



Research Paper

A new method for preconcentration of Th(IV) and Ce(III) by thermophilic *Anoxybacillus flavithermus* immobilized on Amberlite XAD-16 resin as a novel biosorbent



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ABSTRACT

A novel approach has been improved for the preconcentration of Th(IV) and Ce(III) by using thermophilic *Anoxybacillus flavithermus* which were immobilized on Amberlite XAD-16 resin and analyzed by Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES). The concentration of Th(IV) and Ce(III), the eluent type and volume, the pH of model solutions, influences of matrix interference parameters, and flow rate were analyzed and optimized extraction conditions were determined. The optimum pH for quantitative sorption of Th(IV) and Ce(III) was found as 7.0 and elution of Th(IV) and Ce(III) from mini-columns was studied with 1.0 mol L⁻¹, 5 mL HNO₃ with recoveries from 100%. The precision of this method was validated by comparing the analytical recovery and certified reference materials (IAEA Soil-7). Finally, this enrichment method was used to determine Th(IV) and Ce(III) in bastnaesite ore sample.

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1. Introduction

Thorium and cerium which are large in the number of radioactive elements in the world have various usages in many different fields like chemical industry, optics, radio, metallurgy, material fields, aeronautics, and aerospace. (He et al., 2007; Shraddha and Singh, 2015; Zhang et al., 2005). In spite of these applications, even very small amounts of thorium, cerium and their compounds have both chemical toxicity and radioactivity that can result in a perennial damage in bone, kidney, and liver. (Shtangeeva et al., 2005; Rao et al., 2006a, 2006b; Shraddha and Singh, 2015). Numerous nuclear accidents (such as that in Chernobyl) have worldwide effects such as the resulted artificial radionuclides reached the stratosphere and caused widespread contamination of lands (Kuwahara et al., 2011; Shtangeeva et al., 2005). In order to minimize their destructive effects to human health and the environment for safety and economy, the recovery of radionuclides is essen-

tial (Gadd 2009). Therefore, determination of these elements from different samples are desired (Sandesh et al., 2015). Due to insufficient sensitivity, the presence of complex matrix, deficiency of selectivity, weak precision, and accuracy; the direct assigment of thorium and cerium is still compelling by instrumental techniques like ICP-OES, ICP-MS, etc. (Shariati et al., 2008; Prabhakaran and Subramanian, 2003). Preconcentration and separation process can easily solve these problems. The common process includes ion exchange, oxidation-reduction, co-precipitation, solid phase extraction, electrodeposition and filtration, etc. (Ozdemir et al., 2014; Gupta et al., 2000; Hussein et al., 2004; Wuyep et al., 2007). However, when metal concentrations are high such as 1–100 mg L⁻¹ (Ozdemir et al., 2014; Ahluwalia and Goyal, 2007; Okumus et al., 2010; Ozdemir et al., 2009) most of these methods are expensive and ineffective excluding solid phase extraction (Sandesh et al., 2015; Rao et al., 2006a,b; Vidal et al., 2012; Spivakov et al., 2006; Turker, 2007; Camel, 2003; Rao and Preetha, 2003). The fundamental working principle of SPE method depends on preconcentration of analytes on the solid sorbent. Various solid sorbents were studied to preconcentrate rare earth elements prior to their instrumental analysis such as activated carbon (Ferreira et al., 2004; Daorattanachai et al., 2005; Karami et al., 2004) silica gel (Akhond

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et al., 2006), polyurethane foam (Anthemidis et al., 2002), Chelex-100 (Ferri and Sangiorgio, 1996), alumina and Amberlite XAD resins (Varhan Oral et al., 2011; Bulut et al., 2007a, 2007b; Duran et al., 2007; Singh and Maiti, 2006; Dolak et al., 2010) and biological materials like bacteria (Robles and Aller 1995), algae (Shunxin et al., 1999), and yeast (Smichowski et al., 2000).

It is known that the biosorption is a technique used for recovery of metals by using microbial cells. Moreover, it is costly more effective method when compared to other solid phase extraction (SPE), due to using living or dead biomass whose cell wall include various functional groups that can be bound to metal, like hydroxyl, amino groups, phosphate, sulphate, and carboxylate (Hussein et al., 2004; Wuyep et al., 2007; Madrid and Camara, 1997; Kotrba et al., 1999; Vijayaraghavan and Yun, 2008). Biosorption enables that metal ions are passively absorbed and complexed to biomass by using methods like complexation, inorganic microprecipitation, ion exchange and physical adsorption, (Okumus et al., 2015; Ginisty et al., 1998; Madrid and Camara, 1997; Kotrba et al., 1999; Vijayaraghavan and Yun, 2008).

