



USE OF *LITHOTHAMNIUM* FOR PHOSPHORUS REMOVAL AND IMPROVEMENT OF THE TROPHIC STATUS OF WATER BODIES

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ABSTRACT

Objective: To evaluate the efficiency of *Lithothamnium calcareum* as an adsorbent for the removal of phosphorus from eutrophicated water bodies.

Theoretical Framework: Conventional wastewater treatment methods, such as activated sludge and biological filters, have low efficiency in removing nitrogen and phosphorus. This highlights the need for alternative strategies, such as the use of adsorbent materials, to mitigate the effects of eutrophication. *Lithothamnium calcareum*, also known as bioclastic granulate (BG), has been the subject of several studies indicating its potential for phosphorus removal and recovery.

Methodology: Phosphorus removal was evaluated using water from Lake Açú, with *Lithothamnium calcareum* (particles of 0.3 to 0.6 mm) as the adsorbent. In 13 experiments, efficiency was analyzed using the Response Surface Methodology (RSM), varying the amount of BG and contact time.

Results and Discussion: The study assessed phosphorus removal by *Lithothamnium calcareum*, achieving 48.8% efficiency with 60 g/L of BG in 180 minutes. Modeling predicted up to 55.14% removal with 68.3 g/L in 204 minutes. Concentration and time significantly influenced the results, demonstrating that conditions with higher amounts of BG and longer contact times led to greater efficiencies.

Research Implications: The results suggest that *Lithothamnium calcareum* offers a promising solution for the treatment of eutrophicated waters, contributing to the reduction of phosphorus in water bodies and the mitigation of eutrophication.

Originality/Value: The study demonstrates the applicability of a natural and sustainable material for phosphorus removal, providing innovative insights for the development of treatment technologies for eutrophicated waters, with potential for subsequent agricultural use of phosphorus-enriched BG.

Keywords: Bioclastic Granulate, Phosphorus Removal, Eutrophication, Response Surface.

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USO DE *LITHOTHAMNIUM* PARA REMOÇÃO DE FÓSFORO E MELHORIA DO ESTADO TRÓFICO DE CORPOS D'ÁGUA

RESUMO

Objetivo: Avaliar a eficiência do *Lithothamnium calcareum* como adsorvente para remoção de fósforo de águas eutrofizadas de corpos d'água.

Referencial Teórico: Os métodos convencionais de tratamento de esgoto, como lodo ativado e filtros biológicos, têm baixa eficiência na remoção de nitrogênio e fósforo. Isso destaca a necessidade de estratégias alternativas, como o uso de materiais adsorventes, para mitigar os efeitos da eutrofização. O *Lithothamnium calcareum*, também conhecido como granulado bioclástico (GB), tem sido objeto de diversos estudos que apontam para seu potencial para remoção e recuperação de fósforo.

Método: A remoção de fósforo foi avaliada com água do Lago Açu, usando *Lithothamnium calcareum* (partículas de 0,3 a 0,6 mm) como adsorvente. Em 13 experimentos, a eficiência foi analisada pela Metodologia de Superfície de Resposta (MSR), variando a quantidade de GB e tempo de contato.

Resultados e Discussão: O estudo avaliou a remoção de fósforo pelo *Lithothamnium calcareum*, atingindo 48,8% de eficiência com 60 g/L de GB em 180 minutos. A modelagem previu até 55,14% de remoção com 68,3 g/L em 204 minutos. Concentração e tempo influenciaram significativamente, demonstrando que as condições com as maiores quantidades de GB e maiores tempos de contato implicaram nas maiores eficiências.

Implicações da Pesquisa: Os resultados sugerem que o *Lithothamnium calcareum* oferece uma solução promissora para o tratamento de águas eutrofizadas, contribuindo para a redução do fósforo em corpos d'água e mitigação da eutrofização.

Originalidade/Valor: O estudo demonstra a aplicabilidade de um material natural e sustentável para a remoção de fósforo, fornecendo insights inovadores para o desenvolvimento de tecnologias de tratamento de águas eutrofizadas com potencial para posterior aplicação agrícola do GB enriquecido com fósforo.

