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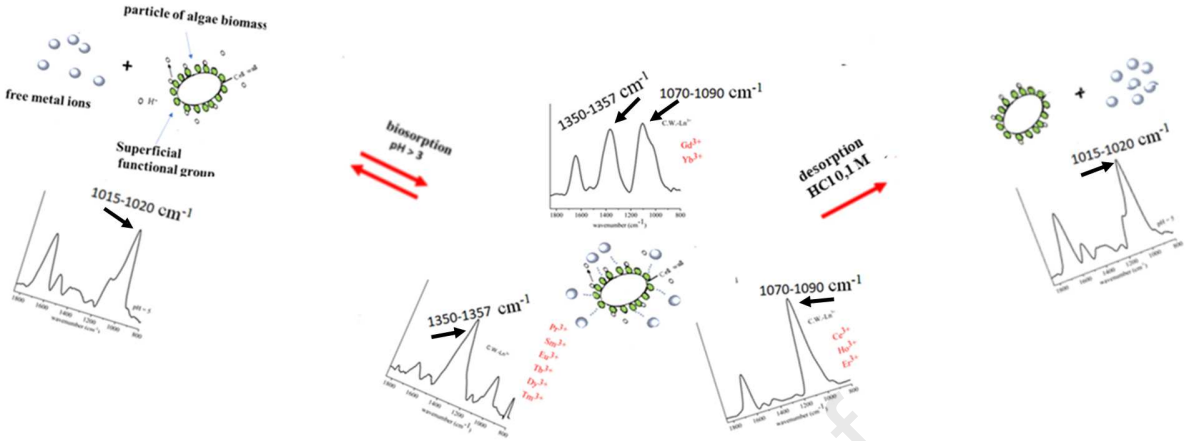

Selective biosorption of Lanthanides onto *Galdieria sulphuraria*

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Abstract

The recovering of trivalent Lanthanides from aqueous solutions, by biosorption process onto *Galdieria sulphuraria* lifeless cells, was investigated. Potentiometry, UV-Vis, FTIR-ATR spectroscopy and SEM-EDS analysis were used. All the experiments were performed at 25 °C, in 0.5 M NaCl. Ln^{3+} biosorption is greater in the 5-6 pH range with values ranging from 80 µmol/g to 130 µmol/g (dry weight). The adsorbed Ln^{3+} ions can be recovered at higher acidity (pH<1) and the biosorbent can be reused. Specific molecular interactions between Ln^{3+} ions and the functional groups on *G. sulphuraria* surface were highlighted. Particularly, proteins are involved if Ln^{3+} = Pr^{3+}, Sm^{3+}, Eu^{3+}, Tb^{3+}, Dy^{3+}, Tm^{3+}, while Ce^{3+}, Ho^{3+}, Er^{3+} form bonds with carbohydrates. Finally, both protein and carbohydrate are involved if Gd^{3+} and Yb^{3+}.

A Surface Complexation approach, with a good graphical fitting to potentiometric experimental collected data, was used to describe the biosorption mechanism. This study

could be of great applicative utility for removing of trivalent actinides, from waste aqueous solutions, by biosorption. As well known the lanthanides were used as model to simulate the chemical behaviour of actinides in the same oxidation state.

Keywords: Lanthanides, *G. sulphuraria,* biosorption, Surface Complexation, FTIR-ATR.

1. Introduction

Rare earth elements (REEs) are a group of chemical elements including Lanthanides (14 chemical elements with atomic number 57-71) and chemically similar elements such as scandium and yttrium. REEs and alloys containing them have a wide variety of high-tech applications such as fluorescent dyes and contrast agents in medical diagnostics and treatments, in wind turbines as permanent magnets, electric car batteries, radar systems, and laser crystals (Chakhmouradian and Wall, 2012; Goodenough et al., 2016; Wall, 2014). That is why they are renamed "vitamins of modern industry" ((http://metalpedia.asianmetal.com/metal/rare_earth/application.shtml; Balaram, 2019). The primary sources of REEs are phosphate or carbonate minerals: bastnäsite, monazite, and xenotime and, in near future, the request of yttrium, lanthanum, praseodymium, as well as europium, terbium and dysprosium it may go over the current supply (Balaram, 2019; Kolodynska et al., 2019; Trifuoggi et al., 2017). As well known, the chemical properties of Ln^{3+} are defined by the ionic radius which gradually decreases from lanthanum to lutetium. This makes it difficult to separate them from each other (if held in naturally occurring ores and other mixtures) and very laborious processes are needed. In this context, the main environmental risks during the extraction and processing of REEs are due to radioactivity of radionuclides, such as thorium and uranium, as well as heavy metals (Balassone et al., 2021; Gupta and krishnamurtihy, 2005; Hu et al., 2018; Xie et al., 2014). Biosorption, on

the other hand, is a cost-effective method for removing metal ions from aqueous solutions (Kumar et al. 2018, Lucaci et al.2020). Mechanisms, such as passive adsorption, surface complexation, absorption, precipitation and ion exchange, on surface of biological materials (as algae, bacteria, fungi, plants, yeasts …), determine biosorption. Biosorbent materials must have structural stability, at high acidity, if it is desired to recycle them. The biosorption of metal ions on living cells can be attributed to the interactions with the cell wall (metabolically passive process in few seconds or minutes) and the intracellular ligands (much slower active process involving the transport of metal ions across the cell membrane into the cytoplasm) (Fu and Wang, 2011; Mehta et al., 2016; Michalak et al., 2013). *Galdieria sulphuraria* is a red alga belonging to Cyanidiophyceae, thriving in geothermal sites, at pHs ranging from 0.5 to 5, temperature up to 55°C and in presence of significant amounts of heavy metals, such as arsenic and mercury (Doemel and Brock, 1971). In the last decade, the interest in using *G. sulphuraria* living cells in the bio-uptake of metals grown very rapidly (Fukuda et al., 2018; Iovinella et al., 2022; Ju et al., 2016; Minoda et al., 2015; Sirakov et al., 2021), even supported by the increasingly comprehensive knowledge of their genomes (Ciniglia et al., 2014; Eren et al., 2018).