Amberlite XAD resins, which are organic polymers, commonly employed to design new preconcentration techniques. Amberlite XAD resins make sorption usually by complexation of functional groups they have and contain nitrogen and sulfur with trace metals. These resins are widely used in SPE, because of their good physical and chemical features such as high surface area, porosity, purity and durability (Varhan Oral et al., 2011; Lemos and Baliza 2005). In these days, removal and enrichment of metals from industrial, natural and radioactive liquid wastes has performed with the help of microorganisms immobilized to suitable support materials such as resin and the obtained results have much greater attention than the conventional preconcentration and separation methods. (Okumus et al., 2015; Ozdemir et al., 2014; Kader et al., 2007; Varhan Oral et al., 2015).

To our knowledge, studies about preconcentration of thorium and cerium are very limited, in which bacterial biomass is used as a SPE. Larch tannin and *myrica rubra* tannin onto collagen fibre matrices were employed as column adsorbents for concentrating thorium (Tsuey-Lin et al., 2008). In the batch experiment conditions, the biosorption of the thorium and cerium were investigated by employing different microorganisms such as *Micrococcus luteus* (Romera et al., 2006), *Streptomyces levoris* (Tsuruta 2006), *Citrobacter* sp. (Yong and Macaskie, 1998), *Pseudomonas* (Sar and D'Souza, 2002), *Micrococcus luteus*, *Bacillus* sp., *Bacillus megaterium*, *Arthrobacter nicotianae*, *Streptomyces levoris* and *Rhodococcus erythropolis* (Tsuruta 2004), *Sargassum filipendula* in a dynamic system (Picardo et al., 2009).

The current study systematically investigates sorption and desorption of Th(IV) and Ce(III) on a column with thermophilic *Anoxybacillus flavithermus* immobilized on Amberlite XAD-16 resin. The immobilization of thermophilic *Anoxybacillus flavithermus* on Amberlite XAD-16 was identified by FT-IR spectroscopy. The optimum preconcentration and determination conditions for the recovery of Ce(III) and Th(IV) was studied with model solutions. The reformed preconcentration protocol was validated by analyzing certified reference materials (IAEA Soil-7) and applied for the determination of Th(IV) and Ce(III) in bastnaesite ore sample.

2. Experimental

2.1. Instrumentation

The amount of Th(IV) and Ce(III) in the aqueous phase was calculated by using a Perkin Elmer Optima 2100 DV Inductively coupled plasma-optical emission spectrometry (ICP-OES). The pHs of the solutions were calibrated with a Metler Toledo

MPC 227 (Polaris Parkway, Columbus) model digital pH meter. The functional groups of thermophilic *Anoxybacillus flavithermus* immobilized on Amberlite XAD-16 were characterized by a Mattson 1000 model Fourier transform infrared (FT-IR) spectrometer (Cambridge, England) as KBr tablets. 1.0 cm × 10.0 cm filtration columns equipped with polypropylene frits were employed for solid phase extraction (SPE). The solutions were passed through the column with Watson Marlow SCI 323 (Milford, MA) model peristaltic pump. The solutions were centrifuged with Sigma Christ 2K15 (St. Louis, MO, USA) microwave pressure digestion system (Speedwave MWS-3, Berghof). Real and certified reference materials were dissolved with Berghof Speedwave TM MWS-3C model microwave (Harretstrasse-Eningen, Germany).

2.2. Reagents and solutions

In this study, all chemicals were analytical reagent-grade. The solutions were prepared with quartz double distilled water by using a Millipore Milli-Q water purification system. Standard cerium and thorium were used by diluting a corresponding 100 µg mL⁻¹ solution (Merck, Darmstadt-Germany). Certified reference materials (CRM) were supplied from National Research Council of Canada. The solutions were centrifuged with Sigma Christ 2K15 (St. Louis, MO, USA). pH was adjusted by the addition of diluted hydrochloric acid or sodium hydroxide to the sample solution. The laboratory glassware was heedfully cleaned and kept in 1.0 mol L⁻¹ nitric acid for at least 24 h and the materials were rinsed three times with double distilled water before all applications.

Adsorber resin (polystyrene divinyl benzene) Amberlite XAD-16 (surface area 725 m²g⁻¹, pore diameter 4 nm and bead size 20–60 mesh) was obtained from Sigma (St. Louis, MO, USA). Amberlite XAD-16 resin obtained from the supplier contained organic and inorganic impurities. In order to remove the contaminants, it was treated with 4.0 mol L⁻¹ HCl. The resin was firstly rinsed with double distilled water until neutral pH, then with an ethanol-water (1:1) solution and finally with double distilled water. Eventually, it was stored in the polyethylene bottle (Erdoğan et al., 2004).

