



Lanthanides biosorption by immobilized microalgae using statistical design

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Abstract

Previous studies showed that *Chlorella vulgaris* microalgae (Chlorophyta) possessed high physiological activity as a biosorbent for rare-earth elements (REE), and in this study, the sorption capacity for both La^{3+} and Sm^{3+} was demonstrated. The microalgae cells were immobilized onto calcium alginate gel aiming at its use in the recovery and separation of studied REE. The biosorption tests were conducted in batch using an incomplete factorial design, and the residual concentrations of REE were determined using ICP-OES. Contact time was the variable that positively influenced La^{3+} biosorption, while the temperature variable showed a positive effect only on the biosorption of Sm^{3+} . Thus, the evaluated conditions seem to present selectivity for separating light REE and heavy REE and may allow the development of a REE separation process on a larger scale based on the biosorption process. This is thought to be the first report on utilization of immobilized *C. vulgaris* cells as a candidate for the bioseparation of REE from diluted liqueurs.

Keywords: *Chlorella vulgaris*, calcium alginate, biosorption, lanthanum, samarium

Introduction

Rare-earth elements (REEs) are a group of 17 elements from periodic table which comprises the lanthanide series (lanthanum (La, $z = 57$) to lutetium (Lu, $z = 71$)), including yttrium ($z = 39$) and scandium ($z = 21$), which exhibit similar chemical behaviors. REE have numerous industrial applications. REEs can be further divided into two groups: the light ones (from La to Sm) and the heavy ones (from Gd to Lu) [Balaram, 2019; Souza *et al.*, 2019] [5, 28]. REEs have been extensively used as catalysts, battery alloys, ceramics, microwaves, sensors, dyes, coatings, electronic conductors, cracking, and oxidation catalysts and permanent magnets, possessing high demand worldwide [Akah, 2017; Giese, 2018; Giese, 2019] [3, 15, 8]. The high market value of REE is due to the high cost of extraction, separation, and recovery of the purest compounds of REE oxides. The hydrometallurgical processes are commonly employed for this purpose and involve a series of extraction steps using solvents and ion-exchange resins [Jha *et al.*, 2016; Ang *et al.*, 2017].

Biosorption has been described as a bioprocess with the potential to recover REE and an alternative to the classical ion-exchange method for REE separation [Giese, 2020a; Giese, 2022] [18]. The biosorption mechanisms include adsorption, uptake, reduction, methylation, and oxidation. In this way, biosorption is similar to an ion-exchange mechanism; the REE can bind to oxygen donor atom groups present in the cell wall (cells) of microbial species, such as carboxylic or phosphoric groups [Giese, 2020b; Giese *et al.*, 2020] [19, 18].

Many microbial species have been used in the biosorption of REE [Das *et al.*, 2008; Das & Das, 2013; Sadovsky *et al.*, 2016; Coimbra *et al.*, 2019; Giese *et al.*, 2019; Giese & Jordão, 2019; Giese, 2022] [10, 11, 8]. Different studies have shown that one can have better efficiency in the biosorptive process when microorganisms are immobilized in porous matrices [Aria *et al.*, 2004; Correa *et al.*, 2017; Coimbra *et al.*, 2019; Giese *et al.*, 2020] [8, 18]. Microalgae have also been studied for their biosorption of REE capacity [Klimmek *et al.*, 2001; Correa *et al.*, 2017; Heidelmann *et al.*, 2017] [25, 22]. They have a cellular coating consisting of polysaccharides and proteins, which have anionic carboxylic groups, sulfate groups, and phosphate groups that can passively bind to these elements. For REE/seaweed bonding it is necessary also to shift cations, such as Ca^{2+} ions [Klimmek *et al.*, 2001; Diniz & Volesky, 2005] [25, 12].

Given the possibility of applying biosorbents in the recovery and separate REE from diluted solutions, this study aimed to investigate the effect of different parameters on the adsorption process of lanthanum (La^{3+}) and samarium (Sm^{3+}) ions by the microalgae *Chlorella vulgaris* (Chlorophyta) cells immobilized in calcium alginate gel using a factorial design experiment.

Materials and Methods

Microalgae cultivation and biosorbent preparation

The microalgae *C. vulgaris* used in the biosorption experiments was obtained from the culture collection from Laboratório de Ecofisiologia e Toxicologia de Cianobactérias, Instituto de Biofísica, Centro de Ciências da Saúde, UFRJ (Brazil). The cultivation of both microalgae was performed according to ABNT NBR 12648 [ABNT, 2018] [1] in L. C. Oligo medium. For the biosorption experiments, the harvested microalgae after 7 days of growth were first freeze-dried overnight (24 h) at $-10\text{ }^{\circ}\text{C}$, followed by lyophilization.

