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BIODEGRADATION OF ETHYL ACETATE IN RADIOACTIVE LIQUID ORGANIC WASTE BY BACTERIAL COMMUNITIES

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ABSTRACT

The research and development program in reprocessing of low burn-up spent fuel elements began in Brazil in 70's, originating the lab -scale hot cell, known as CELESTE located at IPEN-CNEN/SP. The program was ended at the beginning of 90's and part of the radioactive waste generated mainly from the analytical laboratories is stored at the Waste Management Laboratory. Among various types of radioactive waste generated, the organic liquid represents a major problem for its management, because it can not be directly solidified with cement. The objective of this work is to develop a pretreatment methodology to degrade the ethyl acetate present in organic liquid waste so that it can subsequently be immobilized in cement. This work was divided into two parts: i) selection and adaptation of three bacterial communities for growth in medium containing ethyl acetate. ii) degradation experiments of ethyl acetate present in radioactive organic liquid waste. The results showed that from bacterial communities the highest biodegradation level observed was 77%.

1. INTRODUCTION

The radionuclides have been widely used in the industrial, agricultural, medicine and, mainly in the research area. The detriment of the use of this technology to the society is the generation of radioactive wastes that require special treatment to prevent environmental and human contamination.

The International Atomic Energy Agency defines radioactive waste as "material that contains or is contaminated with radionuclides at concentrations or levels of activity higher than the limits for exemption established by the competent authority" [1].

In Brazil, the Nuclear Energy Commission defines the radioactive waste as "any material resulting from human activities, containing radio nuclides in quantities that exceed the limits specified in the exemption standard CNEN -NE-6.02 - Licensing of Radioactive Installations and for which the reuse is inappropriate or not provided" [2].

Before sending to the temporary storage or final disposal, all radioactive waste must be processed and packed properly and in some cases solidified with materials that vary with the type of waste.

The immobilization is the process after the treatment and it is applied to radioactive waste that still does not me et the requirements for the storage, transport and final deposition. The objective of immobilization is to convert the waste into a monolithic solid to create a barrier against the release of radionuclides into the environment. The common matrixes used for immobilization are cement, bitumen and polymers. The most appropriate choice of matrix depends on various factors such as chemical compatibility with the waste, the commercial availability, cost, technology related and quality of final product.

In Brazil, the regulation CNEN-NN-6.09 "Acceptance Criteria for radioactive waste disposal" [3], states that the radioactive liquid waste must be incorporated in a matrix, homogeneously distributed and with a minimum of segregated material, forming a monolithic product. However, it is difficult to meet this standard for organic liquid waste , since it is incompatible with the cement which is main matrix of immobilization used in the country. Pre-treatment is required to obtain a final product with good quality characteristics or destroyed by incineration [4-5].

The Radioactive Waste Management Laboratory at the Instituto de Pesquisas Energéticas e Nucleares (IPEN-CNEN/SP), São Paulo, Brazil is responsible for the treatment and storage of the radioactive wastes generated by IPEN-CNEN/SP itself and those received from radioisotope users in many states of the country. Among the radioactive wastes stored at IPEN/CNEN-SP, there are organic liquid with high quantities of ethyl acetate which was widely used as a solvent for the analytical activities.

The ethyl acetate ($CH_3COOC_2H_5$) is an ester produced in large scale due to its high solvency power. It is a clear liquid, colorless and with a strong fruity smell, obtained by reaction of acetic acid with ethanol. It is widely used in industries of paints, varnishes, adhesives, thinners, removers, photographic film, explosives, coatings for paper, pharmaceuticals, food processing, perfumes, flavorings and synthetic leather finishing [6-7]. The main risks surrounding the use of ethyl acetate are related to their capacity to be partially miscible in water and can contaminate drains, rivers, streams and other currents of water, reacting with oxidants, acids and alkali s [6-8].

The biotreatment of radioactive liquid organic waste containing ethyl acetate stored at IPEN- CNEN/SP, appears to be a very promising technology. This approach is based on the use of microorganisms able to metabolize and digest the ethyl acetate present in the wastes making them compatible with the matrix of cement

2. OBJECTIVE

The objective of this work is the development of an alternative methodology to degrade ethyl acetate from the radioactive liquid organic waste using bacterial communities.

3. EXPERIMENTAL WORK

This work was divided into two parts. i) Selection and adaptation of three bacterial communities for growth in medium containing ethyl acetate the bacterial communities were obtained from soil and water samples collected in the uranium mine of Poços de Caldas (Minas Gerais, Brazil) and in sediments of the São Sebastião channel (São Paulo, Brazil). Both places are known as environmentally impacted areas, the first one by the extensive uranium mining and milling operations, and the second one by several oil spills. The uranium mine of Poços de Caldas was the first facility to produce uranium concentrate in Brazil and operated from 1982 to 1995. The wastes generated during the mining activities are now a source of acid drainage which promotes solubilization of uranium, thorium, radium and stable elements as manganese, iron, zinc, fluorine [9; 10]. São Sebastião channel, on the north coast of São Paulo State, locate the most important oil terminal in Brazil and from its inauguration in 1974 until 1997, occurred 305 oil spills which exposed coastal ecosystems [11]. ii) testing of degradation of ethyl acetate present in radioactive organic liquid waste by bacterial communities obtained in the previous step.