2.3. Growth and preparation of dried dead cells for solid phase extraction (SPE)

In the current study, the thermophilic *Anoxybacillus flavithermus* was isolated from thermal springs mud sample of Ömer, Afyonkarahisar, Turkey. Its biochemical and morphological properties were investigated. 16S rRNA was analyzed by Ref-Gen (METU Technocity/Ankara).

The fermentation medium containing peptone (10 g L⁻¹), beef extract (10 g L⁻¹) and NaCl (g L⁻¹) was prepared with tap water. The pH of culture medium was calibrated to 7.0 with using 0.1 mol L⁻¹ NaOH and HCl. All of the fermentation medium cultured using 250 mL bottle flasks including 25 mL of sterile fermentation medium. 1 mL old culture was inoculated in bottle flasks for 8 h and incubated at 55 °C for 24 h in a shaker at 120 rpm. The growth medium was centrifuged at 10,000 rpm for 10 min, and the pellet was used for the proceeding steps. Pellets were treated with saline solution for removing the culture medium contaminants and waited at 80 °C for 24 h in an oven for drying. The dried bacteria were ground in a porcelain mortar to get a fine powder and then put into an autoclave to be sure of the complete death of bacteria.

2.4. Column preparation

For Th(IV) and Ce(III) immobilization of bacteria to substrate, 1000 mg of Amberlite XAD-16 was mixed with 250 mg of dried and dead bacteria powder. The paste was heated in an oven at about 105 °C for 1 h to dry the mixture, which was