Palavras-chave: Granulado Bioclástico, Remoção de Fósforo, Eutrofização, Superfície de Resposta.

USO DE *LITHOTHAMNIUM* PARA LA REMOCIÓN DE FÓSFORO Y MEJORA DEL ESTADO TRÓFICO DE CUERPOS DE AGUA

RESUMEN

Objetivo: Evaluar la eficiencia de *Lithothamnium calcareum* como adsorbente para la remoción de fósforo en cuerpos de agua eutrofizados.

Marco Teórico: Los métodos convencionales de tratamiento de aguas residuales, como los lodos activados y los filtros biológicos, tienen baja eficiencia en la remoción de nitrógeno y fósforo. Esto resalta la necesidad de estrategias alternativas, como el uso de materiales adsorbentes, para mitigar los efectos de la eutrofización. El *Lithothamnium calcareum*, también conocido como granulado bioclástico (GB), ha sido objeto de varios estudios que indican su potencial para la remoción y recuperación de fósforo.

Metodología: La remoción de fósforo se evaluó utilizando agua del Lago Açu, con *Lithothamnium calcareum* (partículas de 0,3 a 0,6 mm) como adsorbente. En 13 experimentos, la eficiencia se analizó mediante la Metodología de Superficie de Respuesta (MSR), variando la cantidad de GB y el tiempo de contacto.

Resultados y Discusión: El estudio evaluó la remoción de fósforo por *Lithothamnium calcareum*, alcanzando un 48,8% de eficiencia con 60 g/L de GB en 180 minutos. El modelado predijo hasta un 55,14% de remoción con 68,3 g/L en 204 minutos. La concentración y el tiempo influyeron significativamente en los resultados, demostrando que las condiciones con mayores cantidades de GB y tiempos de contacto más prolongados resultaron en mayores eficiencias.



Implicaciones de la Investigación: Los resultados sugieren que el *Lithothamnium calcareum* ofrece una solución prometedora para el tratamiento de aguas eutrofizadas, contribuyendo a la reducción del fósforo en cuerpos de agua y a la mitigación de la eutrofización.

Originalidad/Valor: El estudio demuestra la aplicabilidad de un material natural y sostenible para la remoción de fósforo, proporcionando ideas innovadoras para el desarrollo de tecnologías de tratamiento de aguas eutrofizadas, con potencial para su posterior aplicación agrícola del GB enriquecido con fósforo.

Palabras clave: Granulado Bioclástico, Remoción de Fósforo, Eutrofización, Superficie de Respuesta.

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1 INTRODUCTION

Conventional wastewater treatment methods, such as activated sludge processes and trickling filters, are typically not effective in removing nitrogen and phosphorus from wastewater. These processes are effective in removing organic matter and suspended solids, but have limitations in removing nutrients, which can lead to eutrophication of receiving water bodies. Studies indicate that, while conventional technologies can reduce the load of organic pollutants, the removal of nitrogen and phosphorus requires specific additional processes, such as nitrification, denitrification and chemical precipitation (Metcalf & Eddy, 2014; Tchobanoglous *et al.*, 2013).

Furthermore, failure to adequately remove these nutrients can result in significant environmental impacts such as excessive algal growth and degradation of water quality (Von Sperling, 2005).

The seaweed *Lithothamnium calcareum*, belonging to the genus *Lithothamnium* and classified in the *Corallinacea* family, according to the AlgaeBase taxonomic system (Soares, 2016), is also known as bioclastic granulate (GB), due to its composition of fragments of organic skeletal material (Dias, 2000). This material is mostly composed of calcium carbonate (CaCO₃) (Caletti, 2017) and covers a significant part of the Brazilian continental shelf, where one of the largest reserves in the world is located (Dias, 2000; Homero, 2012).