This work was focused on trivalent Lanthanide ions biosorption. The goal was to investigate and understand the interaction mechanisms between *G. sulphuraria* surface and Ln^{3+}. A recent study on living cells of *G. sulphuraria* highlighted that the biosorption of Yttrium, Cerium, Europium and Terbium increases with the pH (Iovinella et al., 2022). These results have suggested a preliminary investigation on the acid-base properties of *G. sulphuraria*. Particularly, in this work, the acid-base properties of the functional groups on the surface of *G. sulphuraria* were evaluated in water suspension by potentiometry and Fourier Transform Infrared-Attenuated total reflection (FTIR-ATR) spectroscopy. The interaction properties of *G. sulphuraria* with Ce^{3+}, Pr^{3+}, Sm^{3+}, Eu^{3+}, Gd^{3+}, Tb^{3+}, Dy^{3+}, Yb^{3+} have been investigated by means potentiometry, UV-Vis spectroscopy, FTIR-ATR

spectroscopy and Scanning Electron Microscope Energy Dispersive X-ray (SEM-EDS) analysis. All the experiments were performed at 25 °C, in 0.5 M NaCl as ionic medium. Furthermore, the experimental potentiometric data, collected in this work, have been interpreted by using a Surface Complexation Model (SCM) approach, that describes sorption in terms of chemical reactions (similar to aqueous complexation), controlled by thermodynamic, between surface functional groups and dissolved chemical species (Stumm et al., 1970; Huang and Stumm, 1976; Davis and Kent, 1990). SCM, as well known, was used to describe the surface properties and sorption reactions. -

2.Experimental

2.1. Reagents

Solutions were prepared using double-distilled water and 0.5 M NaCl (as the ionic medium) (Sigma- Sodium chloride puris, p.a., ACS Reagent C 99.5% (AT)). The acid content was determined by a potentiometric-coulometric titration. HCl solutions (Carlo Erba Hydrochloric acid 37% RPE - for analysis – ISO) were standardized by potentiometric-coulometric titrations. All chemicals were commercial.

2.2. *Metal stock solutions preparation and standardization*

Lanthanide chloride stock solutions were prepared from Ln_2O_3 (Ln^{3+} = Eu^{3+}, Er^{3+}, Dy^{3+}, Ho^{3+}, Yb^{3+}, 99.99% Aldrich Chemical Co.) and HCl acid. The lanthanide content was determined by EDTA titrations (accuracy of 0.2%), using xylenol orange as indicator. Lanthanide chloride stock solutions were also prepared by dilution of Certipur® Single-Element Standards (1000 mg/L) for Inductively Coupled Plasma Spectroscopy (ICP) by MERK.

115 *2.3. Equipment*

116 Potentiometric measurements, at 25.00 ± 0.03 °C, were conducted in an air thermostat,
117 measuring the temperature by means a Pt100 TERSID thermocouple. A Hewlett-Packard
118 (HP) instrumentation was used: an automatic potentiometric data acquisition system and
119 a HP DC power supply (to perform coulometric titrations). Emf measurements (precision
120 ±0.03 mV) of the cell (I)

121 $$G.E. \mid TS \mid R.E. \qquad (I)$$

122 were carried out by using operational amplifiers. The glass electrodes reversible to protons
123 (*G.E.*) were supplied by Metrohm. A reference electrode (R.E.), 0.5 M
124 NaCl/Hg_2Cl_2/Hg(Pt), placed outside, was electrically connected to the test solution (TS)
125 by means a Wilhelm-type salt bridge. A calibrated resistance coil was connected in series
126 to the coulometric device (II) to measure the potential drop and thus calculate the current
127 intensity.

128

129 $$(-)\ Pt \mid TS \mid A.E.\ (+) \qquad (II)$$

130 constant current source

131

132 An external auxiliary electrode (A.E.) is a 0.5 M NaCl/$HgO_{(s)}$/$Hg_{(l)}$(Pt), placed outside,
133 was electrically connected to the test solution (TS) by means a Wilhelm-type salt bridge.
134 Pt is a platinum electrode.
135 A combined glass electrode (by Metrohm), reversible to free proton, was also used.
136 FTIR-ATR spectra, in the 4000–700 cm^{-1}range, were recorded on solid phase by Thermo
137 Nicolet 5700 FT/IR Spectrophotometer using a crystal Zinc Selenide cell. Each spectrum
138 was recorded 128 times, with spectral resolution 4 cm^{-1}.

Absorption spectra, with a Varian Cary 50 UV/Vis spectrophotometer, were recorded using a Quartz Cuvette (10 cm Light Path).

Inductively coupled plasma mass (ICP-MS) analysis was carried out by a Bruker Daltonics spectrometer M90 ICP-MS.

SEM-EDS analysis was carried out by a Hitachi TM3000 tabletop Scanning Electron Microscope (SEM), equipped with a 15 kV electron beam and an Oxford Instruments SWIFTED3000 EDS probe. High magnification SEM images (5000x) were also acquired using a Secondary Electron source. Energy Dispersive X-ray (EDS) spectra were acquired with the Aztec Energy® software (data collection time 5 min).

2.4. Microalgal Culture Preincubation

G. sulphuraria strain ACUF 427, collected from the acidic soil of the thermal station in Gunnuhver, Southwest Iceland, was taken from the algal collection of the University of Naples "Federico II" (www.acuf.net) (accessed on 20 June 2022). The microalga was cultivated in Allen medium, acidified with H_2SO_4 at pH 1.5, with the following final composition of macroelements: $(NH_4)_2SO_4$ 24 g/L; K_2HPO_4 12g/L; KH_2PO_4 6g/L; NaCl 2g/L; $MgSO_4(7H_2O)$ 6g/L; $CaCl_2(2H_2O)$ 0,2g/L; $FeSO_4(7H_2O)$ 0,2g/L; microelements (mg/L): 31 H_3BO_3, 1.25 $CuSO_4 \cdot 5H_2O$, 22.3 $MnSO_4 \cdot 4H_2O$, 0.88 $(NH_4)6Mo7O_{24} \cdot 4H_2O$, 2.87 $ZnSO_4 \cdot 7H_2O$, 1.46 $Co(NO_3)_2 \cdot 6H_2O$, 0.014 $V_2O_4(SO_4)3 \cdot 16H2O$, $0.3Na_2NO_4 \cdot 7H_2O$, 1.19 KBr, 0.83 KI, 0.91 $CdCl_2$, 0.78 $NiSO_4$, 0.12 CrO_3, 4.74 $Al_2(SO_4)_3K_2SO_4 \cdot 24H_2O$ (all chemicals were purchased from Sigma Aldrich) in distilled water autoclaved for 20 minutes (Iovinella et al., 2022)

2.5. G. sulphuraria biosorbent powder preparation

G. sulphuraria biosorbent powders, used in all the experiments performed in this work, were obtained as follows: 500 ml of the microalgal culture (optical density = 5) were

centrifuged at 3000g and the resulted pellet was washed twice with ultrapure water at room temperature. After washing, the pellet into a 2 ml tube was transferred and resuspended in 1 ml of acetone (100% v/v); glass beads were added to the suspension and the tubes transferred into a Mixer Mill (MM400, Retsch, Gmbh), to perform cell rupture for 5 minutes at maximum speed. Three cycles of cell rupture were performed. Then, cell debris was suspended in 1 ml 90% DMSO, and homogenized again in Mixer Mill with glass beads. The tubes were then left shaking overnight at 37°C in a thermostable chamber. Samples were then centrifuged, the supernatant discarded, the pellet was dissolved in 1ml 100 % Ethanol; 3 more cycles of cell rupture, by adding glass beads were performed as described above. The final pellet was dried at 37° C.

