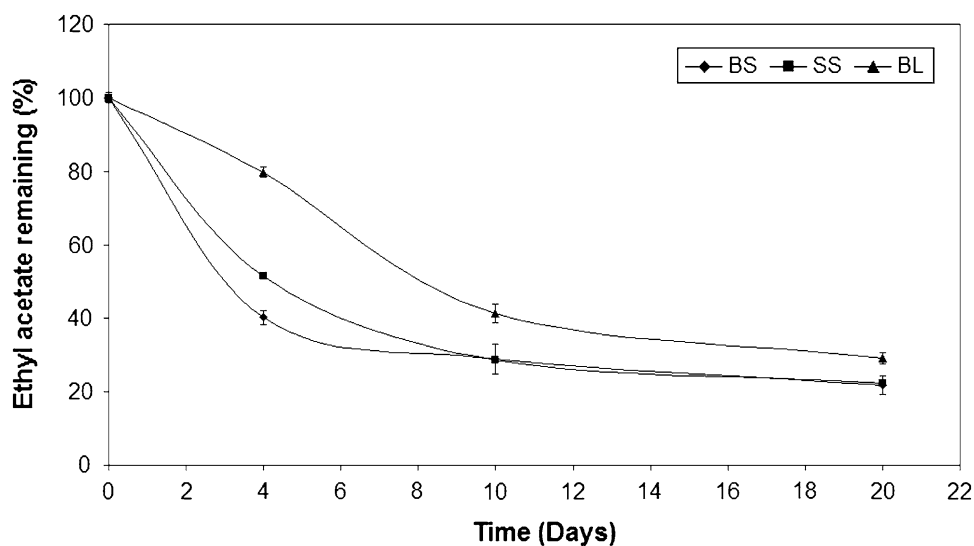


Fig. 2 Degradation rate of ethyl acetate present in raw waste, observed in the test of biodegradation using different bacterial community after 96, 240 and 480 h



ethyl acetate in 20 days. According to Thomas and Macaskie [22], the presence of phosphate in the TBP molecule acts as another source of nutrient for the microorganisms, which may explain the higher degradation values for TBP than ethyl acetate in the BS and SS communities. While these two communities degraded the largest amounts of organic compounds, the BS community demonstrated greater resistance to higher concentrations of the waste in the MIC assays.

The capacity of bacteria to uptake uranium and cesium has been reported [9, 23–29]. The ability of the three communities analyzed (BL, BS and SS) to remove Cs^{137} , Am^{241} and U from the liquid organic waste was evaluated in the present study. The maximum biosorption capacity of each community for uranium, cesium and americium are shown in Figs. 3, 4, 5.

The maximum biosorption capacity of each community for uranium was $27.42 \pm 0.15 \text{ mg g}^{-1}$, $11.75 \pm 0.30 \text{ mg g}^{-1}$ and $11.01 \pm 0.30 \text{ mg g}^{-1}$ for BS, BL and SS communities, respectively.

The biosorption of cesium and americium was efficient in all communities, which removed these radioisotopes within 4 days of contact time. The maximum biosorption capacity of each community for Am^{241} and Cs^{137} was found to be $2.59 \times 10^{-07} \pm 1.81 \times 10^{-09} \text{ mg g}^{-1}$ and $1.09 \times 10^{-09} \pm 2.18 \times 10^{-11} \text{ mg g}^{-1}$ for BS, $1.33 \times 10^{-07} \pm 2.35 \times 10^{-09} \text{ mg g}^{-1}$ and $5.72 \times 10^{-10} \pm 1.14 \times 10^{-11} \text{ mg g}^{-1}$ for BL and finally $1.33 \times 10^{-07} \pm 2.28 \times 10^{-09} \text{ mg g}^{-1}$ and $5.72 \times 10^{-10} \pm 1.14 \times 10^{-11} \text{ mg g}^{-1}$ for SS communities.