Several studies have investigated the surface of this seaweed. According to Carlos *et al.* (2011) and Dias (2000), *Lithothamnium* has a porosity of over 40%, associated with a high specific surface area. Veneu *et al.* (2017) and Zhu *et al.* (2014) described an irregular surface of the material, with a predominance of cavities ranging from 7.1 to 9.5 μm , in addition to



small fragments around and inside the samples. Da Silva *et al.* (2021) classified this material as ultra-microporous, with pores smaller than $7\text{\AA}$ and a surface area of $8.1\text{ m}^2.\text{g}^{-1}$.

Lithothamnium has been widely studied for its use as an adsorbent in water and effluent treatment (Veneu *et al.*, 2017, 2019, 2023; Caletti *et al.*, 2019; Almeida *et al.*, 2021; Portella *et al.*, 2025). The results of these studies indicate a high potential for direct application in the waters of eutrophic lakes, aiming at improving their Trophic State Index (TSI), especially due to their high phosphorus removal capacity. Therefore, the implementation of an additional treatment to the conventional one, which aims to remove nutrients using a methodology based on a calcareous algae with viable and environmentally sustainable exploitation, is a study topic of high scientific interest.

Response Surface Methodology (RSM) has emerged as a powerful statistical tool for optimizing adsorption and removal processes of pollutants from various effluents, allowing the analysis of multiple variables and their interactions. Recent studies demonstrate that RSM is effective in modeling and optimizing parameters such as pH, temperature, adsorbent concentration, and contact time, maximizing contaminant removal efficiency (Foo & Hameed, 2019). In addition, research by Tran *et al.* (2020) highlights its application in the development of sustainable adsorbents, such as biosorbents derived from agricultural residues, for the removal of heavy metals and textile dyes. MSR also facilitates the reduction of the number of experiments required, saving time and resources, as evidenced by Kumar and Jena (2021) in studies on the removal of phosphates from wastewater. These applications reinforce the relevance of MSR as a robust and efficient approach for the improvement of environmental processes.

2 THEORETICAL FRAMEWORK

2.1 *Lithothamnium calcareum*

The *Lithothamnium Calcareum* is a marine algae belonging to the *Corallinaceae* family, according to the taxonomic classification of AlgaeBase (Soares, 2009), and is also known as bioclastic granulate due to its composition formed by fragments of organic skeletal material (Dias, 2000). Mainly composed of calcium carbonate (CaCO_3) (Caletti, 2017), its sediments widely cover the Brazilian Continental Shelf, forming one of the largest reserves of this mineral in quality and quantity (Dias, 2000; Homero, 2012). Compared to CaCO_3 of inorganic origin and that obtained from oyster shells, *Lithothamnium* differs in characteristics such as surface



area, volume and pore size, thermogravimetric profile, morphology, crystalline phase and presence of other elements (Da Silva *et al.* , 2021). Studies indicate that its porosity exceeds 40% (Carlos *et al.*, 2011; Dias, 2000), presenting an irregular surface (Veneu *et al.* , 2017; Zhu *et al.* , 2014) and cavities of 7.1 to 9.5 μ m with small fragments around (Veneu *et al.* , 2017, 2019).

The exploitation of *Lithothamnium* must preserve the living algae banks, as its growth rate is extremely low, around 1 mm per year, making it a non-renewable resource given the annual extraction of thousands of tons (Blake; Maggs , 2003; Bosence ; Wilson, 2003). Thus, only the removal of dead rhodoliths in a subsurface manner is considered economically viable and environmentally responsible (Caletti , 2017). In Brazil, Algarea Mineração S/A holds the right to explore marine biogenic limestone and carries out the monthly removal of 10 thousand tons by means of dredges coupled to ships, with production projections of 54.8 Mt /year in 2022 and 94.1 Mt /year by 2030. After collection, the material goes through washing, drying, grinding and classification stages (Nogueira, 2019).