2.6. *Methodology*

All the experiments have been conducted on *G. sulphuraria* lifeless cells to ensure reproducibility of analytical data.

Acid–base titrations of aqueous suspensions of *G. sulphuraria* powders, in 0.5 M NaCl, at 25.00 ± 0.03 °C, were performed, by monitoring the emf of the potentiometric cell (I).

$$G.E. \mid TS \mid R.E. \qquad (I)$$

Eq. (1) describes the Nernst potential, expressed in mV, of the measuring cell (I).

$$Eg = {}^{*}Eg + 59.16 \log h \qquad (1)$$

where $^{*}Eg$ is the cell constant, h is free protons concentration at equilibrium. Activity coefficients are constants in the presence of large concentrations of the medium ions and thus incorporated in the cell constant (Grenthe, I., 2002). The analytical concentration of protons was decreased stepwise, by means the auxiliary circuit (II):

$$(-)\ Pt \mid TS \mid A.E.\ (+) \qquad (II)$$

constant current source

Pt is a platinum electrode for the reduction of H^+:

$$H^+ + e^- = \frac{1}{2} H_2(g) \qquad (2)$$

The coulometric technique allows for the addition of a precise number of micromoles of electrons without introducing protolitic impurities.

The microfaradays generated by the circuit (II), Eq. (3), after each supply of current of intensity *i*(A), for a time *t*(s), are numerically equal to the number of micromoles of H^+ reduced on the Pt catode.

$$\mu F = \frac{i \cdot t}{0.096487} \qquad (3)$$

The equilibrium was assumed to be reached when the potential *E*g of cell (I) did not change more than ±0.03 mV for at least 15 minutes. A potentiometric-coulometric titration of ionic medium was performed to calculate **E*g, the potentiometric cell constant, as described in the literature (Gran, 1952; Manfredi et al., 2020).

G. sulphuraria suspensions were prepared by resuspending a known amount of biosorbent powders (dry weight ranged from 0.0200 ± 0.0001 g to 0.1200 ± 0.0001 g) in 50.00 ± 0.05 cm^3 of 0.5 M NaCl ionic medium. The suspensions were coulometrically titrated by means the circuit (II).

The pH range investigated went from 2.5 to 9.

Acid-base potentiometric titrations of *G. sulphuraria* -Pr^{3+} suspensions, in 0.5 M NaCl, at 25.00 ± 0.03 °C, were also performed, by monitoring the emf of the potentiometric cell (I). The composition of suspensions was as follows: V = 50.00 ± 0.05 cm^3, $[Pr^{3+}] = 4 \cdot 10^{-4}$ M, 0.5 M NaCl, *G. sulphuraria* 0,1150 ±0.0001 g (dry weight). The reversibility was ensured throughout the pH range 3 - 7 investigated. The lower limit of acidity was chosen

to avoid the precipitation of scarcely soluble oxide of metal ions (Baes and Mesmer, 1977; Biedermann and Sillen, 1953; Ferri et al., 2002; Vasca et al., 2004).

Batch biosorption experiments were carried out at pH ≈5.5. The suspensions were obtained by adding 0.0600 ± 0.0001 g (dry weight) of *G. sulphuraria* biosorbent into 20.00 ± 0.05 cm^3 of solution with composition: 0.001 M Ln^{3+}, 0.5 M NaCl. Suspensions were shaken for 3 hours to achieve adsorption equilibrium, confirmed by the pH stability (±0.01 logarithm unit). Lanthanide concentrations, at equilibrium with metal ion biosorpted onto *G. sulphuraria* surface, were evaluated in supernatant by UV-Vis (for Pr^{3+}, Sm^{3+}, Ho^{3+}, Er^{3+}) spectrophotometric measurements, or ICP-MS, using the external calibration (or standard addition) method.

Desorption experiments, as function of pH, were also conducted. The recovery of Ln^{3+} from *G. sulphuraria* -Ln^{3+} samples were resuspended in a known volume (5-10 cm^3) of HCl. The pH investigated goes from 0.5 to 2. Suspensions were shaken for 2 hours. The amount of metal ions recovered was evaluate by ICP-MS analysis of supernatant.

ATR-FTIR spectra of dried *G. sulphuraria* suspensions, as a function of pH, were recorded. *G. sulphuraria* suspensions were prepared by resuspending a known amount of biosorbent (0.0050 ± 0.0001 g dry weight) in 2 cm^3 of 0.5 M NaCl ionic medium at various pH values (pH ranging from 0.5 to 12) for 3 hours. In order to measure the pH, a calibrated combined glass electrode, reversible to free proton, was used. *G. sulphuraria* samples were collected in the form of wet pastes (after centrifugation and remotion of supernatant) and dried at room temperature. ATR-FTIR- spectra of dried *G. sulphuraria* -Ln^{3+} (Ce^{3+}, Pr^{3+}, Eu^{3+}, Gd^{3+}, Dy^{3+}, Ho^{3+}, Er^{3+}, Tm^{3+}, Yb^{3+}) suspensions, obtained by using a similar procedure, were recorded. Particularly, suspensions were prepared by resuspending a known amount of biosorbent (0.0050 ± 0.0001 g dry weight) in 2 cm^3 of Lantanide solution (0.0008 M Ln^{3+}, 0.5 M NaCl). All the experiments were repeated 3 times.