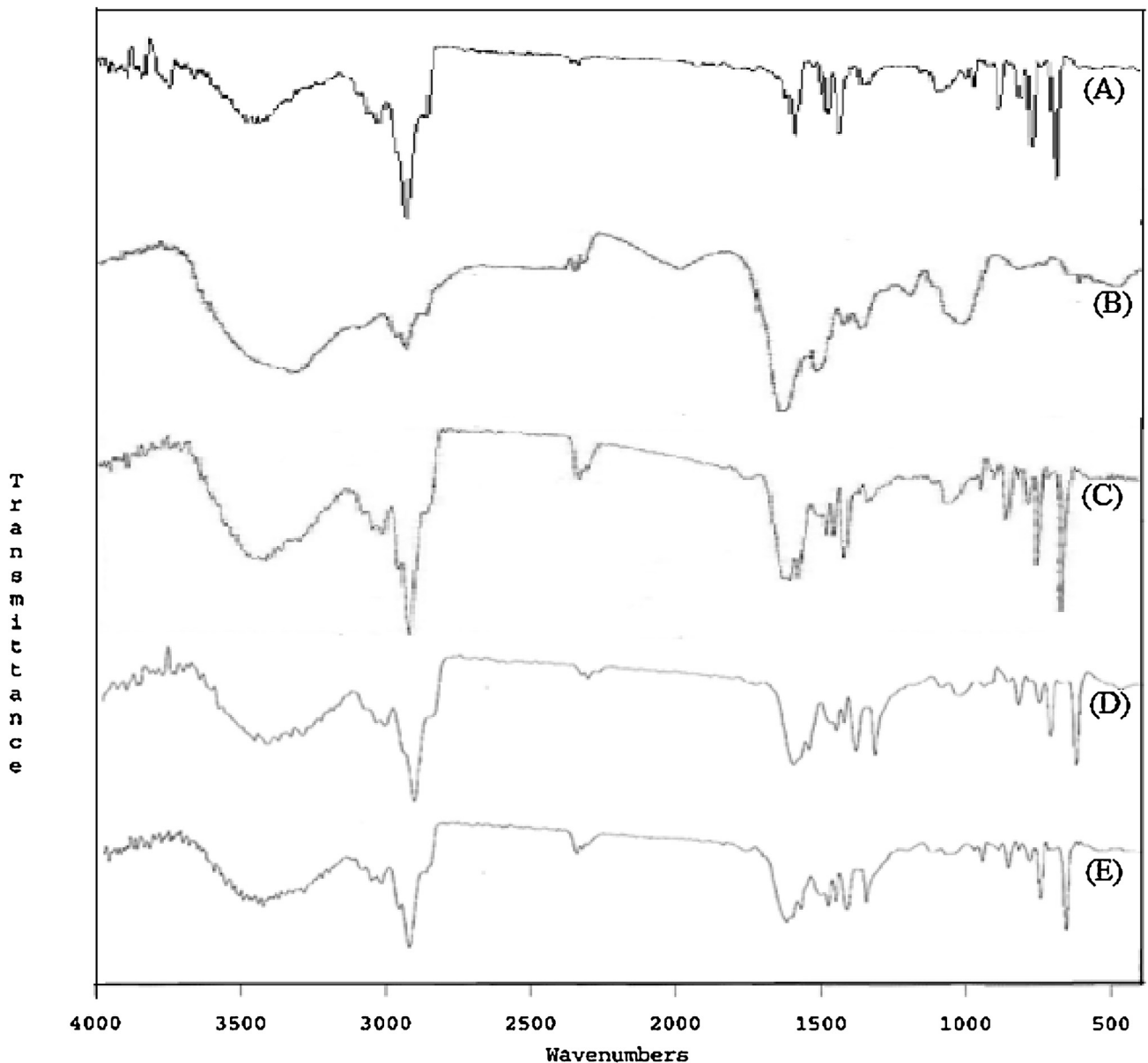


Fig. 1. (A) FT-IR spectrum of Amberlite XAD-16, (B) FT-IR spectrum of thermophilic *Anoxybacillus flavithermus*, (C) FT-IR spectrum of thermophilic *Anoxybacillus flavithermus* immobilized on Amberlite XAD-16, (D) FT-IR spectrum of the thermophilic *Anoxybacillus flavithermus* immobilized on Amberlite XAD-16 after sorption of Th(IV), (E) FT-IR spectrum of the thermophilic *Anoxybacillus flavithermus* immobilized on Amberlite XAD-16 after sorption of Ce(III).

then weighed to calculate the bacteria taken up by the resin. The contact between thermophilic *Anoxybacillus flavithermus* and Amberlite XAD-16 resulted in improved immobilization efficiency. Finally, the handled product was ground for getting its original size (20–60 mesh) and used as an adsorbent. Afterwards, a 1.00 cm × 10.00 cm polyethylene column was packed with the mixture.

2.5. General sorption studies

The developed biosorption method was firstly applied to model solutions before applying to the trace metals in the real samples. For this aim, the metal solutions 250 µg Th(IV) and Ce(III) in 50 mL were prepared. pH of the model solutions was adjusted to the aimed values (in the range 2–7) adding NaOH or HCl solutions. The optimum pH values were obtained as 7.0 for each metal ion. Then, Th(IV) and Ce(III) solutions were passed through the column at the optimum flow rate (4 and 3 mL min⁻¹, respectively).

Finally, the retained Th(IV) and Ce(III) solutions were eluted from the solid phase by 1.0 mol L⁻¹, 5 mL HNO₃. ICP-OES was used for the determination of the concentration of the Th(IV) and Ce(III) in the eluate. Thermophilic *Anoxybacillus flavithermus* immobilized on Amberlite XAD-16 was used repeatedly (55 times) after washing successively with 1.0 mol L⁻¹ HNO₃ solution and distilled water. Having passed this solution completely, the column was rinsed twice with 10 mL distilled water. A 5 mL 1.0 mol L⁻¹ HNO₃ solution was used for elution of the adsorbed Th(IV) and Ce(III) on the bio-sorbent. This solution was diluted for ICP-OES analysis.

2.6. Procedure for certified reference materials

The certified reference material IAEA SOIL-7 was digested as the way recommended. For this purpose, a 1.0 g leave portion was weighted and added to digestion vessel. A 5 mL of concentrated HNO₃ was included and shaken cautiously by clean Teflon bar, and then waited for at least 30 min prior to the vessel was closed. The

vessel was applied the recommended program in microwave pressure digestion system. The volume was completed to 500 mL and the biosorption method was applied.

2.7. Analysis of real samples

The bastnaesite ore sample which includes the rare earth elements were sampled from Sivrihisar-Eskişehir (Turkey), which was ground to a size of 100 mesh after the homogenization. Then 0.1 g of sample was digested with HNO_3 -HF solutions in a microwave oven. After adjusting optimum pH, the volume was completed to 500 mL and the biosorption method was used for the sample.

2.8. Analytical recovery

Various quantities (100 and $200 \mu\text{g g}^{-1}$) of Th(IV) and Ce(III) were put in bastnaesite ore sample solutions. Before solid-phase extraction, the sample solution pH was adjusted to 7.0 and the developed process was used to the sample. The concentrations of the Th(IV) and Ce(III) were determined by ICP-OES.

2.9. Characterization studies

Once the comparison was made between Fig. 1B and C, the peak variation was observed at C=O, N–H and C–N groups. These outcomes could be strong proof of the resin and thermophilic *Anoxybacillus flavitermus*. While carbonyl peak was appeared at 1655 cm^{-1} , N–H peak was determined at 1535 cm^{-1} . In addition to this C–N peak was seen at 1049 cm^{-1} in Fig. 1B. However in Fig. 1C, C=O, N–H and C–N peaks were shifted to 1632 cm^{-1} , 1605 cm^{-1} and 1112 cm^{-1} respectively. Moreover the peak changes in intensity of above mentioned groups were also observed.