The immobilized *C. vulgaris* biomass was obtained from the immobilization of the microalgae cells in sodium alginate. To this end, the solutions containing two different concentrations of microalgae *C. vulgaris* cells were immobilized according to the procedure described by Coimbra *et al.* [2019] [8].

Batch biosorption experiments and factorial design

Batch biosorption experiments were conducted in 250 mL Erlenmeyer flasks containing 100 mL of synthetic solutions of lanthanum (La) and samarium (Sm) at different concentrations according to the factorial planning presented in Tables 1 and 2. The conditions of temperature, initial pH, number of cells, and contact time are described in Table 2. The Erlenmeyer flasks were kept under constant agitation at 100 rpm in a shaker incubator. The determination of the final concentration of REE in solution was performed using plasma optical emission spectrometry (ICP-OES). The biosorption of REE from synthetic solutions was evaluated using an incomplete factorial planning (Table 2). Variance and multiple regression analyses were also performed using STATISTICA software version 13.2.

Table 1: Incomplete factorial design

Exp.	x ₁	x ₂	x ₃	x ₄	x ₅	x ₆	%La (Y ₁)	%Sm (Y ₂)
1	-1	-1	-1	1	1	-1	66.84	N.D.
2	1	-1	-1	-1	-1	1	N.D.	72.53
3	-1	1	-1	-1	1	1	56.59	75.84
4	1	1	-1	1	-1	-1	N.D.	N.D.
5	-1	-1	1	1	-1	1	N.D.	73,72
6	1	-1	1	-1	1	-1	61.4	N.D.
7	-1	1	1	-1	-1	-1	N.D.	N.D.
8	1	1	1	1	1	1	60.17	82.21

N.D. = not detected

Table 2: Variables used in experimental design.

Independent original variables	-1	+1
x ₁ , temperature (°C)	20	40
x ₂ , initial pH	3	6
x ₃ , number of immobilized cells	0.5 x 10 ⁸	1.2 x 10 ⁸
x ₄ , time (min)	30	240
x ₅ , [La], mg/L	0	500
x ₆ , [Sm], mg/L	0	500

Results and Discussion

In this study, an incomplete factorial design totaling 16 experiments was used to evaluate the effect of different parameters on the capacity of a biosorbent formed by microalgae *C. vulgaris* cells encapsulated in calcium alginate gel. According to Table 1, the highest percentage of La³⁺ adsorption (66.84%) was observed in experiment n°1, in a mono-elemental system, with experimental conditions of 20 °C, initial pH of 3.0 and using 1.5x10⁸ immobilized cells, after 240 min of contact time. On the other hand, the highest rate of Sm³⁺ removal (82.21%) was observed in experiment n° 8, in a bi-elementary system, with experimental conditions of 20 °C, initial pH of 3.0, and 1.8x10⁸ of immobilized cells after 240 min.

The biomass of microalgae *C. vulgaris* has been described as a biosorbent material with the potential for adsorption of different REE from diluted solutions. Heidelmann *et al.* [2017] [22] described the biosorption capacity of La³⁺ by free *C. vulgaris* cells, which presented a value of saturation of 1.11 mg/g (0.8 mmol/g). Recently, Birungi and Chirwa [2018] [7] have described a value of 75 mg/g for La³⁺ by free *C. vulgaris* cells in distinct conditions. Kucuker *et al.* [2017] described the use of free cells of these microalgae for neodymium (Nd³⁺) biosorption aiming at the recovery of this REE from the recycling of hard disk magnets. The researchers described biosorption rates of up to 63% when the initial Nd³⁺ concentration was 250 mg/L.

No studies using immobilized *C. vulgaris* cells were found in the literature. The most important aspect in the biosorption process is the separation of the biomass from the aqueous solution after sorption [Michalak *et al.*, 2013] [26]. In this perspective, the use of immobilized biocatalysts is a huge advantage in the process [Giese, 2015].

The Pareto chart determines the magnitude and importance of the effects from different variables studied, where the bars that cross the reference line are statistically significant. According to Figure 1A, the most critical variable for La³⁺ biosorption was the concentration of this element (x₅), followed by contact time (x₄), initial pH (x₃), and Sm³⁺ concentration (x₆). According to the level chart (Figure 1B), higher percentages of La³⁺ biosorption can be found increasing contact time at higher pH values. The contact time is an important parameter for scaling the biosorption process for columns, since the separation of metals in solution depends on the relationship of concentrations at the input and output from the moment of the biosorption process [Michalak *et al.*, 2013] [26].

On the other hand, for the Sm^{3+} biosorption process, represented in Figure 2A, the most significant variable was the concentration of Sm^{3+} (x_6), followed by the initial pH (x_3), La^{3+} concentration (x_5), and initial temperature (x_1). The level graph (Figure 2B) shows that the biosorption of Sm^{3+} by immobilized *C. vulgaris* is favored by the increase of initial pH and the process temperature. Biosorption is a spontaneous process with the change of the values of the thermodynamic functions [Witek-Krowiak, 2012] ^[29]. These results demonstrated that the rate of Sm^{3+} biosorption depends on the temperature of the process.