SEM-EDS analysis were performed by depositing the dried *G. sulphuraria*-Ln^{3+} suspensions (Ln^{3+} = Pr^{3+}, Er^{3+}, Tm^{3+}) on a conductive carbon tape placed on a pin stub. Afterwards, the stubs were sputtered with gold with a Quorum Technologies K650X sputter coater machine (intensity current: 70mA; sputtering time: 750 s; vacuum: $1 \cdot 10^{-2}$ mbar). High magnification SEM images (5000x) were also acquired using a Secondary Electron source. Images were analysed through ImageJ® program, measuring the size and the ratio between two orthogonal dimensions of the particle (for at least three particles).

EDS spectra were acquired with a magnification of 100x, by the Aztec Energy software (data collection time: 300 s).

3. **Results and discussions**

3.1 Acid-base properties of G. sulphuraria in 0.5 M NaCl

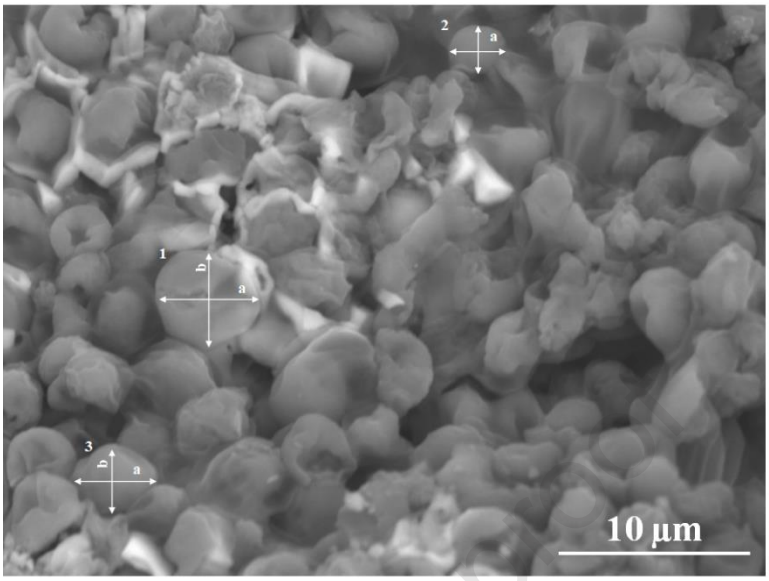


Figure 1: SEM image (magnification 5000x) of dried *G. sulphuraria* suspensions in 0.5 M NaCl, pH≈5.

Figure 1 shows SEM image, with magnification 5000x, of dried *G. sulphuraria* suspensions (in 0.5 MNaCl, pH≈5): the size and the ratio between two orthogonal dimensions of the particle (measured for at least three particles) was ≈ 3µm and 1, respectively.

The number of discrete surface binding sites on the *G. sulphuraria*, the site concentrations and their conditional pKa (valid in 0.5 M NaCl as ionic medium) were evaluated in water suspensions, through potentiometric–coulometric titrations. Primary pH(µF) data of Figure 2a show that the number of weak acid sites increased with the extent of biomass (the number of micromoles of electrons increases with increasing the biosorbent mass). A good fit of experimental data (dashed lines in Figure 2a) was obtained by assuming two protolitic reactions, Eqs. (4) and (5)

$$> R_1H + H_2O \rightleftarrows \ > R_1^- + H_3O^+ \qquad (4)$$

$$> R_2H + H_2O \rightleftarrows \ > R_2^- + H_3O^+ \qquad (5)$$

where R_1 and R_2 indicate two distinct types of acidic groups.

Acidity constants, valid in 0.5 M NaCl, and site concentrations (expressed in mmol/g), calculated by numerical LETAGROP program (Sillen and Warnqvist, 1969), are reported in Table 1.

Table 1: Summary of the equilibrium constants, valid in 0.5 M NaCl, obtained in the present work, for the *G. sulphuraria*. R_1 and R_2 indicate two distinct types of acidic groups on surface. The uncertainties (indicated in parenthesis) are to be intended as 3 σ.

Sites number (mmol/g, dry weight)	reaction	pKa
0.38(2)	$> R_1H + H_2O \rightleftarrows\ > R_1^- + H_3O^+$	4.6(1)
0.43(3)	$> R_2H + H_2O \rightleftarrows\ > R_2^- + H_3O^+$	7.0(2)

FTIR-ATR spectra of dried *G. sulphuraria* suspensions (in 0.5 M NaCl), as a function of pH, were recorded. An enlargement of the infrared absorbance spectra, in the 1850–800 cm^{-1} region, is reported in Figure 2b. FTIR peak assignments were based on spectral values of literature (Barone et al., 2020). Particularly, the most relevant assignments were as follows: protein Amide I band (mainly ϑ(C=O) stretching, 1640 cm^{-1}); protein Amide II band (mainly δ(N-H) bending and ν(C-N) stretching, 1478-1575 cm^{-1}); proteins (δ(CH_2) and δ(CH_3) bending of methyl; ϑ(C-O) of COO- groups, δ($N(CH_3)_3$) bending of methyl, 1380-1320 cm^{-1}); phosphodiesters (>P=O stretching at 1230-1240 cm^{-1}); carbohydrates (ϑ(C-O-C)) in the region 1015-1020 cm^{-1}.

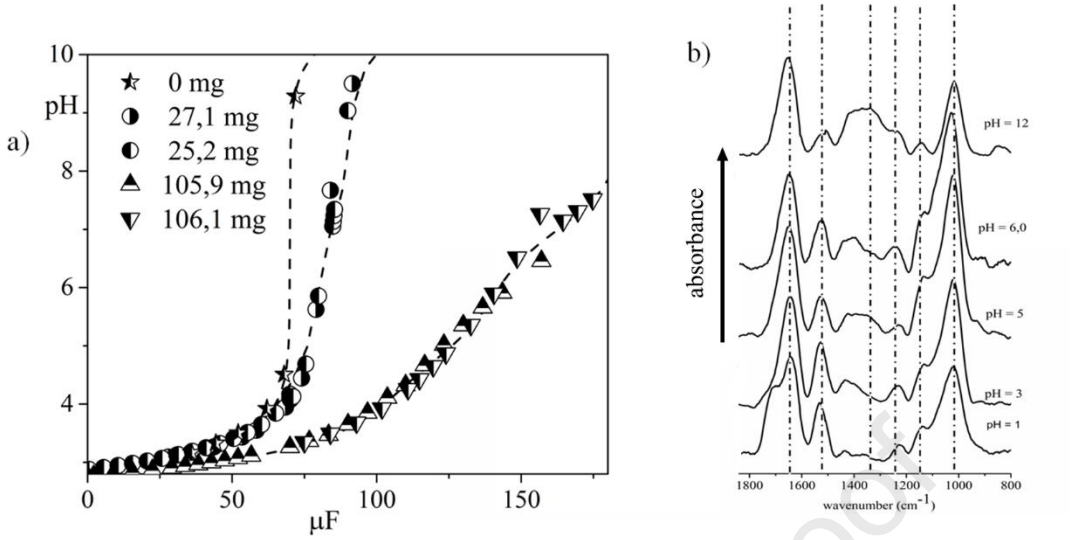


Figure 2: a) pH(μF) experimental data (symbols) collected and fitting curves (dashed lines) obtained by using acid constants of Table 2, for the *G. sulphuraria* suspensions; b) ATR infrared absorbance spectra, in the 1850-800 cm^{-1}region, of dried *G. sulphuraria* suspensions at different pH values.

