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Influence of biosurfactants in the recovery of REE from monazite using *Burkholderia thailandensis*

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ABSTRACT

The demand of rare earth elements (REE) has grown over the past decades due to their importance in high technology devices such as wind turbines, superconductors, rechargeable batteries, autocatalytic converters, magnets, or LED lighting. The development of clean mining processes is gaining interest and the biomining of REE is mainly focused on monazite using phosphate solubilizing microorganisms. The members of the genus *Burkholderia* can dissolve phosphorous from inorganic rocks. Furthermore, several species of *Burkholderia* are able to produce biosurfactants named rhamnolipids. Nevertheless, rhamnolipid interactions with REE have been poorly investigated.

The aim of the present work is the study of the solubilization of monazite and the recovery of REE using the bacterium *Burkholderia thailandensis*, and the influence of the rhamnolipids produced by the bacteria in the REE mobilization. *B. thailandensis* grown in nutrient broth with 1% monazite (*w/v*) reached 8.3 mg·l^{-1} REE after 15 days.

To produce rhamnolipids, *B. thailandensis* was grown in medium supplemented with 10% glycerol and the biosurfactants were extracted. The critical micelle concentration (CMC) was determined: 94.45 mg·l^{-1} for commercial rhamnolipids and 60.41 mg·l^{-1} for purified rhamnolipids. The maximum REE solubilization was obtained at CMC reaching 9.36 mg·l^{-1} with commercial rhamnolipids and 5.13 mg·l^{-1} with rhamnolipids produced by *B. thailandensis* E264.

1. Introduction

The relevance and demand of rare earth elements (REE) have grown over the past four decades because of their extensive use in several fields, such as electronics, renewable energy capture technologies, biomedical devices and other industries (Goodenough et al., 2018). Most of the rare earths are common elements, although they are sparsely concentrated in mineral deposits and this fact hampers their extractive metallurgy. REE resources are mostly present in oxidic form and only three REE ores are considered for economic extraction: bastnaesite (REE-CO_3F), monazite (light REE-PO_4) and xenotime (heavy REE-PO_4). REE primary ores are conventionally leached using concentrated acidic and/or alkaline reagents at high temperature (Peelman et al., 2016).

Biohydrometallurgy is generally considered as an environmentally friendly technology to recover some valuable metals using microorganisms reducing at the same time the toxicity of the waste materials and the energy costs (Kaksonen et al., 2020; Vera et al., 2022). Bioleaching has been commercially applied to obtain metals from low-grade sulfide ores and in the pre-treatment of sulfide refractory gold ores. Nowadays, the potential of biohydrometallurgy to extract other raw materials, such as rare earth elements, is being investigated. The biomining of REE is mainly focused in monazite using phosphate solubilizing microorganisms able to dissolve phosphorous from inorganic rocks (Fathollahzadeh et al., 2018a). Phosphate solubilizing microorganisms have been previously used as biofertilizers in agriculture; nevertheless, there are few works related to the extraction of valuable metals from minerals. Several bacteria, such as *Enterobacter aerogenes*, *Pantoea agglomerans* and *Pseudomonas putida*, play an important role key role in the release of phosphorous, REE, iron and thorium by the production of organic acids and microbial processes (Corbett et al., 2017). Not only heterotrophic but also autotrophic bacteria has been used in the release of REE from monazite (Fathollahzadeh et al., 2019). For instance, *Acidithiobacillus ferrooxidans* has been demonstrated to leach REE. Furthermore, other phosphate solubilizing microorganisms such as

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the fungus *Aspergillus niger* were capable to solubilize REE from monazite due to the production of organic acids and simultaneous advancement in redox potential (Castro et al., 2020; Keekan et al., 2017).

In regard to solubilization of phosphorus from inorganic rocks, the genus *Burkholderia* has demonstrated to grow in presence of phosphate minerals converting different forms of insoluble P to its soluble form (Delvasto et al., 2009; Delvasto et al., 2008). Furthermore, a number of species of *Burkholderia* are able to produce rhamnolipids (Díaz De Rienzo et al., 2016; Hörmann et al., 2010; Wittgens et al., 2018). Rhamnolipids present effective tensioactive properties and low toxicity and they are stable within a wide range of temperatures and pH values. In consequence, these molecules are attracting interest from industry because of their structural diversity and potential for use in areas such as enhanced oil recovery, environmental bioremediation, food processing and pharmaceuticals (Chong and Li, 2017; Gudiña et al., 2015). Limited research has been done on rhamnolipid interactions with REE; however, recent studies indicated that monorhamnolipids selectively complex REE with potential application on the recovery of these critical metals (Hogan et al., 2017; Zhou et al., 2017). Other biosurfactants such as tea saponin have been used to recover light/medium/heavy REE (La, Dy and Er) from contaminated soils (Li et al., 2020).

The main objective of the present work is to characterize the solubilization of inorganic phosphorous and the recovery of rare earth elements from monazite using the bacteria *Burkholderia thailandensis* E264. The importance of rhamnolipids in the bioleaching of REE was studied using a scmR- mutant that overproduces rhamnolipids (ED1023) and adding glycerol to the medium to increase the biosurfactants production. In addition, the rhamnolipids were purified and put in contact with the monazite to examine their ability to bind to REE in abiotic experiments.

2. Materials and methods

2.1. Mineral ore

The REE mineral ore was collected from the Morro dos Seis Lagos deposit (Brazil). The elemental composition of the natural phosphate minerals was determined by X-ray fluorescence chemical analysis. The main elements in the monazite were (% wt.): Al, 16.7; Si, 16.5; Fe, 4.95; S, 4,59; P, 1.42; Ce, 1,23; Nd, 0.69; La, 0.62; Th, 0.54; O, 46.8.

2.2. Bacterial strain and bioleaching experiments

The bioleaching experiments were performed with the wild type strain *B. thailandensis* E264 (University of Salford, UK) and the scmR-mutant strain ED1023 (Armand-Frappier Santé Biotechnologie Research Centre, Institut National De La Recherche Scientifique, Canada). The bioleaching experiments were carried out in 250-ml Erlenmeyer flasks in triplicate. Each flask containing 1% mineral ore (*w/v*) was autoclaved for 30 min at 121 °C. A volume of 100 ml of sterile nutrient broth (Oxoid) was added to each flask and the initial pH of the experiment was pH 5.3 ± 0.1. To grow the strain ED1023, 100 μg·ml^{-1} of the antibiotic kanamycin was added. Flasks were inoculated with aliquots to a concentration of 10^7 cells ml^{-1} and then cultures were incubated aerobically on an orbital shaker at 150 rpm and 30 °C for 21 days. Uninoculated flasks were used as controls. 5 ml was periodically withdrawn during the experiments for further analysis (phosphorous and REE concentrations and pH).