The FT-IR analysis comparison of Fig. 1D–E with Fig. 1C revealed that at $\nu(\text{C}=\text{O}) = 1632 \text{ cm}^{-1}$ and $\nu(\text{C}-\text{N}) = 1112 \text{ cm}^{-1}$ vibrations of thermophilic *Anoxybacillus flavitermus* loaded on resin were changed at $\nu(\text{C}=\text{O}) = 1656 \text{ cm}^{-1}$ and $\nu(\text{C}-\text{N}) = 1102 \text{ cm}^{-1}$ for Th(IV) biosorption and $\nu(\text{C}=\text{O}) = 1653 \text{ cm}^{-1}$ and $\nu(\text{C}-\text{N}) = 1100 \text{ cm}^{-1}$, for Ce(III) biosorption, respectively. The peak intensities for both the C=O and C–N groups varied after Th(IV) and Ce(III) biosorption with the observation of weaker peak intensities than for those at Fig. 1C. These important variations in peak intensity and shifting at peak positions at C=O and C–N peaks could be due to the complexation with Th(IV) and Ce(III). In addition to this the peaks between 500 and 800 cm^{-1} were also determined as weaker due to the complexation.

3. Result and discussion

3.1. Influence of pH

pH is among the major parameters in biosorption investigations since it has significant effects on equilibrium by changing the metal ion(s) speciation and solubility, surface wall of functional groups on the biosorbent and extent of ionization of the biosorbent during operation (Bhatti et al., 2009; Ozdemir et al., 2010). 50 mL of the metal ion solutions including $5.0 \mu\text{g mL}^{-1}$ Th(IV) and Ce(III) was passed from the SPE column at 1 mL min^{-1} flow rate at different pH ranges (pH: 2.0–7.0) to study the influence of pH on the extent of Th(IV) and Ce(III) biosorption,

The effect of pH on the biosorption of Th(IV) and Ce(III) on Amberlite XAD-16 immobilized with *Anoxybacillus* sp. SO B1, was investigated and the results are demonstrated in Fig. 2. Optimum pH was found as 4.0–7.0 for Th(IV) and 7.0 for Ce(III), respectively. Due to high proton concentration at lower pH, biosorption of heavy metal owing to the competing of hydrogen ions with heavy metal

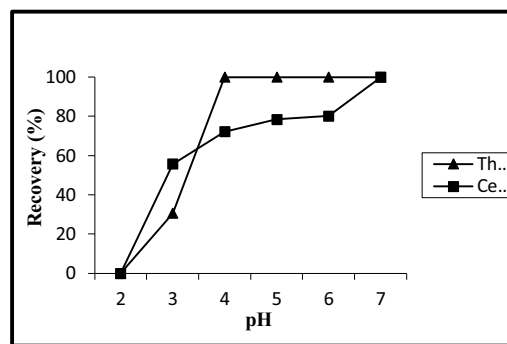


Fig. 2. Effect of pH on the recovery $5.0 \mu\text{g mL}^{-1}$ Th(IV) and $5.0 \mu\text{g mL}^{-1}$ Ce(III), 50 mL, Eluent: 1.0 mol L^{-1} , 5 mL HNO_3 , Flow rate sorption: 1 mL min^{-1} .

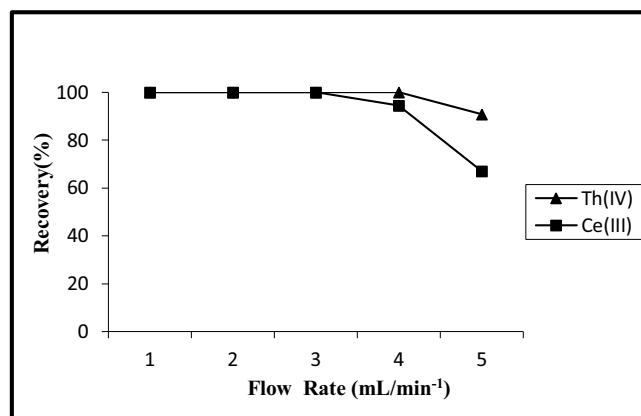


Fig. 3. Effect of flow rate on biosorption $5.0 \mu\text{g mL}^{-1}$ Th(IV) and $5.0 \mu\text{g mL}^{-1}$ Ce(III), 50 mL, Eluent: 1.0 mol L^{-1} , 5 mL HNO_3 , pH: 7.0 for each metal.

on the surface wall of biosorbent functional groups. Due to deprotonation of the metal binding groups, the negative charge quantity on the cell wall of biomass raises with pH increment. Therefore, the metal ions compete more efficiently for present binding groups and so biosorption rises (Kapoor and Viraraghavan 1997). All in all, upcoming experiments can be designed pH 7.0 for each metal.