The temperature-dependence of the rates of Sm^{3+} biosorption and the time-dependence of La^{3+} biosorption appears to be essential to the development of a separation process where it could be possible to separate light REE species from heavy REE species.

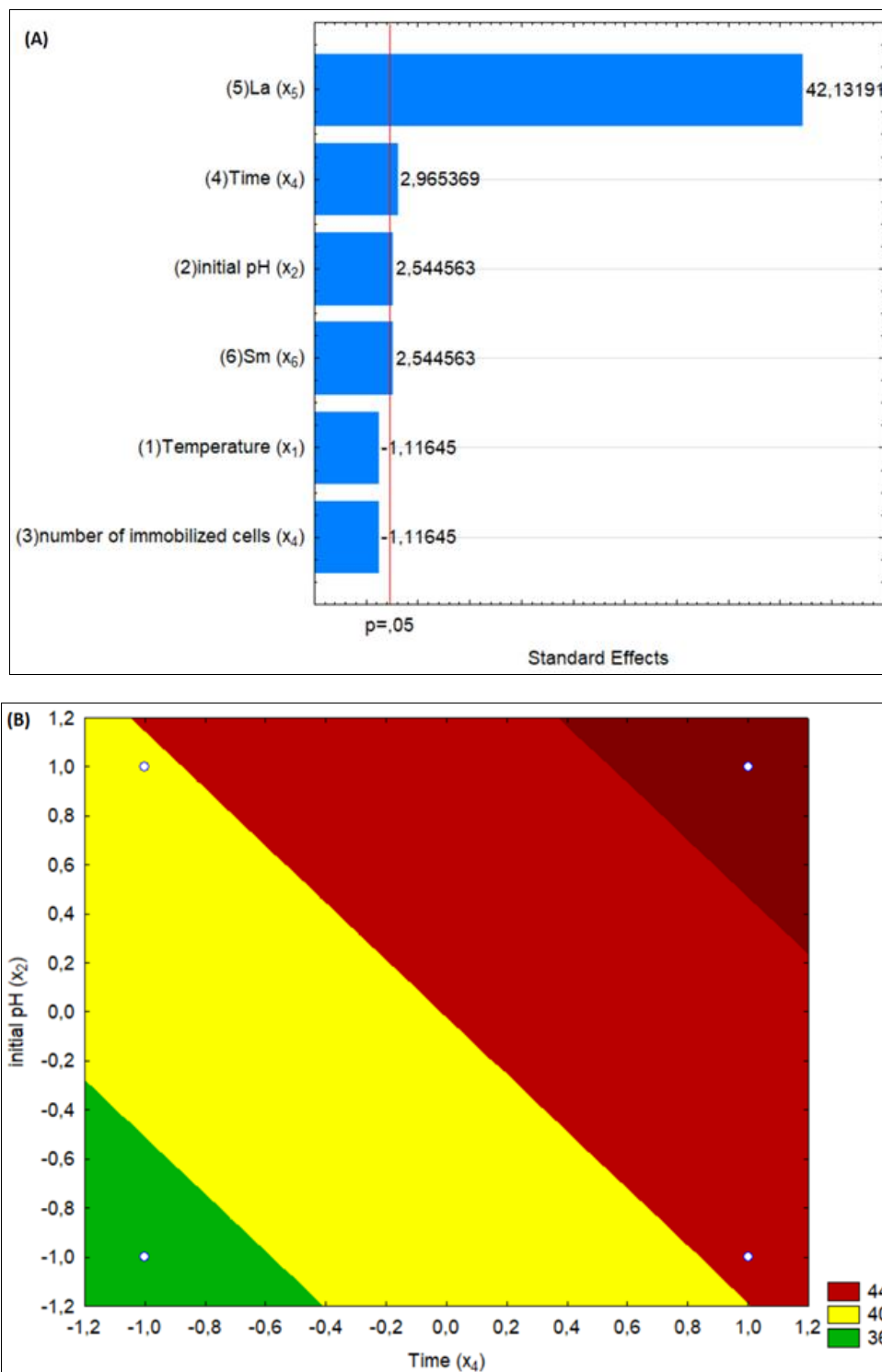


Fig 1: (A) Pareto diagram of the estimated effects. (B) Graph of levels for the lanthanum (La^{3+}) biosorption considering the variables pH x time. Conditions: Temperature of 30 °C; 1.6×10^8 cells; $[\text{La}] = 500$ mg/L and $[\text{Sm}] = 100$ mg/L.