2.3. Rhamnolipid production and extraction

To produce rhamnolipids from *B. thailandensis* E264, 600 ml of culture was grown in nutrient broth supplemented with 10% glycerol at 25 °C during 11 days in the shake flask experiments. The rhamnolipids extraction was based in the method previously developed (Funston et al., 2016). The culture was centrifuged at 10,000 ×*g* for 15 min to remove cells. The supernatant was collected, and the pH was adjusted to 2.0 with concentrated HCl and remained at 4 °C overnight. The supernatant was extracted three times by adding an equal volume of ethyl acetate. The organic phase was collected, dried by adding 0.5 g $MgSO_4$ per 100 ml ethyl acetate, and filtered. The rhamnolipids extract was obtained rotary evaporating the solution in a rotavapor Büchi R-210 with a heating bath Büchi B-491 at 65 °C.

2.4. Determination of critical micelle concentration (CMC) and REE mobilization using rhamnolipids

The CMC of the rhamnolipids (commercial and purified from *B. thailandensis* culture) was determined with a Krüss K10T digital tensiometer, with a platinum plate (20 × 10 × 0.1 mm) and an accuracy of 0.1 mN·m^{-1}, by measuring the surface tension of a concentration series. Solutions were placed in a thermostated flask at 25 °C. Commercial rhamnolipids (90% pure) produced by AGAE Technologies, LLC (USA) were also tested.

Solutions with commercial rhamnolipids and purified rhamnolipids from *B. thailandensis* culture at ½ CMC, CMC and 2CMC were put in contact in flasks containing 1% mineral ore (*w/v*) during one week.

2.5. Analytical methods

The concentration of phosphorous and REE (cerium, lanthanum and neodymium) was evaluated during the bioleaching experiments using inductively coupled plasma optical emission spectrometry (ICP-OES, Perkin Elmer Optima 2100 DV). Samples collected from the biological leaching experiments were centrifuged at 12,000 ×g for 10 min prior to the ICP-OES analysis. Each sample was measured in triplicate with relative standard deviation (RSD) <3%.

The pH of the samples was measured immediately using a Crison Basic 20 pHmeter sensitivity: 98%).

To analyze the presence of organic acids (oxalic, citric and gluconic acids) in the samples, supernatant samples collected were filtered and directly analyzed by high performance liquid chromatography (HPLC) in an Agilent Technologies 1260 Infinity II machine using a Aminex HPX-87H (7.8 × 300 mm, 9 μm, BioRad) column. The organic acids were determined at 210 nm in a diode array type of UV/VIS detector. The solvent used for HPLC analyses was water acidified with 5 mM sulfuric acid. Flow rate was maintained at 0.6 ml/min, and column temperature at 60 °C. Chromatograms generated from samples were compared to that of authentic standards.

2.6. Field Emission Scanning Electron Microscopy (FE-SEM)

B. thailandensis and the solid residues obtained after bioleaching were filtered onto 0.2 μm pore-size polyamide membranes (Sartorius). The samples were successively dehydrated with acetone (30, 50 and 70% acetone) and stored overnight at 4 °C in 90% acetone. The samples were critical-point dried and coated with graphite or gold for 2 min at a Quorum Q150R S equipment. The examination of solid phase specimens and the spatial distribution of bacteria on mineral were performed with a JEOL JSM-6335 F microscope at 20 kV coupled to an electron dispersive spectroscopy (EDS) detector X-Max 80 (Oxford Instruments).

3. Results and discussions

3.1. Bioleaching of REE with B. thailandensis E264

This study was focused on the ability of *B. thailandensis* to dissolve REE from monazite. The microbial leaching was evaluated overtime following the changes in phosphorous and REE concentration and in pH value (Fig. 1). The measured values in abiotic experiments remained practically unchanged in the evaluated conditions. Instead, the bacterial growth of the wild type strain (E264) in presence of monazite ore led to a