3.2. Influence of flow rate

It is known that sample solutions flow rate which affects the duration of the analysis and the biosorption rate of the complexes on the resin is significantly important. The sample flow rates were studied at 1 – 5 mL min^{-1} . As seen in Fig. 3, the flow rate for Th(IV) and Ce(III) has become decrease after the 4 and 3 mL min^{-1} , respectively. Eventually, it was defined that when the flow rate was higher than the determined values for both metals, the lower biosorption efficiency was obtained. This result was caused from short interaction time between metal-ion and biosorbent.

3.3. Influence of the eluent type, concentration and volume

Among the parameters in biosorption from aqueous solutions, the type, concentration and volume of the eluent are important ones. The concentration level of the eluent acid must be as lowest as possible to prevent biomass deterioration. Two distinct acidic solutions which have different volumes and concentrations were analyzed at the optimum conditions in order to choose the most suitable eluent (Table 1). Different volumes of HNO_3 and HCl (0.5 and 1.0 mol L^{-1}) solutions were passed through column under the determined optimum conditions, respectively. The higher recovery values were obtained by increasing concentrations of HNO_3 and

Table 1
Effect of Type, Concentration and Volume of the Eluent on the Recoveries of Th(IV) and Ce(III).

Acid	Volume (mL)	Recovery of Th(IV)	Recovery of Ce(III)
0.5 mol L ⁻¹ HCl	2	37.79 ± 0.54	40.54 ± 0.31
	5	55.76 ± 0.95	55.35 ± 1.06
	10	68.35 ± 0.87	65.00 ± 1.21
1.0 mol L ⁻¹ HCl	2	85.12 ± 1.76	80.73 ± 1.02
	5	91.15 ± 1.33	88.58 ± 0.84
	10	97.75 ± 1.05	95.44 ± 1.43
0.5 mol L ⁻¹ HNO ₃	2	60.85 ± 0.78	58.90 ± 1.13
	5	70.76 ± 1.61	71.39 ± 1.27
	10	88.67 ± 1.89	86.41 ± 1.07
1.0 mol L ⁻¹ HNO ₃	2	76.75 ± 0.98	69.17 ± 1.76
	5	100.0 ± 2.75	100.0 ± 1.54
	10	100.0 ± 2.16	100.0 ± 2.21

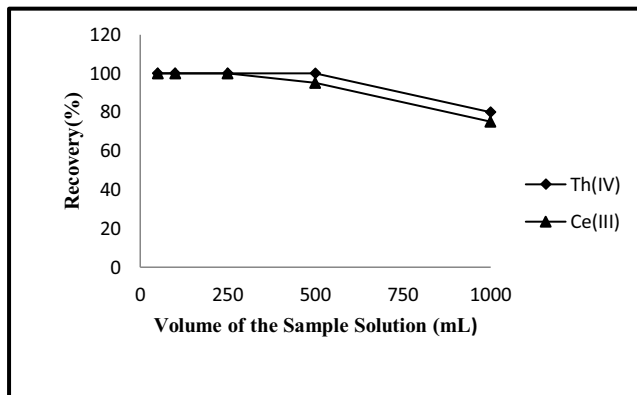


Fig. 4. Effect of Volume of the Solution on the Bisorption of Th(IV) and Ce(III), pH: 7.0, Eluent: 1.0 mol L⁻¹, 5 mL HNO₃, Flow rate: 4.0 and 3.0 mL min⁻¹ for Th(IV) and Ce(III), respectively.

HCl. As a result of that, the ideal HNO₃ concentration was found to be 1.0 mol L⁻¹ for both metals. Besides that, the effect of different volumes (2, 5 and 10 mL) of HNO₃ (1.0 mol L⁻¹) was also studied and the optimum eluent volumes of two metals were determined as 5 mL for the proceeding steps.

3.4. Influence sample volume

To get high preconcentration factor, determination of the optimum sample solution volume is important. By passing various sample volumes (50, 100, 250, 500 and 1000 mL) which includes 5.0, 2.5, 1.0, 0.5 and 0.25 μg mL⁻¹ of metals through the SPE column at determined ideal conditions, respectively, the influence of sample volume on the biosorption of tested metal ions was studied. It was resulted that Th(IV) and Ce(III) were recovered quantitatively (≥99%) until 500 mL of the solution volume (Fig. 4). Therefore, the preconcentration factor was determined as 100 since the elutions solutions volume was used 5 mL for each metal.