2.2 PHOSPHORUS

Total phosphorus in domestic sewage occurs as inorganic and organic phosphate, originating, respectively, from detergents and physiological sources, with soluble polyphosphates being converted to orthophosphates by hydrolysis in the collection system itself (IAQW, 1995; Mota; Von sperling , 2009). Both phosphorus concentration and phosphorus are essential for microbial and algal growth, but its excess in water bodies can result in eutrophication, causing impacts such as aesthetic manipulation, anaerobiosis, fish mortality, difficulties in water treatment and problems in industrial supply (Von sperling , 1995; Branco; Rocha, 1979; Von sperling , 2005). Conventional sewage treatment systems prioritize the removal of organic matter, maintaining nitrogen and phosphorus concentrations similar to raw sewage, but strategies such as nutrient removal by physical-chemical or biological processes can mitigate eutrophication (Von sperling , 1995, 2009; Jordão; Pessôa , 2014). In addition, methodologies have been developed to recover and reuse these nutrients in the form of slow-release fertilizers, promoting their efficient capture and reuse (Lee *et al.* , 2022; Shao *et al.* , 2022).



2.3 EUTROPHICATION IN LENTIC ENVIRONMENTS

Excess nutrients in lakes and reservoirs can compromise their use and function, as these environments are lentic due to low flow velocity, with functions such as flood control, irrigation and domestic supply (Agostinho *et al.* , 2007). In summer, eutrophic reservoirs can develop anoxic conditions in the deeper layers, releasing phosphorus from the sediments (Lamparelli , 2004), while in winter, temperature homogenization leads to thermal inversion, which can redistribute nutrients accumulated in the hypolimnion and cause a significant deterioration in water quality (Von sperling , 2005).

2.4 NUTRIENT REMOVAL BY ADSORPTIVE PROCESSES

The need to remove various organic and inorganic pollutants has driven the search for new technologies for effluent treatment, since many of these substances, even at low concentrations, are resistant to biological degradation or are not efficiently eliminated by physical-chemical processes (Vidal *et al.* , 2014). In this context, adsorption has gained prominence in recent decades, becoming a focus of global research and an essential tool for both industrial applications and environmental protection.

2.5 ADSORPTION

Adsorption is a mass transfer phenomenon in which certain substances accumulate on the surface of porous materials, which, due to their high surface area, act as adsorbents, while the retained substances are called adsorbates (Ruthven , 1984). Adsorbents have different chemical compositions, pore sizes and structures, and can be found in various formats, such as pellets, grains and spherical particles, as well as forms derived from the reuse of waste (Geankoplis , 1993). The choice of adsorption system depends on the volume of fluid to be treated, its continuity and composition, and batch experiments are limited to small volumes and do not provide accurate data for continuous systems (Gaspar, 2003).

2.6 RESPONSE SURFACE METHODOLOGY

This method combines statistical and mathematical techniques to model and analyze how a response variable is influenced by several independent variables. It allows us to



understand the relationship between the factors involved and predict the behavior of the system under different experimental conditions. The main objective is to find the best conditions in which the independent variables maximize or minimize the desired result, optimizing processes and aiding decision-making. In addition, this approach can be applied in various fields, such as engineering, chemistry, biotechnology and environmental sciences, providing greater efficiency and control over the analyzed processes (Chelladurai *et al.* , 2021; Hadiyat ; Sopha & Wibowo , 2022).

3 METHODOLOGY

To carry out this work, a single 10L water sample was collected from Lake Açú at the Federal Rural University of Rio de Janeiro (UFRRJ) (UTM coordinates -22.7619743, -43.6959412) to perform the tests. Initially, the phosphorus concentration in the sample was determined. The methodologies applied followed the procedures established by the "Standard Methods for the Examination of Water and Wastewater " (Apha , 2018).

The *Lithothamnium calcareum* , supplied by the company PrimaSea ®, located in the Industrial District of Candeias, Bahia, was ground and processed using an electromagnetic stirrer with a set of sieves, in order to separate the material in the particle size range of 0.3 to 0.6 mm (Figure 1).

Figure 1

The left Lithothamnium calcareum grain size range from 0.3 to 0.6 mm and on the right the shape provided by the company PrimaSea ®.





The experiments involved the contact of 125 mL of the water sample from Lake Açu with the bioclastic granulate, in different combinations of concentration and time. The Response Surface Methodology (RSM) chosen in this study analyzed the variation of two factors with 5 levels each (Table 1), generating 13 combinations (Table 2), with 5 repetitions at the central point. The response variable considered was the percentage of phosphorus removal (R%).

