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VALORIZATION OF THE MACROALGAE *Solieria filiformis* USING A BIOREFINERY CONCEPT

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ABSTRACT

Solieria filiformis is a red macroalgae cultivated along the coast of Ceará, which comprises various bioactive compounds of high added value. The sequential extraction of these biocompounds and the reuse of the generated residue can be approached through the concept of biorefinery, ensuring the comprehensive utilization of the resource. Thus, this study aimed to develop a valorization strategy for this species to obtain R-phycoerythrin (R-PE) and iota-carrageenan (i-CA) sequentially. Additionally, the solid residues were utilized for biogas production, thereby addressing the biorefinery concept. The yields of R-PE and i-CA reached 0.406 ± 0.044 mg/g (PI=0.798 ± 0.044) and 319.60 ± 82.21 mg/g, respectively. The solid residue resulting from carrageenan extraction achieved the highest specific biogas production (37.3 mL gSV^{-1}). Overall, the proposed biorefinery strategy proved capable of producing significant bioactives for cosmetic, nutraceutical, and functional food applications.

Keywords: Biorefinery. Sequenced extraction. R-phycoerythrin. Carrageenan. Biogas

1 INTRODUCTION

The red macroalgae *Solieria filiformis* is a native species found along the entire Brazilian coastline. It represents a promising raw material for obtaining high-value compounds with potential development within the scope of the blue economy. It contains high levels of protein, with R-phycoerythrin (R-PE) being the major component, and synthesizes high levels of sulfated polysaccharides, primarily composed of carrageenans[1]. When extracted, R-PE exhibits fluorescence and numerous important biological activities, such as antioxidant and anticancer properties, making it widely applicable in industrial settings. Iota-carrageenan (i-CA) is used as thickeners and gelling agents in the food and cosmetic industries[2], and it also holds potential for health benefits due to its anticoagulant and antibiotic actions.

The acquisition of these bioproducts can be achieved through a sustainable alternative approached by the concept of biorefinery. This concept facilitates the efficient conversion of biomass into a wide range of products and energy, aiming to optimize the utilization of natural resources and minimize environmental impacts. By embracing this approach, not only do we maximize the efficiency of biomass valorization processes, but we also mitigate waste and promote sustainability throughout the production chain[2,3]. In addition to directly obtaining these bioproducts, the *Solieria filiformis* biorefinery aims to enhance efficiency by converting the remaining solid residues into biogas. This waste valorization process is crucial for minimizing environmental impact and optimizing resource utilization, thereby transforming byproducts into renewable energy sources. The analysis of this waste-to-biogas conversion underscores the holistic and sustainable approach of the biorefinery, fostering circular economy principles and contributing to the reduction of fossil fuel dependency.

In light of this context, this study aims to assess the potential of the macroalgae *Solieria filiformis* in obtaining R-PE and i-CA through sequential extraction, followed by analyzing the biogas production from the residues resulting from the individual extractions of R-PE and i-CA, as well as from the residue resulting from the sequential extraction of both products. This process seeks to maximize the benefits and profits derived from the biomass, adding economic, social, and environmental value to it.

2 MATERIAL & METHODS

The macroalgae *Solieria filiformis* was cultivated on Flecheiras Beach, in the municipality of Trairi (Ceará). The algae were harvested and washed with running and distilled water to remove unwanted contaminants. The extraction of R-PE was carried out by grinding the macroalgae in an electric grinder for 6 min, immersed in phosphate buffer (25 mM) at a ratio of 1:2 (w/v), followed by homogenization for 4 h. The extract was centrifuged, and the supernatant was analyzed in a UV-VIS spectrophotometer to obtain total protein content[4], R-PE concentration[5], and purity index[6]. The concentration of R-PE, purity index, and yield (Yield R-PE) were determined using equations 1, 2, and 3, respectively.

$$[R_{PE}]\,(mg/mL) = 0.1247 \times [(Abs_{564} - Abs_{730}) - 0{,}4583 \times (Abs_{618} - Abs_{730})] \quad (1)$$

$$PI = Abs_{564}/Abs_{280} \quad (2)$$

$$Yield\ R-PE\ (mg/g) = [R_{PE}] \cdot V/mass \quad (3)$$

where [R_{PE}] is the R-phycoerythrin concentration (mg/mL), and Abs_{564}, Abs_{618}, and Abs_{730} are the absorbances of samples at 564, 618, and 730 nm, respectively. The absorption of total protein is represented by Abs_{280}.