3.5. Influence of interfering ions

Since they interfere with the determination of Th(IV) and Ce(III) analyses, the influence of foreign ions (Na⁺, K⁺, Mg²⁺, Cl⁻ and SO₄²⁻) is also the important parameter for metals biosorption. 500, 1000 and 5000 μg mL⁻¹ of Na⁺, K⁺, Mg²⁺, Cl⁻ and SO₄²⁻ ions had not significant interference with the determination of the analytes. As seen in Table 2, quantitative recovery was obtained for both metals although there is high interfere in the ion concentrations excluding magnesium ion which had an impact on the recovery efficiency. The recovery efficiency in the presence of this ion (Mg²⁺)

Table 2
Effect of the Interfering Ions on the Recovery of Th(IV) and Ce(III).

Ions	Concentration (μg mL ⁻¹)	Recovery of Th(IV)	Recovery of Ce(III)
Na ⁺	500	101.33 ± 2.87	103.65 ± 3.03
	1000	100.81 ± 2.08	100.17 ± 2.55
	5000	99.27 ± 3.47	100.85 ± 1.94
K ⁺	500	100.41 ± 2.52	102.54 ± 2.72
	1000	100.05 ± 1.79	100.33 ± 2.18
	5000	98.66 ± 1.55	99.45 ± 3.56
Mg ²⁺	500	94.45 ± 1.72	96.37 ± 2.85
	1000	88.12 ± 1.83	86.47 ± 2.30
	5000	75.86 ± 1.47	70.90 ± 1.12
Cl ⁻	500	99.66 ± 1.57	101.78 ± 2.34
	1000	100.95 ± 0.01	102.54 ± 2.13
	5000	98.45 ± 1.23	99.46 ± 1.57
SO ₄ ²⁻	500	100.15 ± 1.34	101.43 ± 2.01
	1000	103.57 ± 1.67	102.55 ± 1.54
	5000	100.70 ± 2.45	101.61 ± 1.89

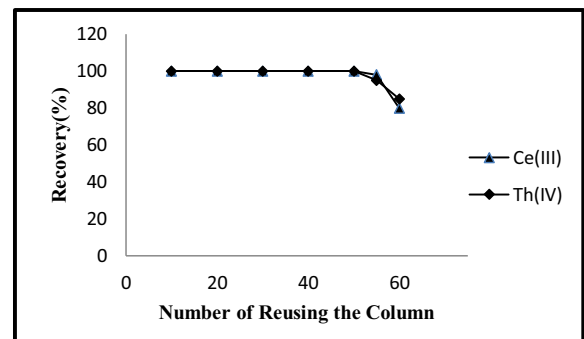


Fig. 5. Effect of the Reuse of the Column, 5.0 μg mL⁻¹ Th(IV) and Ce(III), 50 mL, pH: 7.0, Eluent: 1.0 mol L⁻¹, 5 mL HNO₃, Flow rate sorption: 4.0 and 3.0 mL min⁻¹ for Th(IV) and Ce(III), respectively.

decreased depending on concentration. Our results indicate that the new method shows a good tolerance to matrix interference.

3.6. Influence of column reuse

In evaluating the efficiency of the solid phases used in biosorption studies, regeneration of the biosorbent is one of the significant parameters (Chen et al., 2010). The SPE column stability and potential reusability, including thermophilic *A. flavithermus* immobilized on XAD-16 were defined by observing the change in the uptakes of Th(IV) and Ce(III) ions through many biosorption–desorption cycles. All cycle was evaluated by passing 250 μg 50 mL of Th(IV) and Ce(III) through the SPE column and then eluting the tested metal ions by appropriate eluent. The experimental results showed that thermophilic *A. flavithermus* immobilized on Amberlite XAD-16 can be used maximum 55 times without a decrease in the recoveries of Th(IV) and Ce(III), respectively (Fig. 5).

3.7. The capacity of the thermophilic bacteria loaded resin

The breakthrough curve plot of a method was used to calculate the biosorption capacity of the thermophilic *A. flavithermus* immobilized on XAD-16 (Bag et al., 1998). A 0.25 g of thermophilic *A. flavithermus* loaded on resin was packed in an SPE column, and then various amounts (5–40 μg mL⁻¹) of tested metal ions were passed through the SPE column at 1 mL min⁻¹ flow rate and optimum pH. The amount of the studied metal ions in the eluate was evaluated by ICP-OES until resin loaded with thermophilic *A. flavithermus* was saturated. The previously mentioned curve was used to calculate

Table 3
Analysis of the Th(IV) and Ce(III) in Certified Reference Material (IAEA Soil 7).