The characterization of the acid-base properties of the *G. sulphuraria* was preliminary to investigation on her Lanthanides adsorptive ability.

3.2 *Lanthanides biosorption onto G. sulphuraria*

3.2.1 *FTIR-ATR and SEM-EDS characterization*

FTIR-ATR spectra were also recorded to highlight spectral features of *G. sulphuraria* surface after contact with lanthanides solutions.

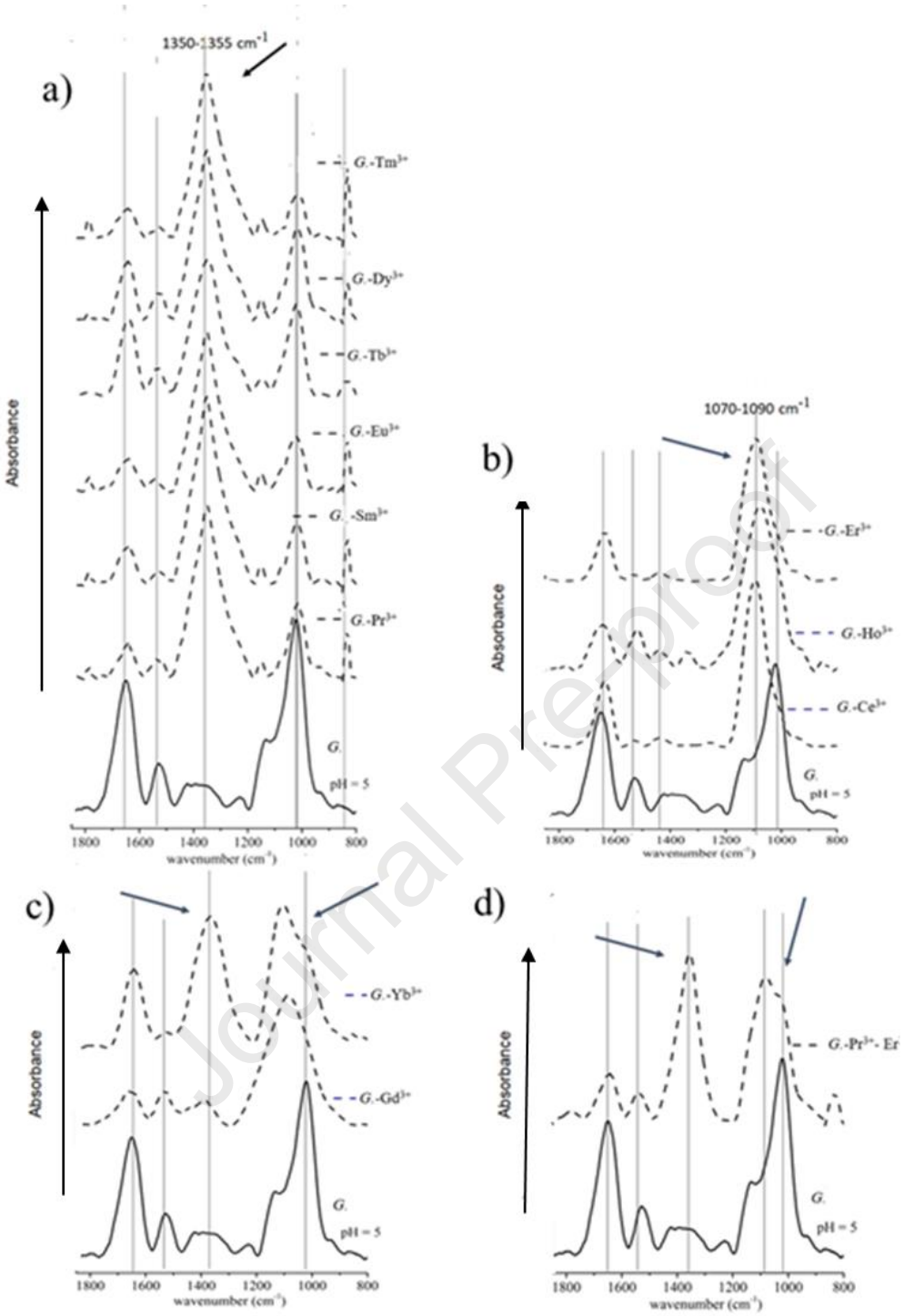


Figure 3: ATR infrared absorbance spectra, in the 1850 -800 cm^{-1}region, of dried *G. sulphuraria* (lines) and *G. sulphuraria* -Ln^{3+} (dashed lines) suspensions at pH ≈ 5. Changes in position and intensity of specific bands (1020 cm^{-1} and 1350 cm^{-1}) are highlighted by arrows.

In Figure 3 experimental FTIR-ATR spectra, in 1850–800 cm^{-1} region, of dried *G. sulphuraria* and *G. sulphuraria*-Ln^{3+} suspensions (pH ≈5), are compared. Remarkable changes in position and intensity of specific bands, in response to equimolar Ln^{3+}

solutions (Ln^{3+}= Ce^{3+}, Pr^{3+}, Sm^{3+}, Eu^{3+}, Gd^{3+}, Tb^{3+}, Dy^{3+}, Ho^{3+}, Er^{3+}, Tm^{3+}, Yb^{3+}), were detected. More specifically, changes were remarked in the 1000 -1100 and 1340 -1360 cm^{-1} regions: the band at 1020 cm^{-1} (assigned to the C-O-C bending of carbohydrates and/or polysaccharides) was shifted to a higher wavenumber, thus highlighting interactions of the functional group with metal ion, if Ln^{3+} = Ce^{3+}, Ho^{3+}, Er^{3+}. In this context, it is reported in literature that Er^{3+} complexes with carbohydrates. Moreover, the extent of the displacement and the intensity of the band also seem to depend on the amount of metal bound to the surface, as well as on the strength of complexation bond (Yang et al., 2003). A sharp increase in the peak intensity at 1340-1360 cm^{-1} region (assigned to protein), if Ln^{3+} = Pr^{3+}, Sm^{3+}, Eu^{3+}, Tb^{3+}, Dy^{3+}, Tm^{3+}, was also detected. Finally, both regions of the spectrum appear modified if Ln^{3+} = Gd^{3+} or Yb^{3+} highlighting for the two lanthanides the ability to interact with both protein and carbohydrates.