Table 1

Response Surface Methodology with 2 factors and 5 levels each.

<i>Factors</i>	<i>Levels</i>				
	-1.41	-1	0	+1	+1.41
<i>t(min)</i>	35.15	60	120	180	204.85
<i>cGB (g/L)</i>	11.72	20	40	60	68.28

Table 2

Experiments performed with their determined time and concentration.

<i>Experiment</i>	<i>t (min)</i>	<i>cGB (g/L)</i>
1	60	20
2	180	20
3	60	60
4	180	60
5	35.15	40
6	204,85	40
7	120	11,72
8	120	68,28
9	120	40
10	120	40
11	120	40
12	120	40
13	120	40



This contact was maintained in Erlenmeyers using a " shaker " type agitator at 450 RPM. At the end of each determined time, the sample was filtered using a vacuum suction pump and fiberglass filter, to quantify the remaining concentration of phosphorus in the solution.

For each test, the removal percentage (R%) was calculated using Equation 1, where c_0 represents the initial phosphorus concentration and c_f the final concentration of the nutrient in the solution after the test.

$$\%R = \frac{(c_0 - c_f)}{c_0} \cdot 100 \quad (1)$$

Minitab 17 software was used to perform the statistical analysis and generate the aforementioned experimental design.

4 RESULTS AND DISCUSSIONS

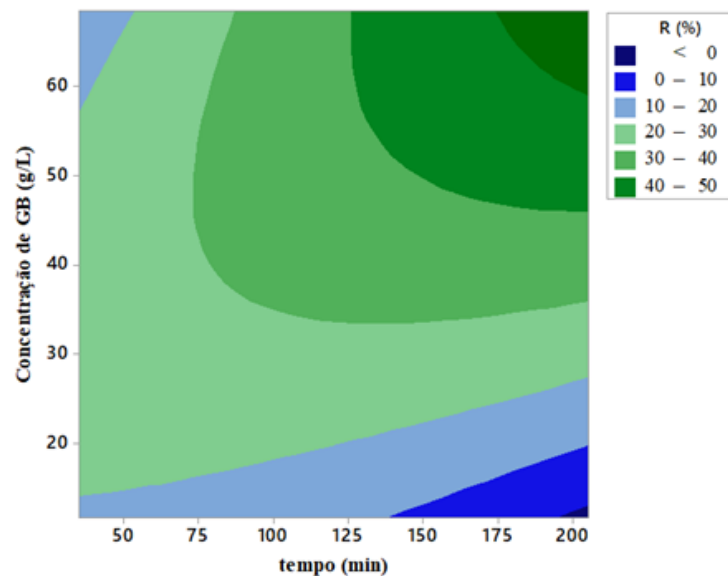
The water sample from Lake Açú used in this study showed a phosphorus concentration of 8 mg/L, a value significantly high in relation to the limits established by the environmental legislation of the State of Rio de Janeiro. According to the State Environmental Institute (INEA, 2021), the maximum permitted concentration of phosphorus for effluents discharged into lentic environments is 1 mg/L, while for lotic environments the limit is 4 mg/L.

bioclastic granulate showed a removal effect even in the shortest contact times, as can be seen in the contour surface graph (Figure 2), where even with a time of 125 to 150 minutes it was possible to remove 30-40% of phosphorus from the analyzed sample.



Figure 2

Contour surface of phosphorus removal in relation to the variables under study.



According to the analysis of variance (Figure 3), only the concentration of bioclastic granulate (GB) and the interaction 'time-GB concentration' showed influence on the response variable percentage of phosphorus removal, R% (95% confidence level). This result is consistent with what can be found in the literature. In the study by Jensen *et al.* (2022), it was observed that the increase in the concentration of modified limestone materials resulted in a greater phosphorus adsorption capacity, reaching maximum efficiencies at specific concentrations, due to the greater availability of active sites. Veneu *et al.* (2023) reported that *Lithothamnium calcareum* showed greater phosphorus removal at higher concentrations, with a direct relationship between the amount of adsorbent and the removal efficiency. Li *et al.* (2024) also demonstrated that modified oyster shells had their phosphate removal efficiency increased proportionally to the adsorbent concentration, highlighting the capacity to recover adsorbed phosphorus for use as fertilizer.