Most of the biosorption studies to remove radioisotopes used isolated bacteria and some of them showed higher biosorption capacity for U than the all communities that we

Fig. 3 Cesium removal efficiency by microbial community

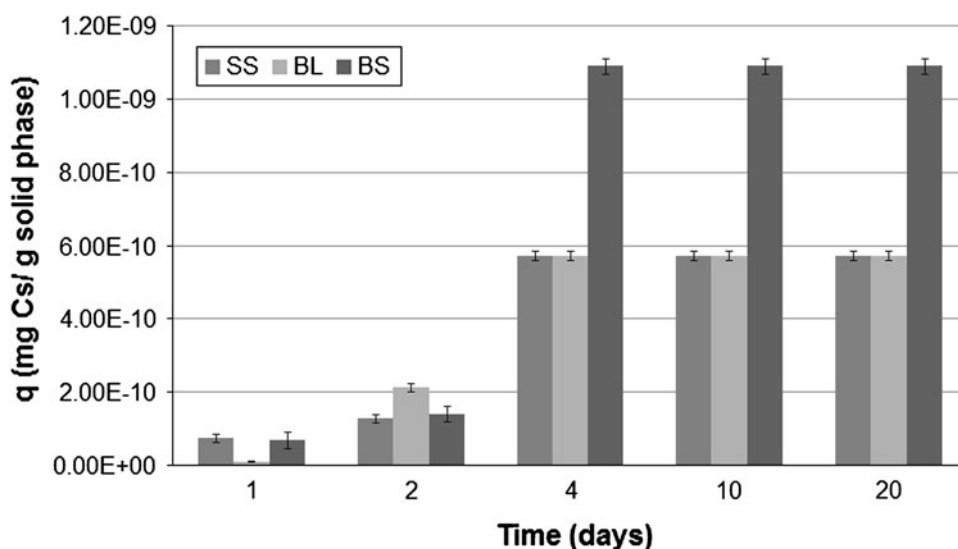


Fig. 4 Americium removal efficiency by microbial community

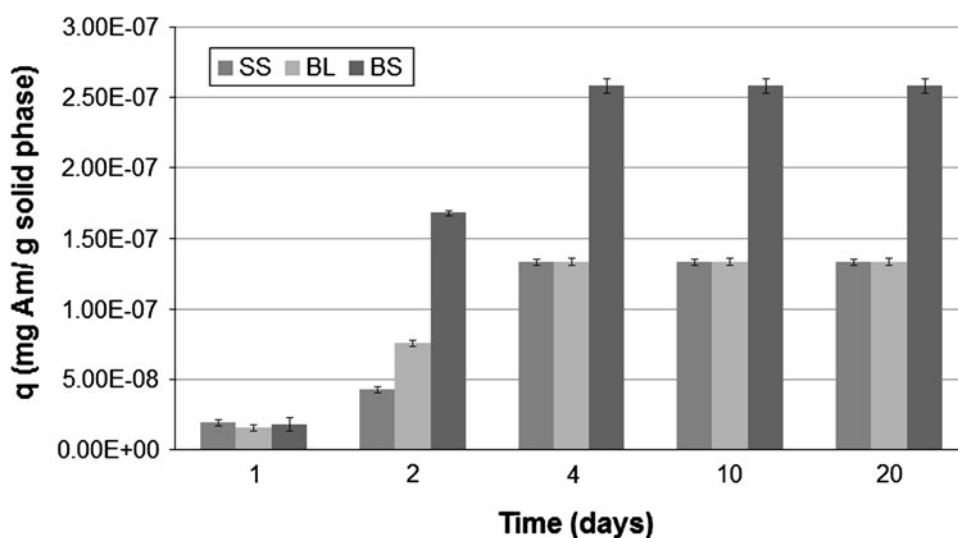
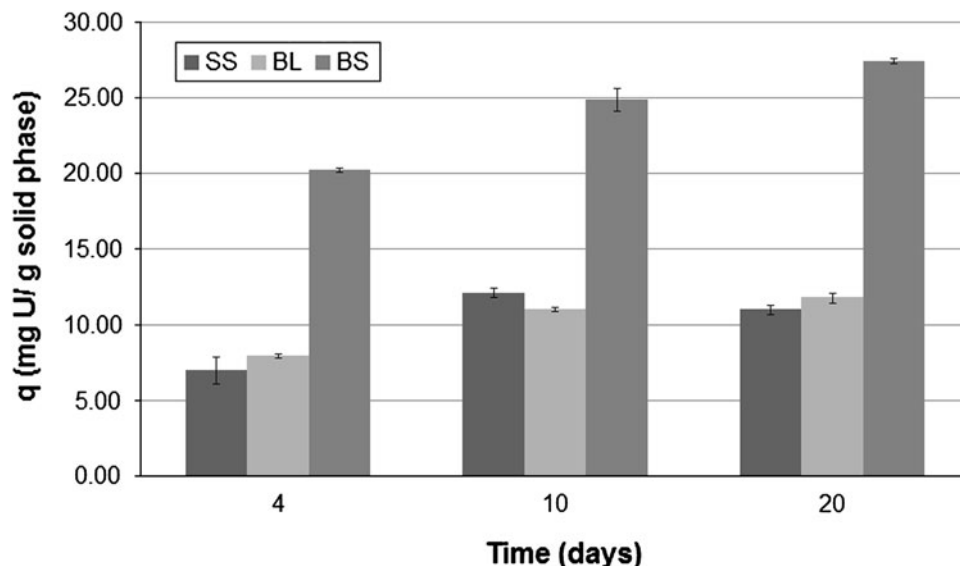