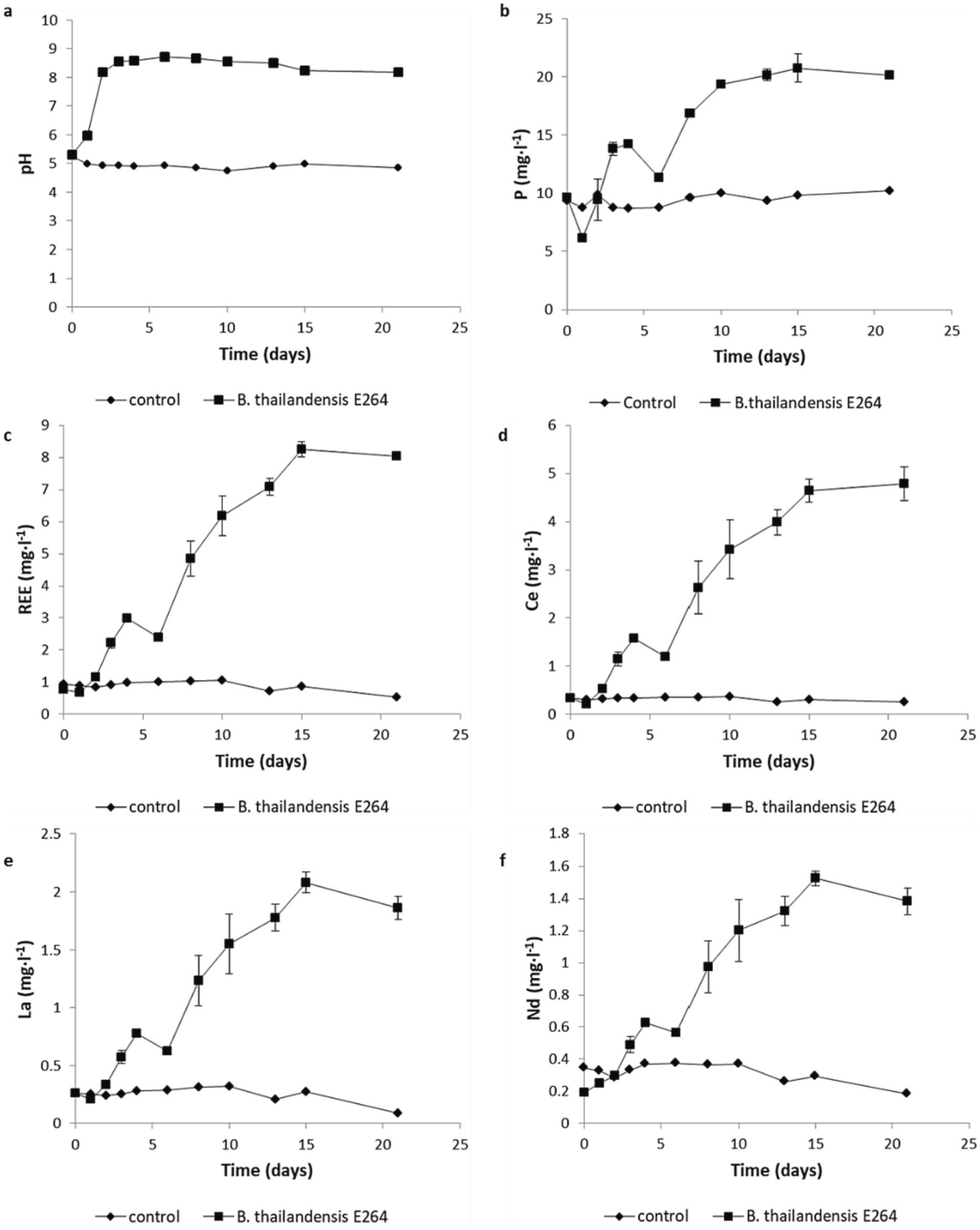

Fig. 1. Evolution of (a) pH, and (b) phosphorous, (c) REE, (d) Ce, (e) La and (f) Nd concentration in abiotic and biotic tests inoculated with *B. thailandensis* E264 in presence of monazite ore (1% pulp density).

decrease of phosphorous concentration due to the increase of the number of cells followed by a gradual increase of phosphorous in solution and the release of REE (Ce, La and Nd). In addition, the pH of the solution in the inoculated flasks increased from 5.3 ± 0.1 to 8.6 ± 0.1. Commonly, it is considered that the release of phosphate and hence REE is enhanced by microorganisms that acidify the medium (Fathollahzadeh et al., 2018b; Fathollahzadeh et al., 2019); nevertheless, *B. thailandensis* is able to solubilize REE by increasing the pH. The maximum amount of REE dissolved by *B. thailandensis* grown on monazite ore was 8.25 ± 0.04 $mg{\cdot}l^{-1}$ after 15 days.

The unreacted monazite ore and the residue from inoculated flasks were observed by FE-SEM (Fig. 2). The brighter areas of the back-scattered electron image of monazite correspond to mineral phases with a high content of heavier elements (Fig. 2a). EDS analyses were performed on the bright areas evidencing the presence of REE, as shown in Fig. 2b. Furthermore, it was observed that cells grew on monazite

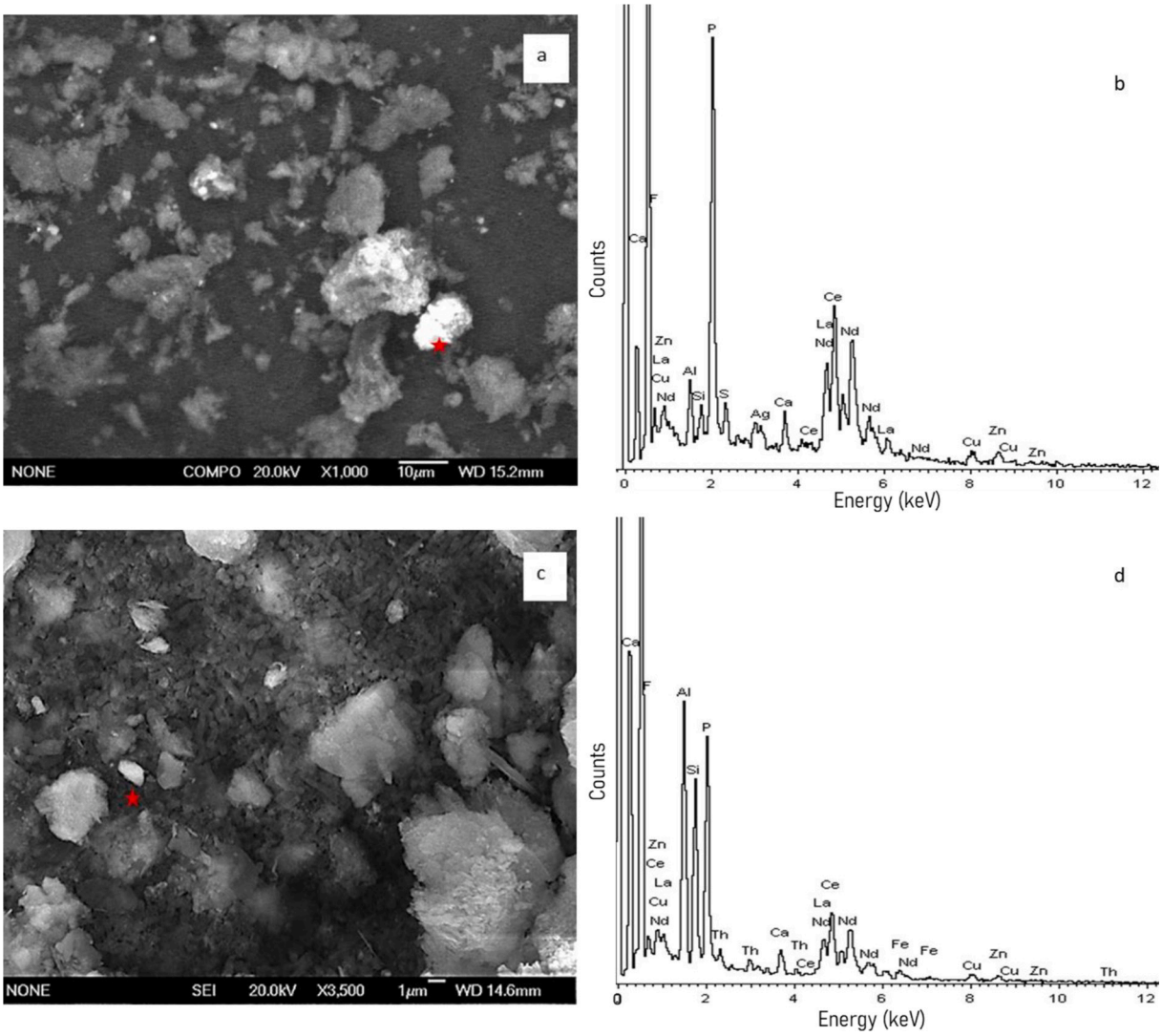

Fig. 2. a) Backscattered electron image and b) EDS of the raw monazite ore before interaction with *B. thailandensis*. c) Secondary electron microscopy and d) EDS of the mineral residue in presence of *B. thailandensis*. EDS were performed on area marked with stars.

modifying the mineral composition. The EDS of the mineral residue after bacterial growth showed a decrease in the phosphorous and REE content (Fig. 2d).

Microorganisms produce several compounds that may be involved in the inorganic phosphate solubilization, such as organic and inorganic acids, siderophores, extracellular polymeric substances or CO_2 (Prabhu et al., 2019; Rodriguez and Fraga, 1999). The release of organic acids has been reported as the most common mechanism in phosphate solubilization by heterotrophic bacteria and fungi, supplying both protons that reduce the pH and complexing compounds. However, it was evidenced that there is no correlation between the pH and the amount of phosphorous solubilized indicating the complexity of P release in soil systems (Asea et al., 1988).