In the analysis of variance (ANOVA), the factors that presented P values lower than 0.05 were considered statistically significant for the response variable, as shown in Figure 3. These results indicate that the factors tested, including the bioclastic granulate, the contact time and the concentration of the material, exerted a relevant impact on the behavior of the variable analyzed. In addition, the interactions between these factors were also evaluated, demonstrating that their combinations significantly influence the results obtained, as indicated by the corresponding coefficients.

**Figure 3***Analysis of variance (ANOVA) of the experiment.*

Analysis of Variance					
Source	DF	Adj SS	Adj MS	F-Value	P-Value
Model	5	1155,94	231,19	11,10	0,003
Linear	2	788,40	394,20	18,92	0,002
t (min)	1	99,77	99,77	4,79	0,065
cGB (g/L)	1	688,63	688,63	33,05	0,001
Square	2	124,97	62,49	3,00	0,115
t (min)*t (min)	1	27,95	27,95	1,34	0,285
cGB (g/L)*cGB (g/L)	1	109,31	109,31	5,25	0,056
2-Way Interaction	1	242,57	242,57	11,64	0,011
t (min)*cGB (g/L)	1	242,57	242,57	11,64	0,011
Error	7	145,84	20,83		
Lack-of-Fit	3	68,75	22,92	1,19	0,420

The regression equation obtained from the coefficients calculated for each of the parameters is presented below (Equation 2).

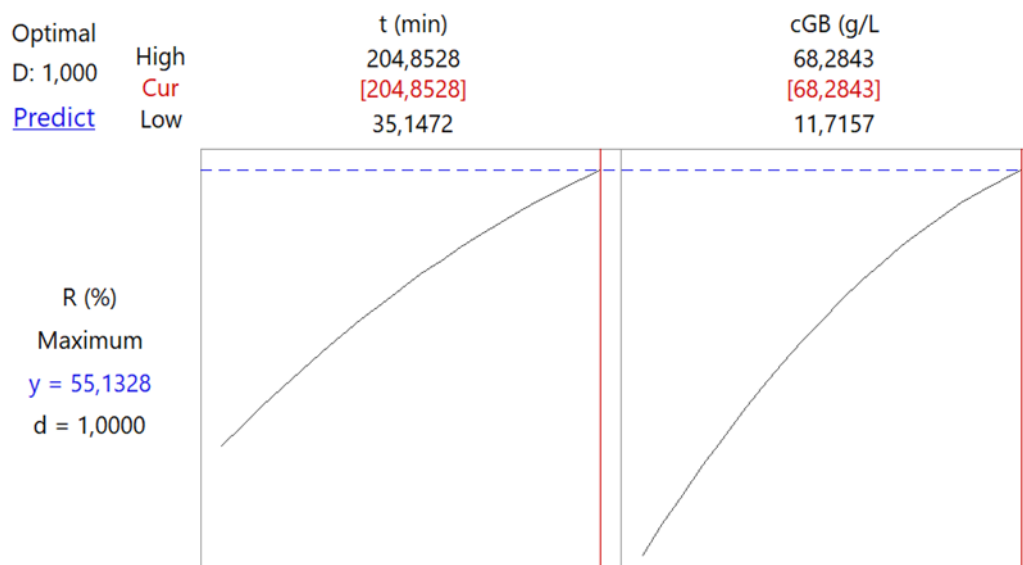
$$R (\%) = 15.0 - 0.067t + 0.478cGB - 0.000557t^2 - 0.00991cGB^2 + 0.00649 t * cGB \quad (2)$$

Using the equation/model obtained, it was possible to predict the values of each of the factors studied that maximized phosphorus removal. The maximum removal value R% estimated was 55.14% for a bioclastic granulate concentration of 68.3 g/L and a time of 204 minutes. The maximum removal observed experimentally was 48.8% for a bioclastic granulate concentration of 60 g/L and a contact time of 180 minutes. Figure 4 shows the result of the optimization performed from the maximization of the linear regression equation obtained.