Figure 3d shows the experimental spectrum of dried *G. sulphuraria*- Er^{3+}-Pr^{3+} suspensions (mixture with $[Pr^{3+}]/[Er^{3+}]$ = 1): two bands, at 1090 e 1355 cm^{-1}, were detected, respectively. ATR-FTIR measurements highlight the suitability of functional groups on *G. sulphuraria* surface to selectively sorption of Lanthanides ions.

SEM-EDS analysis of dried *G. sulphuraria* - Ln^{3+} suspensions (pH ≈ 5, Ln^{3+} = Pr^{3+}, Tb^{3+}, Er^{3+}) were performed. Figure 4 shows high magnification SEM images (left) and related EDS spectra (right). For the latter, the arrows highlight the characteristic peaks of the lanthanides, whose ionization energy values are also reported to highlight their presence, as also demonstrated by the previous tests. The results suggested that the solid particles, after contact with Ln^{3+}, preserved their hollow aspect, as for the ones barely dispersed in the NaCl medium, and it was not highlighted a significant variation in the aspect ratio.

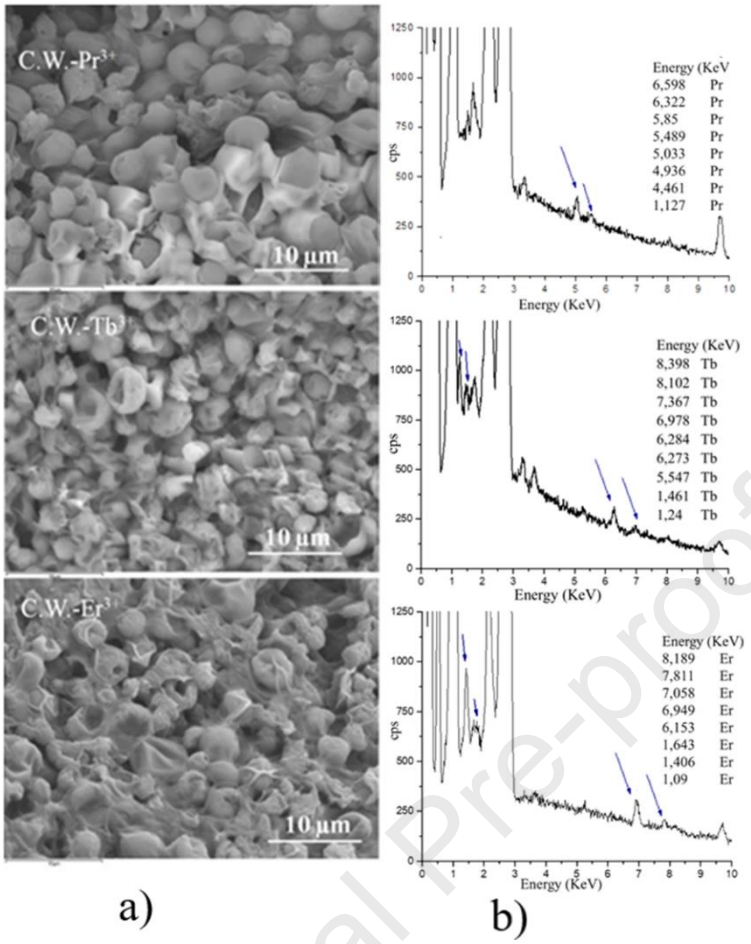


Figure 4: a) High magnification SEM images (magnification 5000x) of dried *G. sulphuraria* - Ln^{3+} suspensions, in 0.5 M NaCl (pH ≈ 5, Ln^{3+} = Pr^{3+}, Tb^{3+}, Er^{3+}). b) EDS spectra (magnification 100x): arrows show the characteristic peaks of the lanthanides, whose ionization energy values are also showed.

3.2.2 *Biosorption experiments*

Batch biosorption experiments were performed. The amount of Ln^{3+} on the *G. sulphuraria* surface, *q*, expressed as µmol/g (dry weight), was evaluated from the difference between analytical (total) concentration of metal ion, *B*, and the concentration of free ion, *b,* at equilibrium with metal ion onto *G. sulphuraria* surface, as follows:

$$q = \frac{V(B-b)}{m} \qquad (6)$$

where V is the volume of the suspension, m is the mass (dry weight) of *G. sulphuraria*, respectively. The concentrations of free metal ions, b, were evaluated by UV-Vis spectrophotometry (if Ln^{3+}= Pr^{3+}, Sm^{3+}, Ho^{3+}, Er^{3+}) or ICP-MS using the external calibration (or standard addition) method. The UV-Vis spectra recorded in this work and Beer-Lambert's plot (Absorbance *vs* concentrations (M)) for Praseodymium, Samarium, Holmium, Erbium chloride stock solutions are show in the Figure 5.