The resulting biomass was dried at 40 °C to proceed with the sequential extraction. The extraction of iota-carrageenan (i-CA) commenced with the rehydration of the biomass with 0.1 M KOH and 1 % (w/v) under agitation for 24 h. The resulting extract was filtered and then heated with 100 mL of distilled water for 3 h and precipitated with ethanol. The obtained i-CA was lyophilized, and its yield was determined. The biogas production from the residues of the individual extractions of R-PE and i-CA, as well as the residue resulting from the sequential extraction of both products, was evaluated. The inoculum was sludge collected from an Upflow Anaerobic Sludge Blanket (UASB) reactor, acclimatized for seven days at 37 °C. The ratio between the mass of volatile solids (gSV) of the residue and the inoculum in the reaction mixture was 1:2. The biogas production potential of the ground macroalgae, residues from the individual extractions of R-PE and i-CA, and the residue resulting from the sequential extraction of both products (PECA) was assessed through anaerobic digestion (AD) specific biogas production assays. The assays were conducted in duplicate, with each using 20 % by mass of inoculum and a ratio between the mass of volatile solids (gSV) of the residue and the inoculum in the reaction mixture of 1:2, diluting the residue with water to achieve the desired value. Penicillin flasks, hermetically sealed with a volume of 100 mL and 50% headspace, equipped with 60 mL syringes, were used for the assay. The flasks were placed in a shaking incubator at 100 RPM, with temperature control set to 37 °C, and the medium pH adjusted to 7.5. The sequential extraction process can be represented in Figure 1.

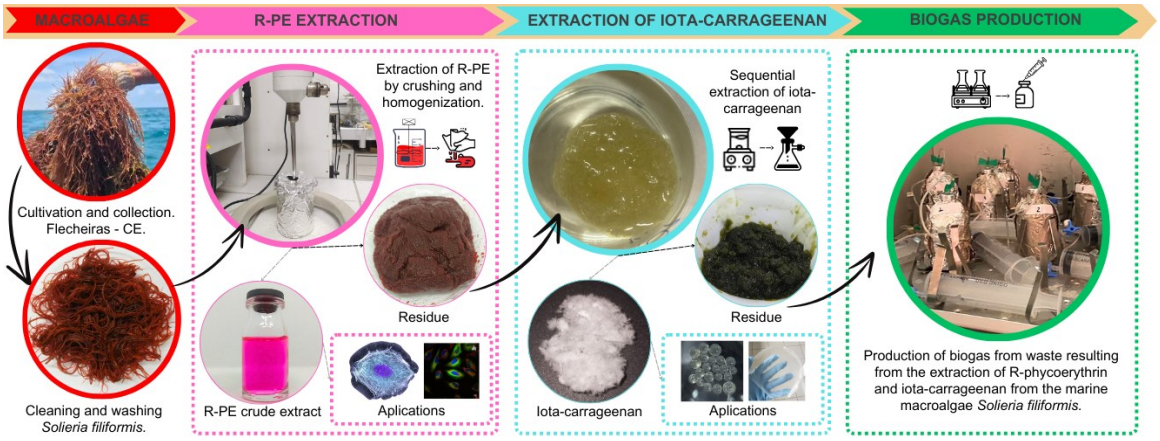

Figure 1. Schematic representation of sequential extractions using a biorefinery approach.

3 RESULTS & DISCUSSION

R-PE is the primary phycobiliprotein found in red algae, serving as an important pink-red, water-soluble pigment that plays a crucial role in photosynthesis[7]. In this study, the initial step of the sequential extraction process involved extracting R-PE from the macroalgae *Solieria filiformis* (300g) in an aqueous medium using homogenization. The concentration of total proteins obtained reached 0.264 ± 0.018 mg/mL, while the R-PE concentration reached 0.203 ± 0.005 mg/mL. A purity index (PI) of 0.798 ± 0.044 was achieved. The low PI may be related to the extraction of other phycobiliproteins, such as phycocyanins, or other protein families present in *Solieria filiformis*. The yield reached 0.406 ± 0.044 mg of R-PE per gram of wet biomass in a 4 h extraction period. These values were comparable, or even superior, to those reported for other Rhodophytas. Bastos Filho (2016)[8] extracted R-PE from *Solieria filiformis* using the homogenization method and estimated a maximum R-PE yield of 0.031 mg/g of wet biomass. Zhao et al (2020)[9] extracted R-PE from *Gracilaria tenuistipitata* using the homogenization method combined with freezing and thawing, achieving a yield of 0.19 mg/g of wet biomass. The study conducted was able to present an optimization process, as it increased the yield in a shorter period of time.

The grinding process reduces the macroalgae particle size, contributing to a greater exposure of R-PE. The increase in R-PE yield is attributed to the larger surface area, which also exposes other proteins, polysaccharides, and lipids during the extraction process. Concurrently, the homogenization process promotes better system homogenization and greater contact between the solvent and the biomass. In these results, the extracted R-PE exhibited fluorescence, thereby preserving its biofunctionality for pharmaceutical applications. As depicted in Figure 2, the R-PE extract displayed absorption peaks at 499 nm and between 540 and 566 nm, along with a fluorescence peak at 573 nm. The extracts exhibited characteristics consistent with R-PE.