Figure 4

Predicted optimization result to maximize phosphorus removal.

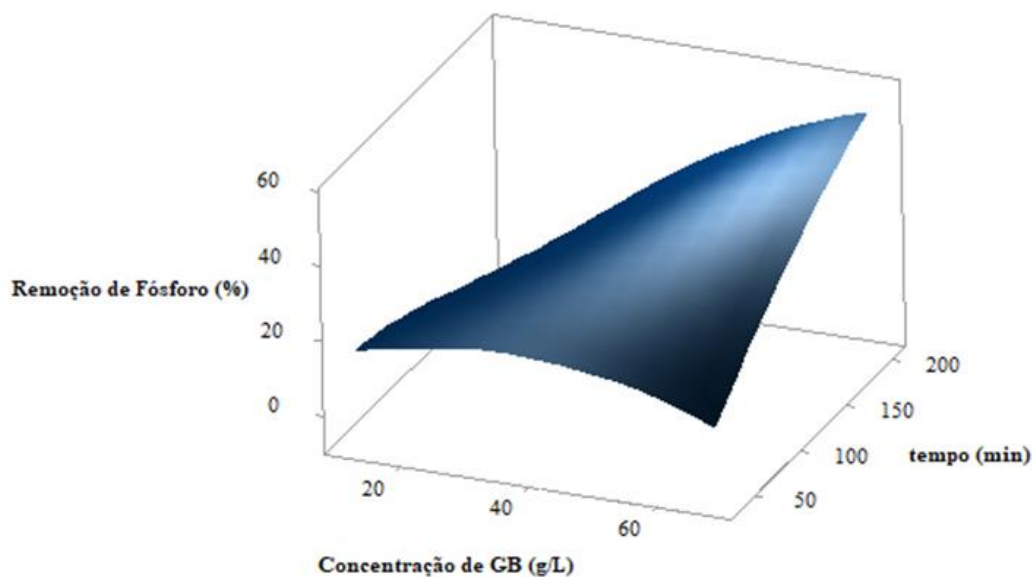


The analysis of the Response Surface (Figure 5) allowed us to observe the phosphorus removal efficiency (%R) in all experiments performed. It is clear that the determining factors for the greater phosphorus removal from the samples were the increase in contact time and the greater concentration of *Lithothamnium calcareum*. These results highlight the significant influence of these variables on the process, reinforcing the importance of their optimization to achieve high efficiencies in phosphorus removal.



Figure 5

Response surface obtained for phosphorus removal.



5 CONCLUSION

The *Lithothamnium calcareum* demonstrated relevant performance in the removal of phosphorus from the water of the eutrophic lake studied, confirming its effectiveness as an adsorbent material under critical environmental conditions. The experimental results indicated that the phosphorus sorption process was highly efficient, reaching a removal rate of 48.8%. This efficiency was maximized with the simultaneous increase of the contact time and the amount of bioclastic granulate .

The predictive analysis, performed using a regression equation that considered the coefficients of each factor studied, suggests the feasibility of reducing the phosphorus concentration in the effluent by approximately half of its initial value, under experimental conditions similar to those adopted in this study. These results demonstrate the potential of *Lithothamnium calcareum* not only on a laboratory scale, but also in larger-scale applications, highlighting its contribution to nutrient control in eutrophic aquatic ecosystems and, consequently, to improving water quality.

ACKNOWLEDGMENTS

The authors are grateful for the financial support provided by the Coordination for the Improvement of Higher Education Personnel (CAPES - master's scholarship), by the Pro-



Management Association of the Waters of the Paraíba do Sul River Basin (AGEVAP - Process 003.017.001.2020) and by the Research Support Foundation of the State of Rio de Janeiro (FAPERJ - JCNE 2019 Proc. E-26/202.750/2019 and APQ1 2023 Proc. -26/210.883/2024), which made this work possible.

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