3.1. Selection and adaptation of bacterial communities

Bacterial communities were selected both from impacted areas, the mining of uranium in Poços de Caldas (FIG: 1) and the São Sebastião channel (FIG: 2). The samples from Uranium mine in the Poços de Caldas were collected in the coordinated BIA SOIL (BS): S 21º 56' 19.0' WO 46º 29' 47. 5' from soil sample and BIA LAKE (BL): S 21º 56' 20.8' WO 46º 29' 13. 5' from water sample. The sediment sample of the São Sebastião channel was donated by the Oceanographic Institute of the University of São Paulo (IO -USP) and collected in SÃO SEBASTIÃO (SS) at coordinated S 23°47' 44. 4' S WO 45° 22 ' 85'.

FIGURE 1: Place of collection of samples: BS and BL

FIGURE 2: Map of the São Sebastião channel [12]

Enrichment of the cultures from water, soil and sediment samples was prepared by adding 1.0 g (soil and sediment) or 1.0 mL (water) sample in mineral salt medium , with 0.5% of ethyl acetate as carbon source. The mineral medium was prepared with 1.0 g $(NH_4)_2SO_4$, 0.2 g KH_2PO_4, 1.6 g K_2HPO_4, 0.2 g $MgSO_4.7H_2O$, 0.1 g NaCl, 0.01 g $FeSO_4.7H_2O$, 0.02 g $CaCl_2.2H_2O$ and 1000 mL of deionized water. The cultures were incubated on a rotary shaker at 28 ºC and 160 rpm for approximately 48 h using cyclohexamide (0.25 mg/L) as antifungal agent. After three serial transfers in the enrichment medium, the cultures were analyzed [13].

3.2. Biodegradation Experiments

The radioactive liquid organic waste evaluated in this work is chemically composed of water and ethyl acetate. The main elements present in this solution were U, Pu, Am and Cs. The minimum inhibitory concentration (MIC) was determined previously according to the method recommended by the US National Committee for Clinical Laboratories Standards [14]. The cultures obtained from BS, BL and SS sites were inoculated in Luria Broth (LB) and incubated at 30°C until $1.5x10^8$ CFU/mL of bacterial density was achieved. This suspension was 10 times diluted in saline sterile (NaCl, 0.85%) and subsequently 1.0 mL aliquots of this dilution were inoculated in Müeller Hinton broth ($1.5x10^7$ CFU/mL) with waste in concentration ranging between 1 to 64% v/v. MIC was evaluated after incubation for 24, 48 and 72 hours at 30ºC, comparing with control tubes and inoculation on nutrient agar plate.

Inocula from each site communities (BS, BL and SS) were prepared for the biodegradability testing of waste samples, incubating 1.0 mL of culture in mineral medium containing 1000ppm of TBP and ethyl acetate, at 30ºC on rotary shaker (150 rpm). When $3x10^8$ CFU/mL of bacterial density was achieved, all cultures were centrifuged at 3.000 rpm for 20 minutes, and the pellet washed twice with saline sterile (NaCl 0.85%). The cells were resuspended in the same solution for bacterial suspensions with 1.5 $x10^8$ CFU/mL. A 1.0mL aliquot of these suspensions was distributed in 50 mL glass vials, previously washed with acetone, containing mineral medium and concentration of the waste close to the MIC values. The cultures were incubated at 30 ºC on rotary shaker (150 rpm) for 96, 240 and 480 hours.

As control, were used cultures heated at 100 ºC for 30 min. All experiments were performed in duplicate.

After the incubation time, the biodegradation reaction was interrupted by heating at 100ºC for 30 min., and 1000 ppm of octane was added, as internal standard, followed by 5 mL of dichloromethane for liquid-liquid extraction of organic compounds. After homogenization on a vortex, a 0.2-µL aliquot was analyzed by gas chromatography (Agilent model 6890N). The separation was carried out using a DB -XLB of J & E Scientific (30m, 0.25mm i.d.; and film thickness 0.25 µm). The GC temperature program was 30ºC for 5 min, up to 180ºC (10°C min^{-1}) kept for 1 min, increased to 230ºC (10°C min^{-1}), this temperature was kept for 3 min to ensure the removal of all compounds. The analysis was done in a split/splitless injector (split ratio 1:14) at 250ºC. The carrier gas was helium at a rate of 1.3 mL min^{-1}. The biodegradation percentage was calculated based on the difference between the concentration of the samples and controls at the end of the experiment [15].