Fig. 5 Uranium removal efficiency by microbial community



investigated, such as *Bacillus pumilus* 200.0 mg g⁻¹, *Bacillus cereus* 190.0 mg g⁻¹ and *Micrococcus lylae* 150.0 mg g⁻¹ [30] and lower biosorption capacity such as *Citrobacter freundii* 16.90 mg g⁻¹ and *Escherichia coli* 17.61 mg g⁻¹ [31].

The percentage of removal is displayed in Table 2. For uranium, maximum uptake was of 92% by the BS community, 81% by the BL community and 76% by the SS community. The BS community was the most effective, uptaking nearly twice the amount of metal adsorbed. The

BL and SS communities achieved similar results to one another in all cases.

Conclusions

All bacterial communities analyzed (BS, BL and SS) demonstrated both biodegradation and biosorption capacity. The BS and SS communities were able to degrade greater amounts of TBP and ethyl acetate than BL community. Moreover, the BS community was able to uptake 92% of the uranium and 100% of the Am²⁴¹ and Cs¹³⁷ at higher concentrations. The results of the present study suggest that the BS community is the most viable for the treatment of large volumes of radioactive liquid organic waste.

Table 2 The percentage of uranium, cesium and americium remaining in solution after the biosorption assays

Radionuclide ^a	Time (days)	BS (%)	BL (%)	SS (%)
Uranium (total)	4	32.5	44.9	51.5
	10	16.4	23.5	16.2
	20	7.7	18.4	23.5
Cesium-137	1	93.8	98.4	87.0
	2	87.2	62.8	78.0
	4	<0.1	<0.1	<0.1
	10	<0.1	<0.1	<0.1
	20	<0.1	<0.1	<0.1
Americium-241	1	93.0	88.3	85.8
	2	35.0	43.2	68.0
	4	<0.1	<0.1	<0.1
	10	<0.1	<0.1	<0.1
	20	<0.1	<0.1	<0.1

^a The initial concentration of Am²⁴¹, Cs¹³⁷, and U (total) in the community BS was equivalent to 32% of the initial concentration of the waste (U (total) 5.6 × 10¹ mg L⁻¹; Am²⁴¹ 5.1 × 10⁻⁴ mg L⁻¹; Cs¹³⁷ 2.1 × 10⁻⁶ mg L⁻¹) and for communities BL and SS was equivalent to 16% (U (total) 2.8 × 10¹ mg L⁻¹; Am²⁴¹ 2.5 × 10⁻⁴ mg L⁻¹; Cs¹³⁷ 1.0 × 10⁻⁶ mg L⁻¹)

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