Analysts	Certified ($\mu\text{g g}^{-1}$)	Found ^a ($\mu\text{g g}^{-1}$)
Th(IV)	7.9	7.6 ± 0.2
Ce(III)	53.6	55.2 ± 2.2

^a Average of 5 determinations with 95% confidence level, $R \pm ts/\sqrt{N}$.

Table 4
Concentrations ($\mu\text{g g}^{-1}$) of the Th(IV) and Ce(III) in Bastnaesite Ore Sample.

Samples	Added ($\mu\text{g g}^{-1}$)	Th(IV) $R \pm ts/\sqrt{N}$	Ce(III) $R \pm ts/\sqrt{N}$
Batnaesite Ore	–	727.0 ± 5.6	5132.0 ± 23.4
	100.0	825.8 ± 6.3	5230.7 ± 25.7
	200.0	930.2 ± 7.5	5325.4 ± 26.6

$N = 5$, Initial sample volume 500.0 mL, final volume 5 mL for Th(IV) and Ce(III).

Table 5
Analytical Figures of Merits.

Parameters	Th(IV)	Ce(III)
Linear Range ($\mu\text{g mL}^{-1}$) ^a	0.25–2.00	0.25–2.00
Calibration equations (y) ^a	604670X + 12708	123850X + 16976
Correlation Coefficient (R^2) ^a	0.9994	0.9945
LOD ($\mu\text{g mL}^{-1}$) ^a	0.0141	0.0195
LOQ ($\mu\text{g mL}^{-1}$) ^a	0.0470	0.0652
Linear Range ($\mu\text{g mL}^{-1}$)	0.005–0.04	0.005–0.04
Calibration equations (y)	1628X + 5127	8019X + 3973
Correlation Coefficient (R^2)	0.9918	0.9913
LOD ($\mu\text{g L}^{-1}$)	0.1335	0.1597
LOQ ($\mu\text{g L}^{-1}$)	0.4442	0.5325

^a For ICP-OES.

the biosorption capacity of the resin for Th(IV) and Ce(III). The biosorption capacity was determined as 28.40 and 20.82 mg g^{-1} , respectively.

3.8. Determination of Th(IV) and Ce(III) in real samples and accuracy of the method

For validation, methods which were proposed for Th(IV) and Ce(III) were applied to IAEA Soil-7 (CRM) according to the mentioned procedure in Section 2.6. The handled results showed that there is a good relation between the observed certified amounts (Table 3). The bastnaesite ore sample dissolved by recommended process in Section (2.7.) was also studied by the developed biosorption process. Then, the Th(IV) and Ce(III) concentrations in eluent was determined with the ICP-OES. Besides that, the standard addition process was also used for verification of proposed process. The handled results were showed in Table 4.

3.9. Analytical figures of merits

The linear concentration curve was drawn in the range of 0.25–2.00 $\mu\text{g mL}^{-1}$. The lowest concentration determined was read ten times and the corresponding intensity values were determined and the standard deviation was calculated. The detection limit (LOD) value for ICP-OES was calculated to be three times the standard deviation and the limit of quantification (LOQ) value ten times that of the standard deviation. 0.50141 and 0.0195 $\mu\text{g mL}^{-1}$ values was calculated for LOD (3 s, n = 10) and LOQ (10 s, n = 10), respectively.

The concentration ranges for the method were determined (0.25–2.00 $\mu\text{g mL}^{-1}$) by dividing by the enrichment factor and the linear concentration curve was drawn. Calibration equation was given in Table 5. The determined concentration was passed through the column and the lower concentration value was read ten times and the standard deviation was calculated. The detection limit

(LOD) and the limit of quantification (LOQ) values for the method were determined as described above. The LOD (3 s, n = 10) and LOQ (3 s, n = 10) for Th(IV) and Ce(III) found to be 0.135, 0.442 $\mu\text{g L}^{-1}$ and 0.1597, 0.5325 $\mu\text{g L}^{-1}$, respectively.

4. Conclusion

A new method was developed by using thermophilic *A. flavithermus* immobilized on Amberlite XAD-16 as a solid phase extractant for the pre-concentration of Th(IV) and Ce(III). The proposed method is simple, has a short analysis time, decreases contamination risk and its analytical cost for consumed reagent lower. The recoveries of Th(IV) and Ce(III) were highly quantitative ($\geq 98\%$). It was determined that the loading capacity after 55 biosorption and elution cycles did not change more than 2.0% for Th(IV) and Ce(III). High preconcentration factors (100 for each metal) were obtained. Moreover, the analytes may be directly pre-concentrated so there is no need to use any chelating or complexing agent, which is the most important advantage of the suggested method. As a result, the metal contents at very low concentration ($\mu\text{g L}^{-1}$) should be defined easily by this new process.

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