4. RESULTS

The MIC of radioactive liquid organic waste was performed in order to determine the maximum concentration of the waste that could be used in the biodegradation experiment tests, without inhibiting the bacterial growth. The concentrations, capable of inhibiting the growth ranged from 32% to 50% for the communities BS, and 16% to 32%, for BL and SS cultures.

Biodegradation level of ethyl acetate in radioactive waste by bacterial communities BS, BL and SS cultivated in mineral medium after 96, 240 and 480 hours is shown in Fig. 4. Results were compared with the initial concentration (Fig. 3), according to the statistic Student's t - test with significance level of 95%.

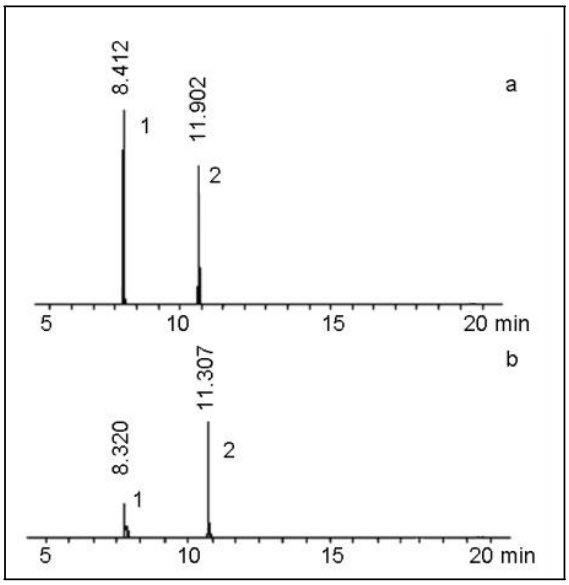

FIGURE 3: Control Chromatogram (a) and chromatogram after the biodegradation experiment (b). Ethyl acetate (1), Octane (2)

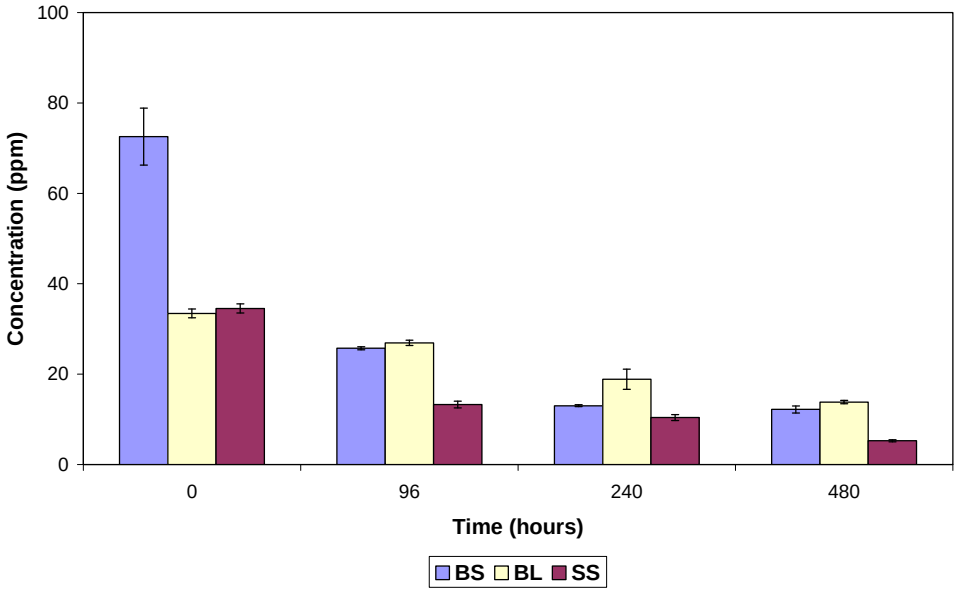

FIGURE 4: Degradation level of ethyl acetate prese nt in radioactive liquid organic waste after 96, 240 and 480 hours.

Figure 4 shows a decreasing of the ethyl acetate concentration as a function of time, after treatment with bacterial communities. The concentration of ethyl acetate, in 96 hours, decreased 59.2%, 14.1% and 45.3% for BS, BL and SS, respectively. This higher ability to degrade the ethyl acetate by communities BS and SS can be explained by the greater diversity of microorganisms in soil than in water [16].

Communities BS and SS degraded the highest amounts of ethyl acetate 77,8% and 76,2% respectively, however BS showed resistance to higher concentrations of the waste observed in MIC assays. These results suggest that the community BS is more viable for treatment of large volumes of radioactive liquid organic waste.

5. CONCLUSIONS

The results obtained in this work demonstrated that the method proposed is viable and efficient to treat the liquid organic radioactive waste containing ethyl acetate. All bacterial communities (BS, BL and SS) showed degradation capacity, but the BS and SS communities were capable to degrade higher levels ethyl acetate. For a large-scale treatment, community BS is more viable.

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