B. thailandensis E264 produces rhamnolipids, which are low molecular weight glycolipid biosurfactants that exhibit an amphipathic property, their structure comprises one hydrophobic and one hydrophilic moiety (Funston et al., 2016). Furthermore, it was demonstrated that rhamnolipids selectively complex REE and may be involved in the mobilization of these critical metals (Hogan et al., 2017).

3.2. Bioleaching of REE with B. thailandensis ED1023

The rhamnolipids production by *Pseudomonas aeruginosa* has been extensively investigated; however, *P. aeruginosa* is an opportunistic human pathogen. *Burkholderia thailandensis* is a non-pathogenic bacterium and produces rhamnolipids; consequently, it could be considered an interesting candidate for use in industrial applications.

Nevertheless, rhamnolipid production using *B. thailandensis* presents low yields. In this study, a formerly characterized scmR- mutant ED1023 was used to bioleach the monazite ore. Previous studies of random transposon mutagenesis screening to identify genes directing rhamnolipid production in *B. thailandensis* E264 showed that the most efficient rhamnolipid producer (ED1023) harboured an inactivating transposon insertion in the scmR gene. It was evidenced that the production of rhamnolipids is downregulated by ScmR during the logarithmic growth phase by affecting the expression of rhl biosynthetic operons (Guillouzer et al., 2020; Martinez et al., 2020).

Fig. 3 shows a comparison in the changes in phosphorous and REE concentration and in pH value when *B. thailandensis* E264 and ED1023 are in contact with monazite. There were not major differences in the pH values reached with E264 and ED1023 and the pH rose up to 8.6 and 8.8, respectively. In addition, ED1023 showed a lower ability to release phosphorous than E264 and, despite the initial delay in the solubilization of REE by the mutant strain, both E264 and ED1023 reached a similar concentration in solution after 21 days, 8.0 ± 0.1 and 7.1 ± 0.3 $mg{\cdot}l^{-1}$ respectively.

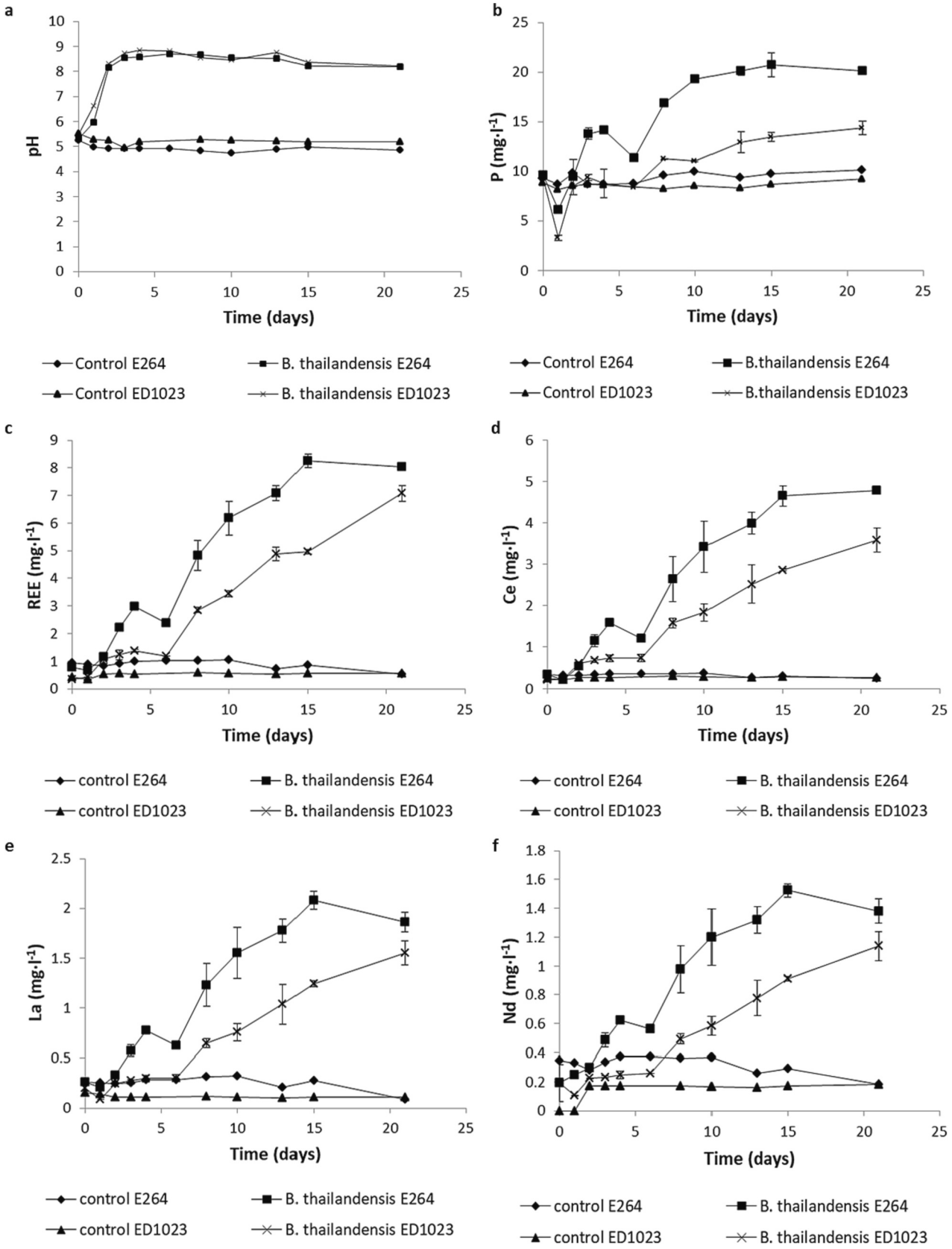

Fig. 3. Comparison of the evolution of (a) pH, and (b) phosphorous, (c) REE, (d) Ce, (e) La and (f) Nd concentration in abiotic and biotic tests inoculated with *B. thailandensis* E264 and ED1023 with monazite ore (1% pulp density).