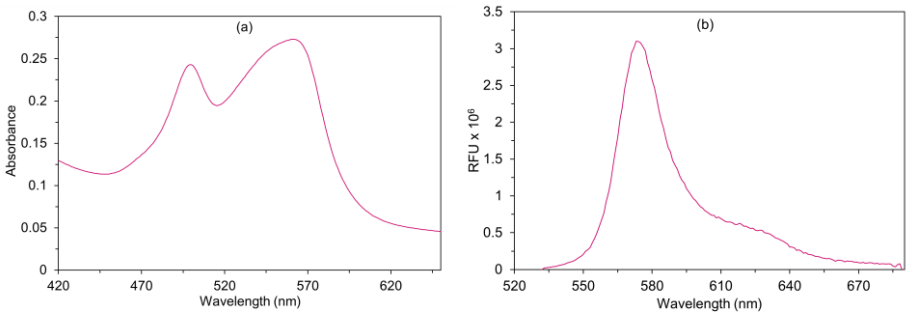

Figure 2. Spectrophotometric absorption analysis (a) and fluorometric analysis (b) were obtained by extraction of *Solieria filiformis*. RFU = Relative Fluorescence Unit.

In the second stage of the sequential extraction process, the residual biomass resulting from the protein extraction (235g) was subsequently processed for the recovery of iota-carrageenan from *Solieria filiformis*. The extraction yielded 319.60 ± 82.21 mg of carrageenan extract per gram of dried residue. The carrageenan yields obtained in the biorefinery process of *Solieria filiformis*, through sequential extraction, reached 28.43 %, while that obtained by direct extraction from the macroalgae was over 30 %, similar to this study. Penuela et al. (2018)[2] analyzed the sequential extraction of carrageenan from *Solieria filiformis* using the microwave-assisted extraction method, within the biorefinery concept, and the yields varied between 17.1 % and 29.7 %. Sousa (2016)[1] extracted sulfated polysaccharides from *Solieria filiformis* by enzymatic digestion with papain, achieving a yield of 21.3 %. The sequential extraction in this study demonstrated efficiency, as the yield achieved is comparable to the direct extraction from the macroalgae in the presented studies.

It is worth noting that the yields of carrageenan from sequential extraction can be significantly influenced by the extraction conditions of the first sequential stage. During the R-PE extraction process, the grinding process stimulates the rupture of the cell wall, facilitating the mass transfer of active compounds into the aqueous medium. In this process, a percentage of sulfated polysaccharides may be extracted and consequently lost for the subsequent stage. However, this weakening of the cell wall could potentially be a positive point for the sequential stage, as it facilitates the removal of bioactives that are more difficult to extract. However, in the present study, the use of this pretreatment before carrageenan extraction did not significantly improve the yield. Additionally, the carrageenan extraction process reaches high temperatures (80 °C), capable of reducing the viscosity of the medium, thereby accelerating the diffusivity of the bioactive from the matrix to the aqueous medium.

The extracted bioactives are part of a larger study whose research group analyzes their applications. The extracted R-PE is being studied for biological application as a fluorescent dye[10], anti-inflammatory activity and in vitro cytotoxicity in cancer cells[11]. As well as carrageenan for the production of biofilms and adsorbents.

In the last stage of the biorefinery process, biogas production took place at the latest until the end of the fifth day of experiments. The residue from i-CA extraction stood out with the highest specific biogas production (37.3 mL gSV^{-1})). However, a reduction in pH was observed due to the formation of fatty acids and an increase in viscosity and gelatinous appearance of the reaction medium, probably due to the ability of carrageenan to form gels and stabilize emulsions in aqueous solutions in the presence of divalent cations, damaging the diffusion of nutrients and consequent production of biogas. This event was not observed in any other experimental condition evaluated. There was no biogas production in the AD of the PECA, possibly due to the low concentration of volatile solids resulting after the sequenced extractions of the compounds. The results of biogas volume per gram of volatile solids and the final pH of the reaction are described in Table 1.

Table 1 – Specific biogas production and final pH.

	R-PE	i-CA	PECA
Specific Biogas Production (mL gSV^{-1})	13,0 ± 1,2	37,3 ± 0,9	0,0 ± 0,0
pH_{final}	7,4	4,45	6,4

4 CONCLUSION

Based on the findings of this study, it can be concluded that the macroalgae *Solieria filiformis* exhibits significant potential for sequential extraction of molecules such as R-phycoerythrin and iota-carrageenan, yielding high extraction efficiencies that are advantageous for sustainable development. Furthermore, the production of biogas from the residue resulting from carrageenan extraction was successfully demonstrated. These results underscore the viability of utilizing *Solieria filiformis* within a biorefinery framework, highlighting its capacity to not only yield valuable bioproducts but also to generate renewable energy from waste streams, thus contributing to the advancement of sustainable practices in bioprocessing.

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