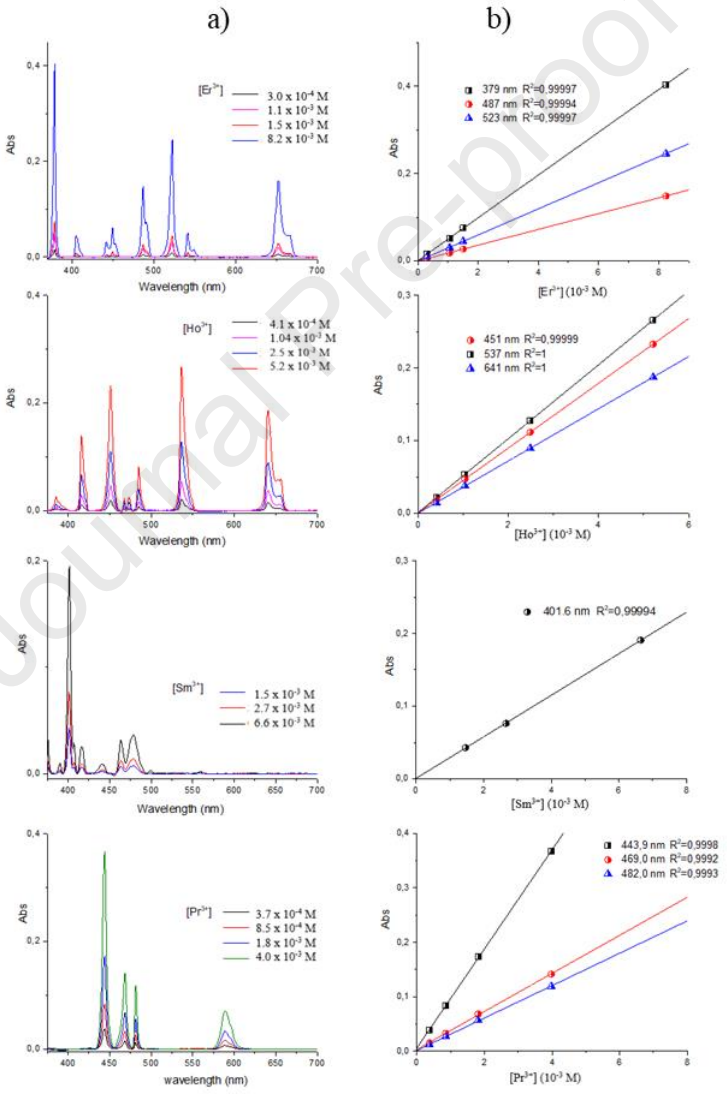


Figure 5: a) Absorbance vs wavelength (nm) and b) Beer-Lambert's plot (Absorbance vs concentrations) for Praseodymium, Samarium, Holmium, Erbium chloride stock solutions.

Table 2: Experimental amount of Ln^{3+} biosorpted on the *G. sulphuraria* surface, q, expressed as μmol/g (dry weight). The uncertainties (indicated in parenthesis) are to be intended as 3 σ.

Pr^{3+} *q* (μmol/g)	Sm^{3+} *q* (μmol/g)	Eu^{3+} *q* (μmol/g)	Gd^{3+} * *q* (μmol/g)	Ho^{3+} *q* (μmol/g)	Er^{3+} *q* (μmol/g)	Tm^{3+} *q* (μmol/g)
140 ± 14 (pH =5)	120 ± 12 (pH =4.5)	140 ± 14 (pH =5)	80 ± 8 (pH =4)	80 ± 8 4<pH <5	80 ± 8 4<pH <5	110 ± 10 (pH =5)
80 ± 8 (pH =3.8)		80 ± 10 (pH =3.8)				

The experimental amount of Ln^{3+} biosorpted on the *G. sulphuraria* surface, *q*, reported in Table 2, highlighted the pH dependency of lanthanides biosorption onto *Galdieria*. Desorption experiments showed the recovery efficiency of more than 90% if pH <1.

Acid-base potentiometric-coulometric titrations of *G. sulphuraria*-Pr^{3+} suspensions, in 0.5 M NaCl, at 25.00 ± 0.03 °C, were performed. The reversibility was ensured throughout the pH range 3-7, investigated. The lower limit of acidity was chosen to avoid the precipitation of scarcely soluble oxide of metal ions (Baes and Mesmer, 1977; Biedermann and Sillen, 1953; Ferri et al., 2002; Vasca et al., 2004). The potentiometric data collected were interpreted by using a SCM approach (Wen et al. 1998; Schindler 1991; Wang and Giammar 2013; Kelly et al. 2002; David Borrok et al. 2004; Borrok and Fein 2004).

In the Figure 6a, the experimental data (pH/μF), collected during titrations of *G. sulphuraria* and *G. sulphuraria*-Pr^{3+} suspensions, respectively, are compared. The potentiometric data have been interpreted by assuming complexation reaction of Eq. (7), with conditional equilibrium constant of Eq. (8). A good fit of the experimental data (dashed curves in Figure 6a) was obtained.

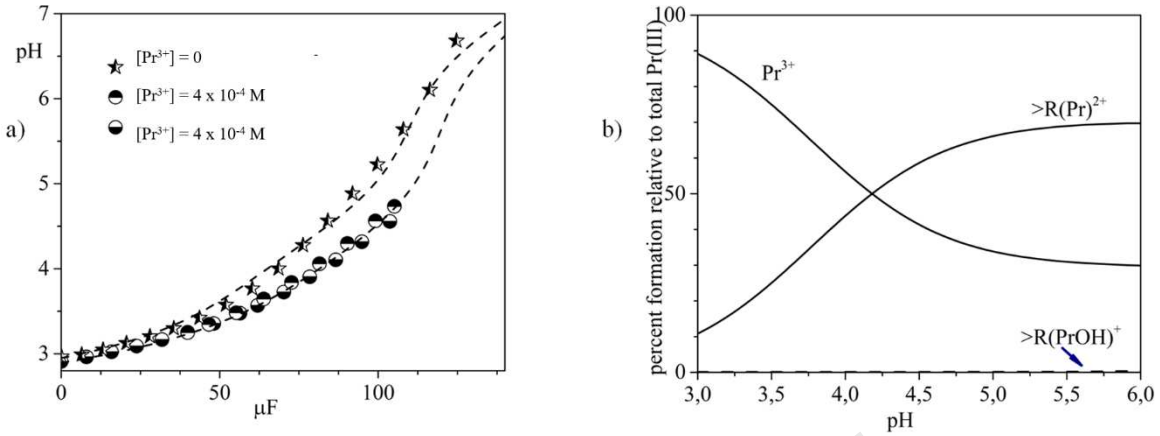


Figure 6: a) pH(μF) experimental data (symbols) and fitting curves (dashed lines) collected for *G. sulphuraria* and *G. sulphuraria*-Pr^{3+} suspensions; b) Equilibrium distribution diagram of G. sulphuraria-Pr^{3+} system, in 0.5 M NaCl, constructed by equilibrium constants of Table 3.

$$Pr^{3+} + > RH \rightleftarrows > R(Pr)^{2+} + H^{+} \quad (7)$$

$$\beta = \frac{[> \mathrm{R(Pr)^{2+}}]\cdot[H^{+}]}{[>RH]\cdot[Pr^{3+}]} \quad (8)$$

The brackets in the Eq. (8) represent the concentrations of the species in chemical equilibrium expressed in molarity. The equilibrium constants, β, calculated by numerical LETAGROP program (Sillen and Warnqvist, 1969), valid in 0.5 M NaCl, are reported in Table 3 and were used to construct the equilibrium distribution diagram (Hyperquad Simulation and Speciation, HySS 2009, program (Gans et al. 1996) of Figure 6b: the surface complexation with Pr^{3+} depends to pH and it appears remarkable at pH > 4.