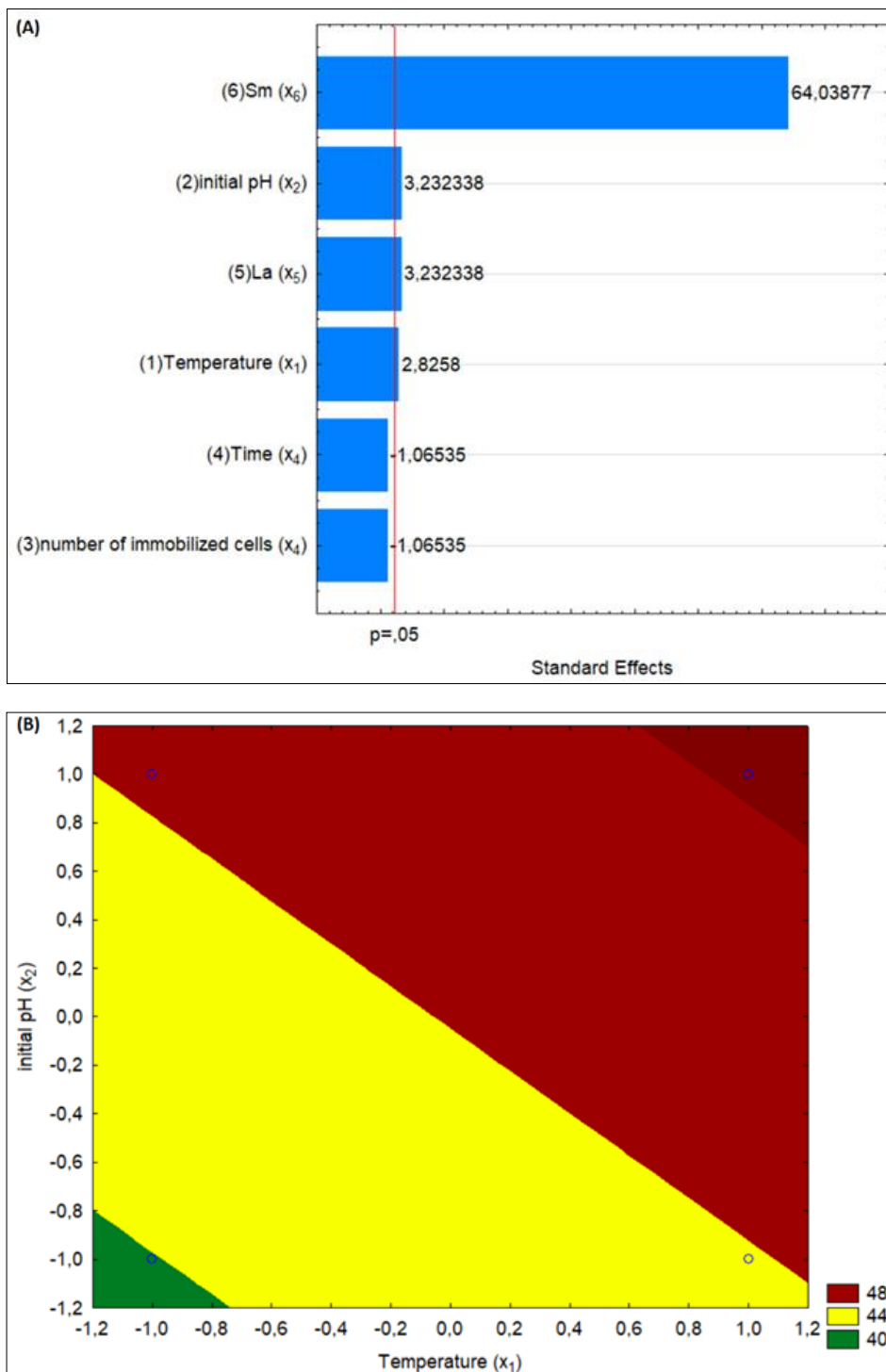


Fig 2: (A) Pareto diagram of the estimated effects. (B) Graph of levels for the samarium (Sm^{3+}) biosorption considering the variables pH x temperature. Conditions: Time of 135 min; 1.6×10^8 cells; $[\text{La}] = 100 \text{ mg/L}$ and $[\text{Sm}] = 500 \text{ mg/L}$.

Conclusion

The results showed the capacity of selective biosorption of La^{3+} and Sm^{3+} by the biomass of *Chlorella vulgaris* microalgae immobilized in calcium alginate. The operating parameters determined by contact time and process temperature were determinant variables for the selective recovery of these REE once mixed in solution. With the increase in temperature, it seems possible to separate the element Sm^{3+} , while the longer contact time with the biosorbent favors the extraction of La^{3+} , the lightest element in the REE group. These findings will help expand the scale and development of the separation of REE based on biosorption process.

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Conflict of Interest

Not applied

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