3.3. Effect of glycerol

B. thailandensis was shown to increase the rhamnolipids production when grown on glycerol (Dubeau et al., 2009) and the effect of glycerol in the bioleaching of monazite using the strains E264 and ED1023 was studied in the present work (Figs. 4 and 5). In case of the wild type strain, the most remarkable change was that in presence of glycerol the pH value rose up to 7.4 and after 2 days the pH began to decrease to pH 4.3, instead of the increase to pH 8.6. In addition, the release of REE during the bioleaching with *B. thailandensis* E264 grown with glycerol

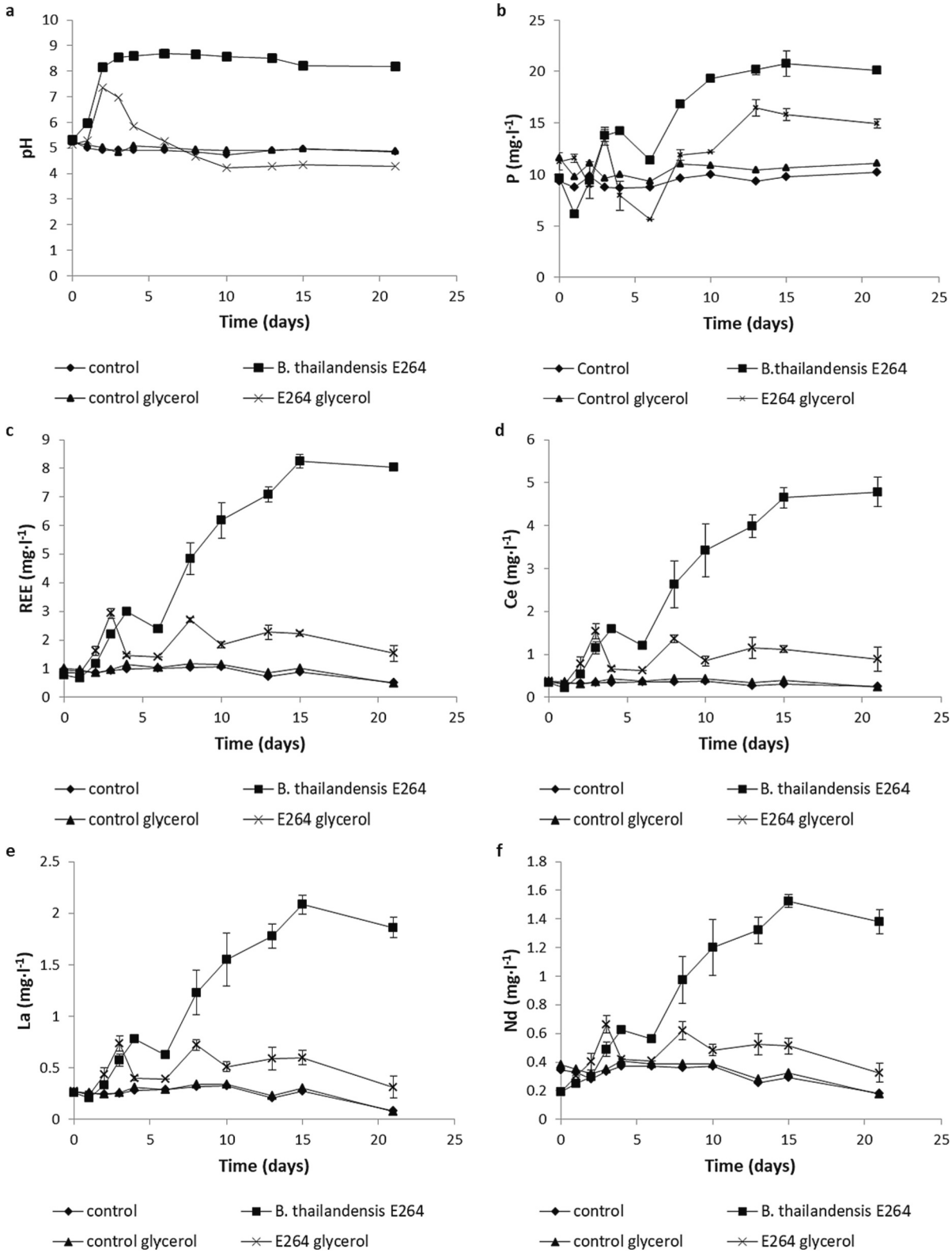

Fig. 4. Effect of glycerol in the evolution of (a) pH, and (b) phosphorous, (c) REE, (d) Ce, (e) La and (f) Nd concentration in abiotic and biotic tests inoculated with *B. thailandensis* E264 with monazite ore (1% pulp density).

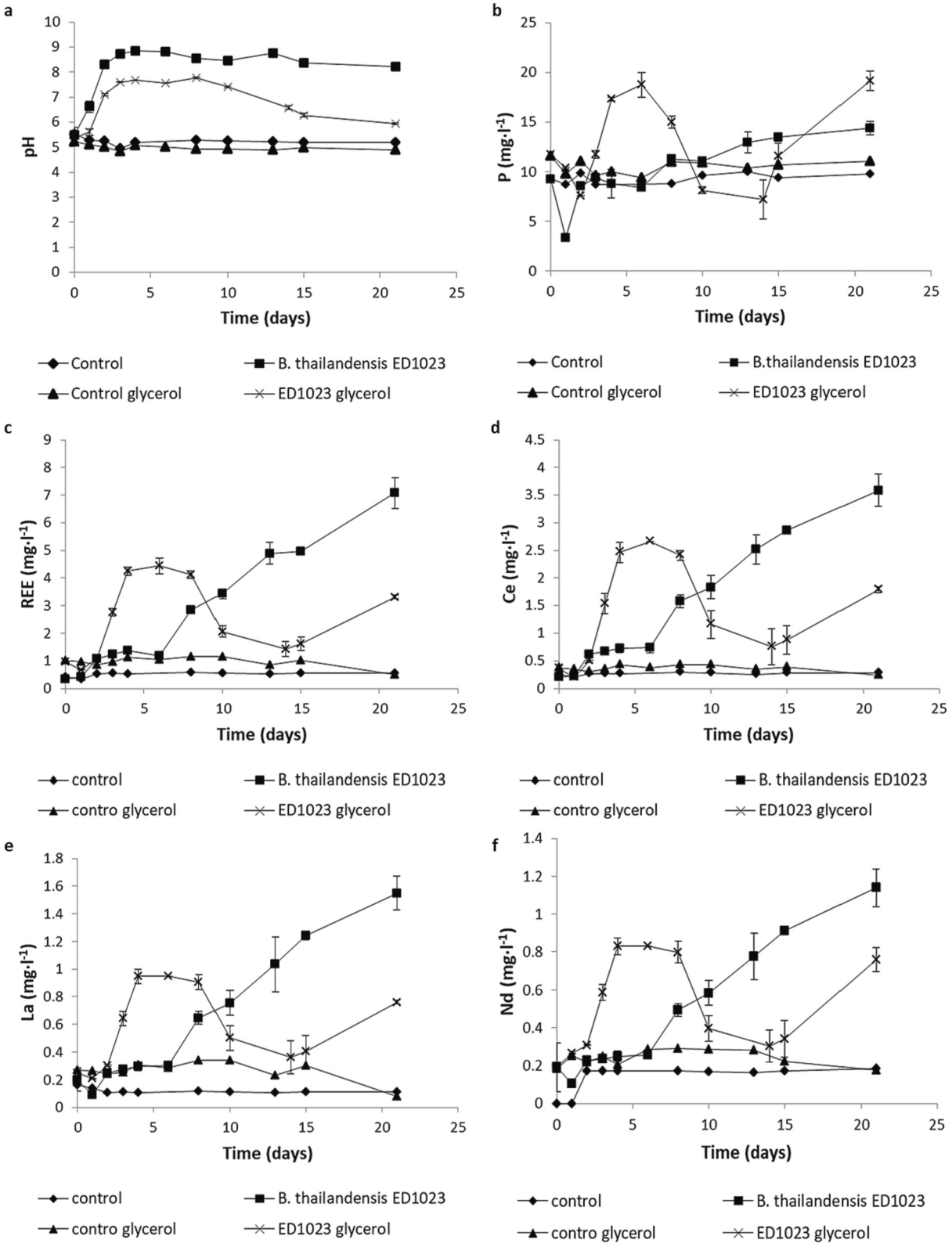

Fig. 5. Effect of glycerol in the evolution of (a) pH, and (b) phosphorous, (c) REE, (d) Ce, (e) La and (f) Nd concentration in abiotic and biotic tests inoculated with *B. thailandensis* ED1023 with monazite ore (1% pulp density).

reached a maximum concentration of 2.93 $mg{\cdot}l^{-1}$ after 3 days followed by a drop of REE in the aqueous phase rather than increase continuously. This behaviour can be explained by the formation of oxalate favored by the presence of glycerol in the medium (Fig. 6a). *Burkholderia thailandensis* produces oxalate by a two-step enzymatic reaction: the formation of a C6-CoA adduct using acetyl-CoA and oxaloacetate as substrates, and the production of three different products, in particular, oxalate, acetoacetate, and CoA. These two reactions are mediated by the bifunctional enzyme Obc1 in *B. thailandensis* and glycerol would have a catalytic role (Oh et al., 2016).

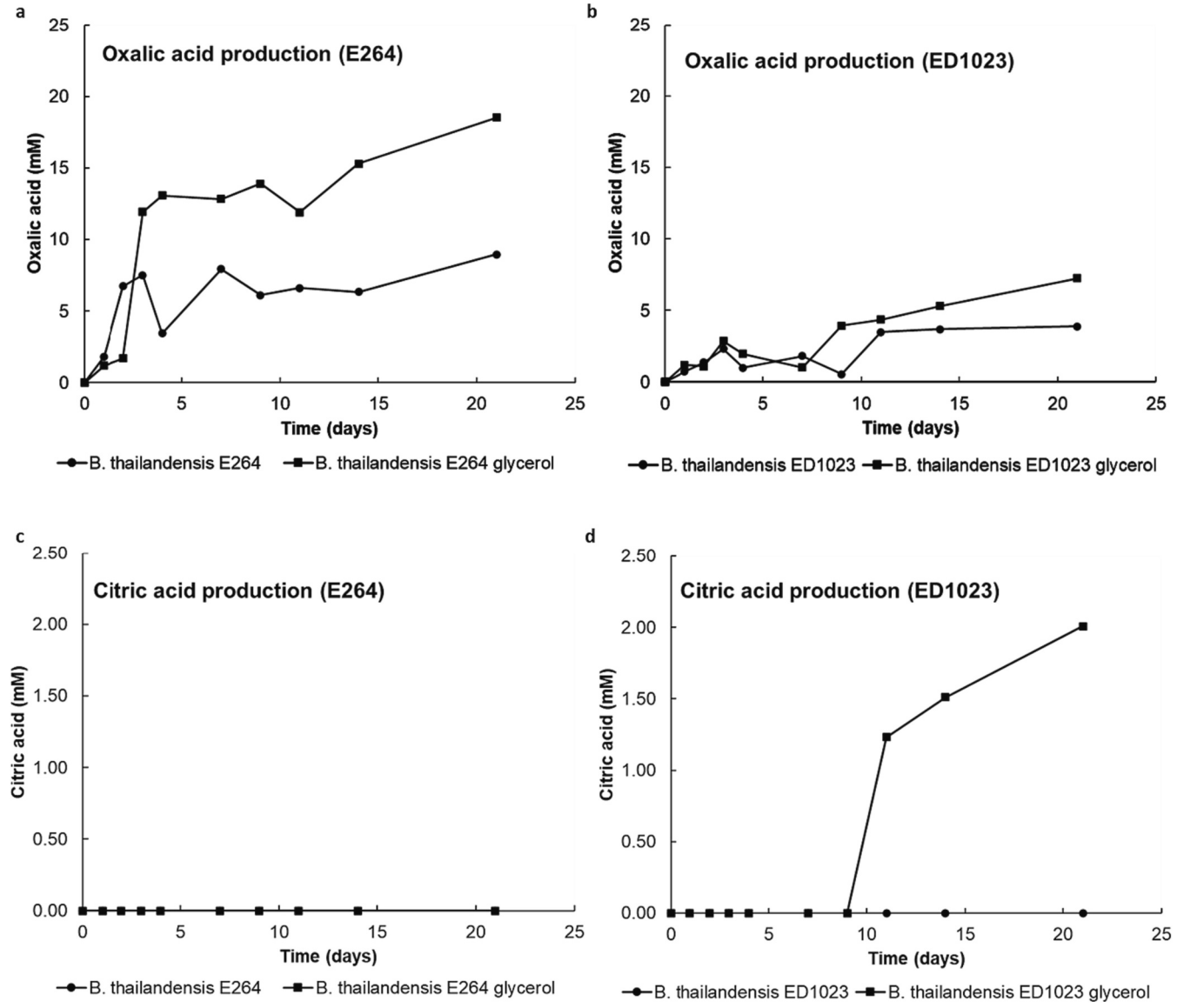

Fig. 6. Evolution of oxalic acid with (a) *B. thailandensis* E264 and (b) *B. thailandensis* ED1023 with and without glycerol in presence of monazite ore (1% pulp density). Evolution of citric acid with (c) *B. thailandensis* E264 and (d) *B. thailandensis* ED1023 with and without glycerol in presence of monazite ore (1% pulp density). Gluconic acid was not detected.

In cultures of the scmR- mutant grown with glycerol, pH remained higher than in cultures of the wild type. The pH value increased up to 7.6 after 3 days, remained constant and began to decrease slowly after 8 days reaching a final pH of 6.0 due to the formation of organic acids (Fig. 5a). The higher pH value is explained by the important reduction in the expression of *obc1* in the *scmR* mutant compared to E264 (Guillouzer et al., 2020). Therefore, the scmR- mutant produces a lower amount of oxalate (Fig. 6a and b). Another consequence of the lower production of oxalate by ED1023 was the higher solubilization of REE in presence of glycerol (Figs. 5 and 6). In addition, the phosphorous and REE concentrations followed a similar trend during the bioleaching experiments. The REE concentration increased faster than in absence of glycerol, up to 4.44 $mg{\cdot}l^{-1}$ during 6 days probably due to the formation of rhamnolipids and a higher pH value that favor their interaction with the metals. The rhamnolipid molecule dissociates with the formation of a stable anion occurring the detachment of a proton from the carboxyl group (Reaction 1).

$$Me^{n+} + n\,R-COOH \rightarrow (R-COO)_n\,Me + nH^{+} \quad (1)$$

where R is a rhamnolipid residue.

The decrease of REE concentration after 7 days in the flasks inoculated with ED1023 in presence of glycerol can be explained by the increase of oxalic acid. Finally, phosphorous and REE concentration rose associated to the production of citric acid (Fig. 6d).

The cultures of *B. thailandensis* E264 and ED1023 grown on monazite with and without glycerol were observed by FE-SEM after 14 days of incubation (Fig. 7). Fig. 7a and b evidenced similar residues in the cultures of E264 and ED1023 grown on monazite. The secondary and backscattered electron images of the residues and the EDS analyses of the areas with high content heavier elements are shown in Figs. S1 and S2, and Figs. S5 and S6, respectively.

When the microorganisms were grown in presence of glycerol higher number of cells was observed (Fig. 7c and d). In addition, E264 generated precipitates containing REE that may correspond to REE-oxalates (Figs. S3 and S4). In case of ED1023 grown with glycerol, a great amount of extracellular polymeric substances was observed as well as some precipitates enclosing REE (Figs. S7 and S8).

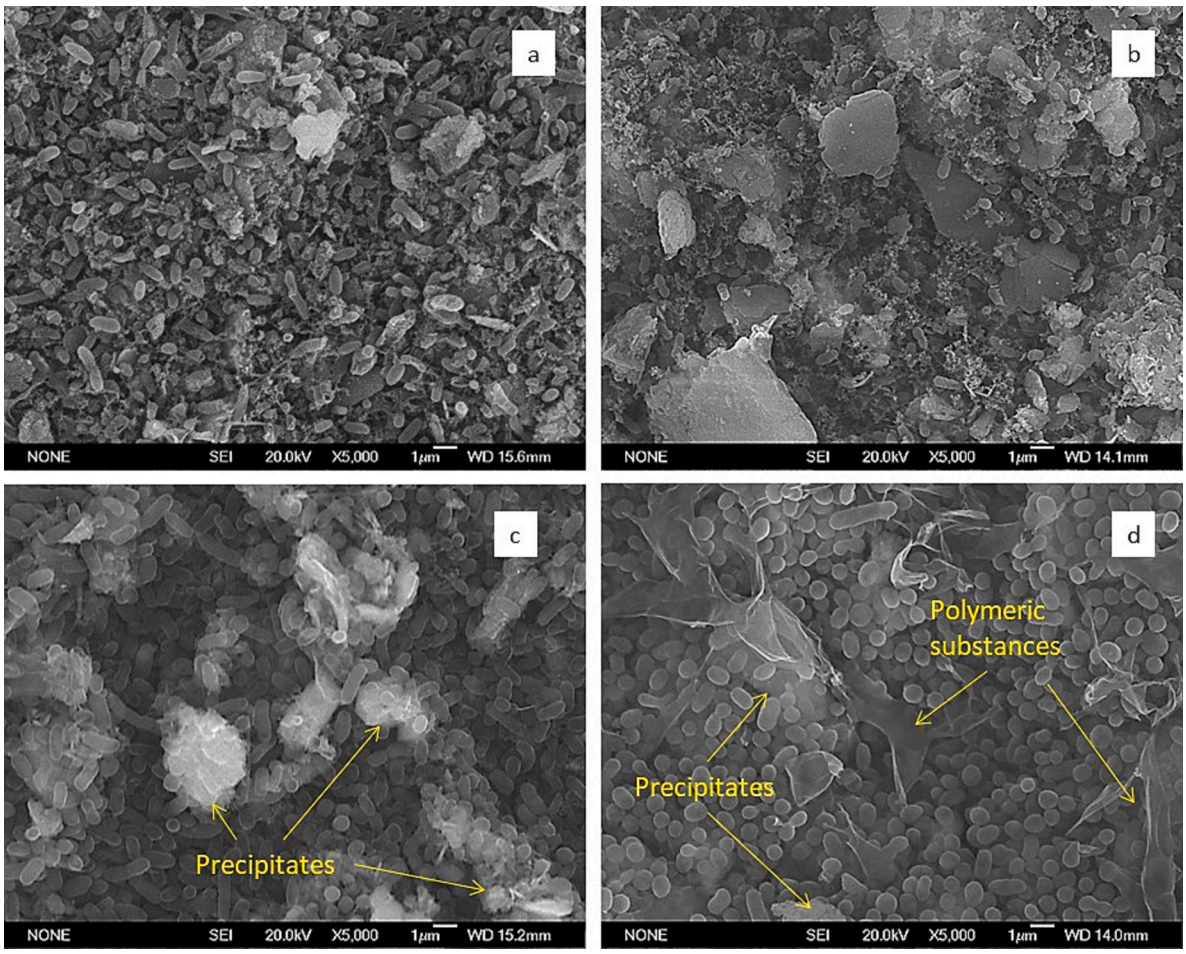

Fig. 7. FE-SEM images of a) E264 and b) ED1023 grown in nutrient broth; and c) E264 and d) ED1023 cultured with supplemented with 10% glycerol with monazite ore for 14 days.

3.4. Effect of rhamnolipids concentration in REE mobilization

The effect of the rhamnolipids concentration in the REE mobilization in abiotic experiments was also studied. Purified rhamnolipids produced by *B. thailandensis* E264 and commercial rhamnolipids produced by *Pseudomonas aeruginosa* were tested.

The critical micelle concentration of the biosurfactants was determined because these molecules are able to form micelles when a specific concentration is reached and the concentration has an effect in the interaction between the rhamnolipids and the minerals (Wang and Mulligan, 2009).

The critical micelle concentration of commercial rhamnolipids was 94.45 $mg{\cdot}l^{-1}$ reaching a surface tension value 38.4 $mN{\cdot}m^{-1}$. Rhamnolipids produced by *B. thailandensis* E264 were extracted after an incubation period of 11 days using nutrient broth medium supplemented with 10% glycerol as an additional carbon source. The critical micelle concentration of the crude extract was 60.41 $mg{\cdot}l^{-1}$.

The potential applications for REE recovery using rhamnolipids have been poorly investigated. Nevertheless, it was evidenced that monorhamnolipids strongly bound to REE (Eu^{3+}, Nd^{3+}, Tb^{3+}, Dy^{3+}, La^{3+}, Y^{3+}, Pr^{3+}, and Lu^{3+}) with log β (complex stability constant) varying from 9.8 to 8.2 (Hogan et al., 2017).

The mechanisms for binding interaction between biosurfactants and metals include ion exchange, counterion association, precipitation–dissolution, and electrostatic interactions (Liu et al., 2018). In the present work, the influence of rhamnolipid concentration in the REE mobilization was studied because this is an important factor in removal efficiencies. Experiments with commercial and purified rhamnolipids were conducted at different biosurfactant concentrations (1/2 CMC, CMC and 2 CMC) during 7 days. The highest extraction yields were obtained at CMC with both commercial and purified rhamnolipids and the best results were reached with the commercial biosurfactant 9.36 $mg{\cdot}l^{-1}$ REE, while with the purified rhamnolipids the concentration was 5.13 $mg{\cdot}l^{-1}$ (Fig. 8).

The results obtained in this study suggest that the REE mobilization increased with the rhamnolipid concentration below the CMC. At very low concentrations of biosurfactant, the monomers start complexation with metal ions. However, high concentrations of biosurfactant led to lower extraction yields indicating that micelles are not directly involved in the process. Furthermore, high surfactant concentration could result in plugging the mineral surface by the dispersion of fine materials or by formation of viscous emulsions (Rothmel et al., 1998).

The linear increase in the removal of metals by rhamnolipids with increasing surfactant concentration below the CMC, followed by a relatively constant removal above the CMC indicates that ion exchange may be the dominant mechanism for enhancing REE extraction from monazite in this study (Doong et al., 1998). On the other hand, counterion exchange could facilitate the dissolution of precipitated metals when the concentration of surfactant exceeds the CMC (Nivas et al., 1996).

4. Conclusions

This work revealed that *B. thailandensis* bioleach monazite increasing the pH up to 8.6, and that the rhamnolipids generated by *B. thailandensis* play a role in the recovery of REE. The strain ED1023, a scmR- mutant

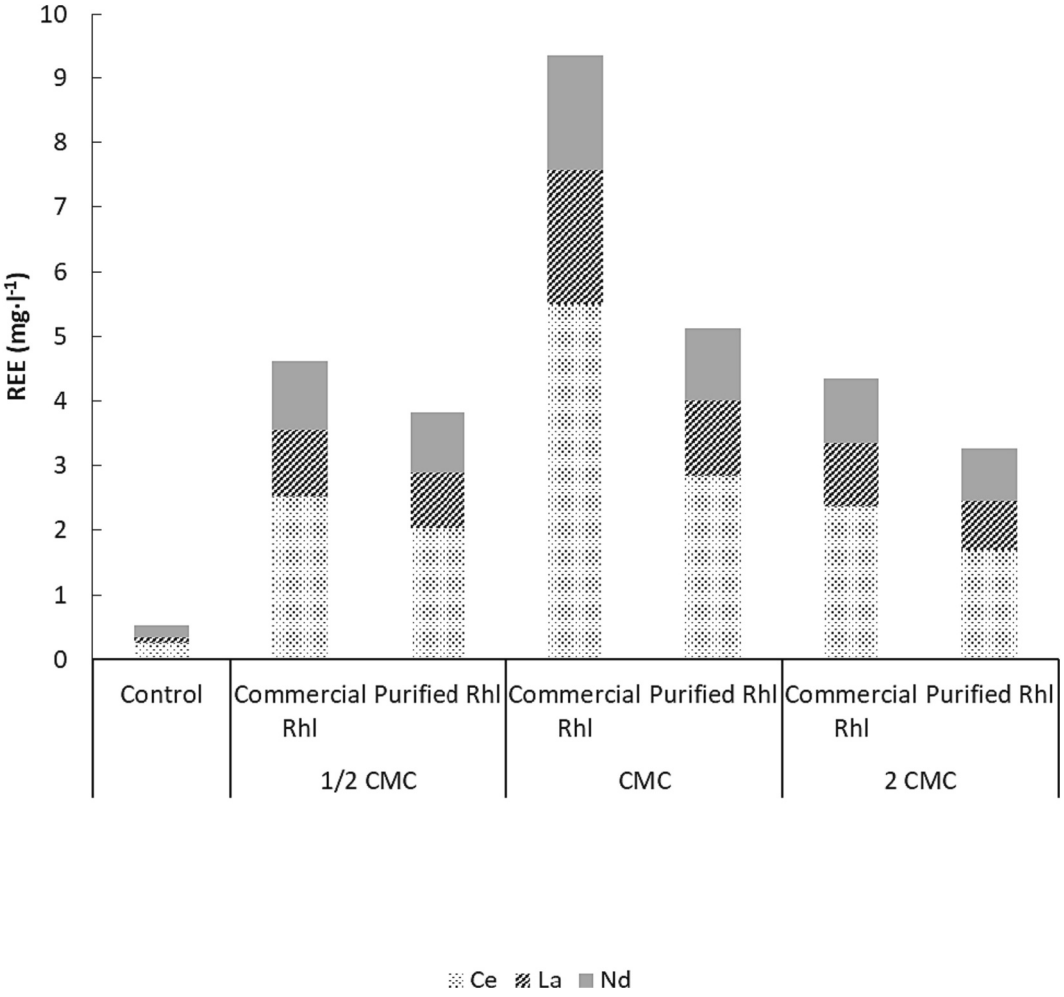

Fig. 8. Effect of rhamnolipids (Rhl) concentration (commercial Rhl produced by *Pseudomonas aeruginosa* and purified Rhl produced by *Burkholderia thailandensis*) on the mobilization of REE (cerium, lanthanum and neodymium) from monazite (1% pulp density) after one week. CMC: critical micelle concentration.

that overproduces rhamnolipids, showed similar yield of REE dissolution compared to the wild type strain. The addition of glycerol to the medium to promote the rhamnolipids formation led to the production of oxalic acid by *B. thailandensis* E264 followed by the REE precipitation. In case of ED1023, the glycerol favored a fast solubilization of REE during 6 days.

The abiotic studies to solubilize REE from monazite ore using commercial rhamnolipids produced by *Pseudomonas aeruginosa* and purified rhamnolipids from *B. thailandensis* evidenced that the highest extraction yields were obtained at CMC and suggest that rhamnolipids are interesting molecules for their potential applications in the recovery of rare earth elements.

CRediT authorship contribution statement

Laura Castro: Conceptualization, Methodology, Investigation, Data curation, Writing – original draft. **Helena Gómez-Álvarez:** Investigation, Methodology. **Manuel Carmona:** Conceptualization, Investigation. **Felisa González:** Conceptualization, Supervision, Validation. **Jesús A. Muñoz:** Conceptualization, Validation, Funding acquisition, Writing – review & editing.

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.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.hydromet.2023.106178.

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