Table 3 Summary of the equilibrium constants, valid in 0.5 M NaCl, obtained in the present work, for the *G. sulphuraria*-Pr^{3+} system. The uncertainties (indicated in parenthesis) are to be intended as 3 σ.

Reaction	-Log β
$> R_1H + H_2O \rightleftarrows > R_1^- + H_3O^+$	4.55(1)
$> R_2H + H_2O \rightleftarrows > R_2^- + H_3O^+$	6.9(3)
$Pr^{3+} + > R_1H \rightleftarrows > R_1(Pr)^{2+} + H^+$	0.90(3)

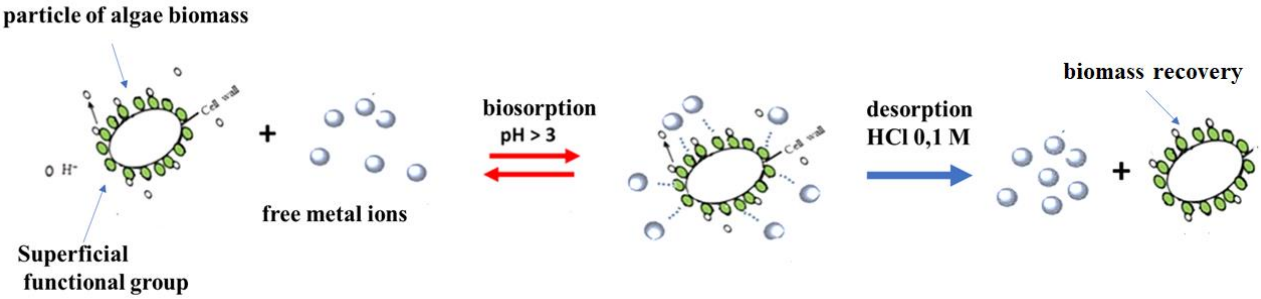
This paper describes a study concerning Ln^{3+} biosorption onto *G. sulphuraria*. A preliminary study to biosorption was the characterization of the acid-base properties of functional groups on *Galdieria* surface. As regards the biosorption, 11 trivalent lanthanides ions (Ce^{3+}, Pr^{3+}, Sm^{3+}, Eu^{3+}, Gd^{3+}, Tb^{3+}, Dy^{3+}, Yb^{3+}) have been considered. Furthermore, the desorption efficiency and the recovering the biosorbent to reuse it, have been also investigated. The study has been performed on *G. sulphuraria* lifeless cells to ensure a reproducibility of analytical results.

SEM-EDS, FTIR-ATR spectrometry, UV-Vis spectrophotometry and potentiometry techniques have been used. A pH-dependent biosorption behaviour, based on surface equilibrium reactions (complexation reactions of lanthanides by functional groups on *Galdieria* surface), with greater adsorption in the 5<pH<6 range, was highlighted.

The lanthanides adsorbed can be easily recovered (90% removal rate) from acid solutions at pH <1, and the biosorbent can be reused (not possible if *Galdieria* living cells are used).

For the Ln^{3+} biosorption-desorption onto *G. sulphuraria* surface, a mechanism based on a surface complexation model, was suggested, as also illustrated in Scheme 1.

Scheme 1: Mechanism for the Ln^{3+} biosorption-desorption on the *G. sulphuraria* surface.



The study suggests that *G. sulphuraria* could be used for the removing of trivalent actinides from aqueous solutions. The Ln^{3+} ions are used as models for trivalent actinides,

similar chemical elements to lanthanides (Baes and Mesmer, 1977; Biedermann and Sillen, 1953; Ferri et al., 2002; Vasca et al., 2004), but too radioactive to be investigated directly.

4. Conclusions

This paper describes a study concerned the use of *G. sulphuraria* lifeless cells as a biosorbent for recovering Ln^{3+} from aqueous solutions. Particularly, the interaction ability of *G. sulphuraria* with Ce^{3+}, Pr^{3+}, Sm^{3+}, Eu^{3+}, Gd^{3+}, Tb^{3+}, Dy^{3+}, Yb^{3+}, was investigated. Biosorption is a cost-effective method for removing metal ions from aqueous solutions and if biosorbent material have structural stability at higher acidity, the latter can be also recycled. In this scenario, *G. sulphuraria*, a red alga thriving in geothermal sites with peculiar ecological conditions (*i.e.*, low pH (0.5-3.0), T≈50°C-55°C), is one of the best candidates for the biological recovery of metals.

G. sulphuraria lifeless cells constitute a good biosorbent to removing Ln^{3+} from aqueous solutions: a greater biosorption in the pH range $5 < pH < 6$ can be gained (ranging from 80 µmol/g to 130 µmol/g); the adsorbed Ln^{3+} ions can be recovered at $pH < 1$ and, thus, the biosorbent can be reused.

The study highlighted, also, that the lanthanides biosorption onto *Galdieria* involves specific interactions between Ln^{3+} and functional groups onto surface: praseodymium, samarium, europium, dysprosium, terbium and thulium form bonds with proteins groups; cerium, erbium, and holmium interact with carbohydrates groups. This specificity in the mechanism of Ln^{3+} biosorption onto *G. sulphuraria* (not described in literature) is surprising considering the systematic and stepwise variation of the chemical and physical properties of Lanthanides along the series, making difficult their separation. Furthermore, this study could be of great applicative utility for the trivalent actinides biosorption from

waste aqueous solutions. Trivalent lanthanides are used to simulate the trivalent actinides. In fact the chemical behaviour of actinides, that are strongly radioactive, is similar to that of lanthanides in the same oxidation state.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper. **Author contributions statement**

Conceptualization: C.M., C.C; Methodology: C.M. Data curation: C.M., A.EH; Resources: M.T., M.I, S.J.D, M.P., C.C, C.L.; Investigation: A.J.A, A. E.H; Formal analysis: C.M; Writing - Original Draft: C.M C.C

All authors have read and agreed to the published version of the paper

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Highlights

Lanthanides biosorption onto *G. sulphuraria* greater at pH > 4.

Specific interactions Lanthanides- functional groups onto *G. sulphuraria* surface.

Lanthanides biosorption mechanism onto *G. sulphuraria* explained by a Surface Complexation Model.

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: