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Structural Characterization of Biomass from *Crassiphycus birdiae in natura* and Residual

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Macroalgae contains micro and macromolecules of great interest for various sectors, for example, food, cosmetics, and pharmaceuticals. These compounds can be obtained through different extraction methods, among them the use of organic solvents with varying polarities. In this study, materials were characterized using scanning electron microscopy and energy-dispersive X-ray spectroscopy, infrared spectrometry, X-ray diffraction and differential scanning calorimetry in the seaweed biomasses before and after an extraction process with organic solvents. The red macroalgae *Crassiphycus birdiae*, cultivated on the northeast coast of Brazil, appeared, after extraction, as an amorphous material with some porosity. Its composition includes the elements carbon, oxygen, sodium, magnesium, sulfur, chlorine, potassium, aluminum, silicon, calcium, and iron. The presence of the agar polysaccharide was also verified by infrared spectroscopy. The decomposition of this polysaccharide was observed using differential scanning calorimetry.

Keywords: macroalgae, red seaweed, residue, biomass, characterization analysis

Introduction

Brazil has an extensive coastline (more than 10,000 kilometeres) with great biodiversity. Macroalgae are found across the coast but mainly in the northeast region of the country. The cultivation of algae must be done in regions that follow criteria for its application, such as socio-economic and environmental factors.

Seaweed is an ideal raw material for obtaining bioproducts because it grows relatively quickly, does not compete with agricultural areas, requires neither inputs nor irrigation, contains compounds with unique chemical structures, and features a cell wall with no lignin.[1-3] Macroalgae are multicellular eukaryotic aquatic organisms that can be classified as green, brown, and red seaweed.[4]

Red macroalgae produce primary and secondary metabolites with great industrial potential. In addition to being evaluated for their nutritional composition,[5,6] they are studied for various applications. They have a high content of proteins and, minerals (e.g., Ca, Mg, Na, K, Fe, Mn, Zn, Cu, Ni, Co, Cr, and Cd)[7] in addition to several halogenated compounds,[8] vitamins, dietary fiber, fatty acids, polysaccharides,[9,10] and bioactive compounds that are promising primarily for pharmaceutical applications, such as anti-inflammatory, antioxidant, antiviral, antifungal, antibacterial, and anticoagulant action, among others.[11,12]

According to Nakhate and Van der Meer,[13] globally, some species of macroalgae are cultivated commercially but with a focus on food applications. However, based on their macro- and micromolecules they may show promise for other biotechnological applications. Cultivation of seaweed should be done in a conscientious and sustainable manner, as it can be used to sequester heavy metals, limit eutrophication, and achieve carbon sequestration.

One of these cultivated algae, *Crassiphycus birdiae* (E. Palestino & E. C. Oliveira) Gurgel, J. N. Norris & Frederick[14] has great added value due to its biomass, which can be used in the production of bioproducts.[15]

The secondary metabolites are bioactive compounds that can be extracted and used for different procedures and as solvents. For example, the fatty acids extracted from seaweed can be used for biodiesel production.[16] Torres *et al.*[17] presented several metabolic approaches in species of the genus *Gracilaria* Greville and their respective

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biological activities, showing the diversity of compounds and their applicability.

The biomass is formed by a fibrillar skeleton with an amorphous matrix.[18] The polysaccharide present in the amorphous matrix of the biomass from *Crassiphycus birdiae* is a known agar and has sulfated groups as its structure.[19]

There is little information about the characterization of residual biomass or the structural verification of the seaweed biomass after modification. For example, Gouveia *et al*.[20] analyzed the biomass of *Gracilaria domingensis* (Kützing) Sonder ex Dickie using different microscopy techniques, such as light microscopy, confocal laser scanning microscopy, transmission electron microscopy, and scanning electron microscopy, where the objective was to verify the modification of morphology in the biomass of algae submitted to different concentrations of lead and copper. In another study, Ayres-Ostrock and Plastino[21] observed changes in different strains from *C. birdiae* after exposure to UV-B radiation. Scanning electron microscopy was used to visualize these morphological changes.

Thus, this work aimed to observe structural changes caused by using the dichloromethane extraction method in *Crassiphicus birdiae* through micrographs from scanning electron microscopy (SEM) and the use of energy-dispersive X-ray spectroscopy (EDX) techniques to qualitatively verify the chemical elements before and after extraction. Other techniques used for biomass characterization were infrared spectroscopy (IR), X-ray diffraction (XRD), and differential exploratory calorimetry (DSC). The structural characterization of biomass is important for understanding its possible applications as a raw material for producing bioproducts.

Experimental

Algal material

The biomass of *Crassiphycus birdiae* (E. Palestino & E. C. Oliveira) Gurgel, J. N. Norris & Frederick was obtained commercially from a culture at Region de Pitangui, Rio Grande do Norte State, Brazil. The samples were ground with a mortar and pestle to obtain smaller particles and biomass powder.

Extraction process

The previously dried, cultivated algae underwent extraction with dichloromethane (CH_2Cl_2) from VETEC in Rio de Janeiro, Brazil, for one week at room temperature and were not shaken. After this period, the biomasses were dried at room temperature.

Biomass characterization utilizing scanning electron microscopy with energy dispersive spectroscopy

Initially, these samples of dried algae both *in natura* (GBI) and after the extraction process (GBEXT) were sputter coated with platinum using LEICA EM ACE-600 (Rio de Janeiro, Brazil) under a current of 50 mA for 2 min, and analyzed with an FEI field emission scanning electron microscope with a secondary electron detector and energy-dispersive X-ray spectroscopy (EDX) model X FLASH 6/60 manufactured by Bruker of Rio de Janeiro, Brazil.

Table 1 shows the parameters used in the scanning electron microscopy (micrographs) and their corresponding figures.

Table 1. Figures and their parameters analysis by scanning electron microscopy (SEM)

Figure	Sample	Voltage / kV	Working distance / mm	Magnification
1a	*in natura* biomass	10.00	18.8	500
1b	*in natura* biomass	10.00	10.3	1000
1c	*in natura* biomass	10.00	18.7	5.000
1d	after extractive process	5.00	15.8	500
1e	after extractive process	5.00	9.9	1.000
1f	after extractive process	5.00	15.9	5.000
2a	*in natura* biomass	10.00	19.2	50
2c	after extractive process	5.00	15.9	50
3		10.00	10.3	20.000

Characterization of algal biomass samples (*in natura* and residual) via infrared

Infrared spectra of macroalgae samples were produced with IRTracer-100, a Fourier transform infrared spectrophotometer, and attenuated total reflectance (ATR) MIRacle (accessory) from Shimadzu Corporation, Niterói, Brazil. The analysis was measured using a DLATGS detector with scan in the range 7800 to 350 cm^{-1} and resolution of 1 cm^{-1}.

The analysis of the solid biomass samples was performed to observe the bands related to the functional groups present.

Characterization of algal biomass samples (*in natura* and residual) by X-ray diffraction

The biomass powder samples were analyzed using X-ray diffraction to identify possible crystalline structures. The Bruker (Niterói, Brazil) D8 Advance diffractometer

with detector LynXeye, equipped Cu Kα radiation (λ = 1.54060 Å). The operating voltage and current were 50 kV and 100 mA, respectively. Scans were collected from 2θ = 10 to 60° with step size of 0.02 at 0.5 s *per* step, and rotation rate of 15 rpm.

Characterization of algal biomass samples (*in natura* and residual) using differential scanning calorimetry

The samples (12 mg) were analyzed using the DSC 404 F1 Pegasus Netzsch (Rio de Janeiro, Brazil), calibrated with an indium sample in a dry atmosphere of passing nitrogen. The temperature was 50 to 300 °C, ranging from 10 °C min^{-1}. Changes were measured as enthalpy changes in temperature function.

Results and Discussion

The red marine macroalgae cultivated on the Brazilian coast was analyzed in this study. The polysaccharides existing in red algae have different applicability in industrial sectors, and, as biosorbents, they are natural environmental indicators.[22] Beyond that, there is little research that uses algae for cultivation.[8]

The *Crassiphicus birdiae* is an important macroalgae because its cell wall is primarily formed by polysaccharides. The micrographs of the *in natura* biomass presented a dense morphology and low porosity, and another sample, the extracted biomass, showed an increase in porosity morphology (Figure 1).

The secondary metabolites found in red macroalgae can be halogenated substances of different chemical classes.[8] The red macroalgae have a high number of polysaccharides incorporated into their amorphous matrix, and these structures are related to the biosorption process. Biosorption is the ability of biomasses in aqueous solutions to remove heavy metals.[18] Several factors can influence this process, such as the type of biomass in the algae, the pH, the concentration of heavy metals, the presence of competitive ions, and the temperature.[23]

The samples of *C. birdiae* were analyzed using EDX. The EDX spectrum identified a qualitative composition that detected carbon, oxygen, sodium, magnesium, silicon, sulfur, chlorine, potassium, calcium, and aluminum. The spectrum of the EDX from the algae biomass extracted with dichloromethane presented the chemical elements previously mentioned, along with iron. The morphology and biomass composition of *C. birdiae* were analyzed using SEM and EDX. Figure 2 presents the SEM of the *in natura* and after extraction process of the biomass (the rectangle marks the analyzed area), and the EDX spectra present chemical elements in the seaweed.

Red macroalgae are composed of cellulose or xylan, hemicellulose, and an amorphous matrix formed of sulfated galactans (e.g., agar, carrageenan, porphyran) that are responsible for most parts of the algal cells. They can produce and store Floridian starch,[18] and they are systems with different functions and complex organizations.[22]

Red macroalgae are excellent producers of halogenated

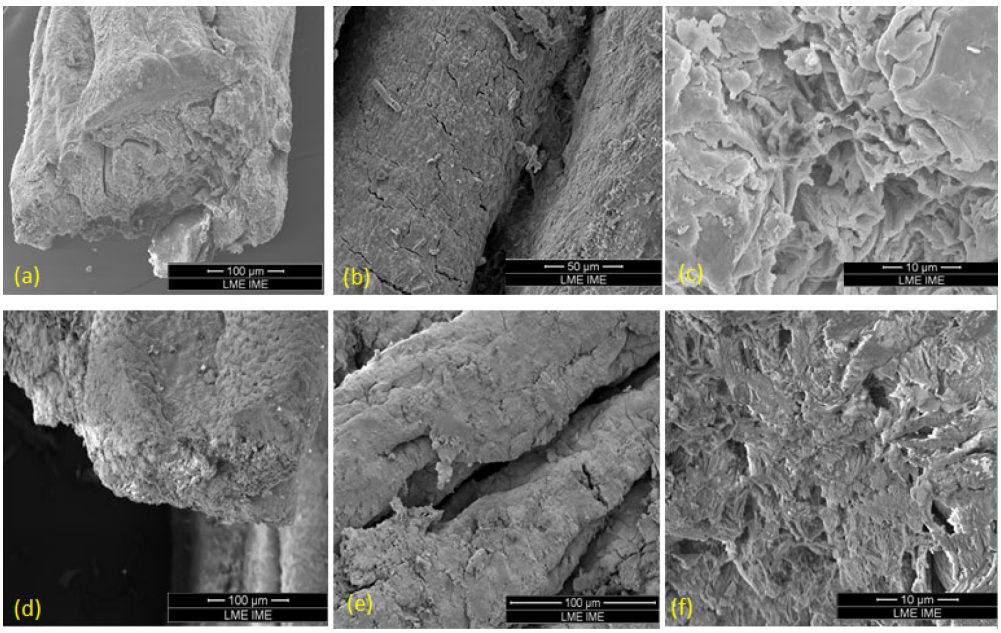

Figure 1. Micrographs of the *in natura* and after extraction process biomass from the *Crassiphycus birdiae*. (a), (b) and (c) micrographs of the *in natura* biomass and (d), (e) and (f) after extractive process of the biomass with 500×, 1000×, and 5000× magnification, respectively.

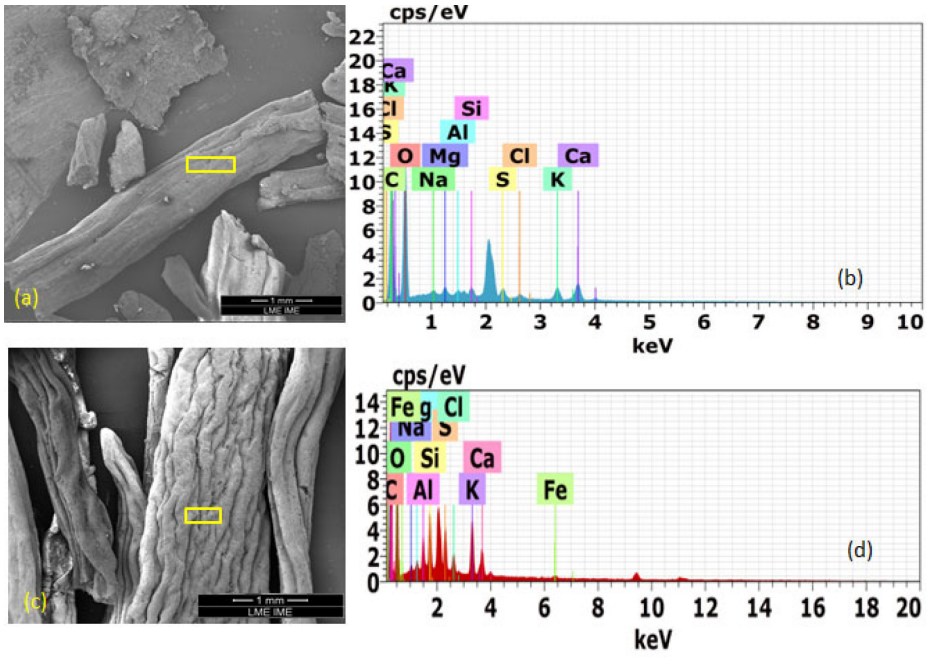

Figure 2. SEM and EDX of the samples *in natura* and after extraction process biomass: (a) SEM *in natura* biomass, (b) EDX spectrum *in natura*, (c) SEM after extractive process, and (d) EDX spectra extracted biomass magnified by 50× in both samples. The unmarked peaks are relative to platinum.

secondary metabolites, and chlorine and bromine can present in different classes of substances.[24]

Crassiphycus birdiae is a red marine macroalga with agar in its cell walls. EDX detected the chemical elements that comprise this biomass in the different conditions of the analysis. Carbon and oxygen, for example, form different classes of organic substances in the primary and secondary metabolites.

The chemical element S is present in the complex substance agar. The presence of the chemical element iron in the algae *C. birdiae*, after dichloromethane extraction, suggests that this element was removed from the environment by the polysaccharides of the cell wall, but the modification in the macroalgae biomass allowed this element to be exposed and thus easier to detect using EDX technology.

It was possible to observe diatoms in the micrographs of *in natura C. birdiae* at a magnitude of 20.000×. Figure 3 shows a micrograph of the diatoms and spectrum of the EDX with the chemical elements carbon and potassium, and high silicon and oxygen content. The element Si was present in the EDX spectra, and the SEM images showed diatoms, which are marine organisms composed of silicon shells.[25,26]

In a study by Costa *et al.*,[27] the residues from brown alga *Sargassum filipendula* C. Agardh after extraction of the alginate, were analyzed using SEM-EDX, which detected the chemical elements Si, Na, Mg, Al, S, K, Ca, and Fe. Lima *et al.*[28] verified, using SEM-EDX, the

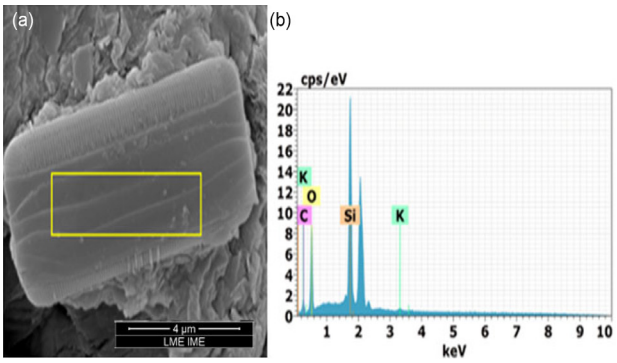

Figure 3. Micrograph and EDX spectra of the diatom present in the *Crassiphycus birdiae*: (a) micrographs of the diatom with regions selected for EDX analyses; (b) EDX spectrum. The unmarked peaks indicate relative platinum.

difference between *in natura* and residual biomass after extraction with organic solvent from the brown alga *Dictyota menstrualis* (Hoyt) Schnetter, Hörnig & Weber-Peukert and diatoms were also observed throughout the macroalgae. The chemical composition detected carbon, oxygen, silicon, sodium, magnesium, aluminum, sulfur, chlorine, potassium, and iron. Risjani *et al.*[29] also used microscopy based on the light and scanning electron to identify different diatoms in Indonesia. Some of these diatom samples were obtained from seaweed.

Biomass from *C. birdiae* was also analyzed using IR, and the spectrum shows bands characteristic of agar in both samples. This compound was extracted not with organic solvent but through hot water extraction. This

is a very important hydrocolloid that can be utilized in pharmaceutics and biotechnological studies.[30]

The IR spectra of the solid samples before and after the dichloromethane extraction process showed similarities. The bands observed refer to the major polysaccharides of the cellular wall, which were not removed in the extraction process. The spectra of Figure 4 show the infrared of the *in natura* and residual biomass analyzes both presented bands with wavelengths at 1630 cm^{-1} referring to C=O. Bands absorbing 1367 and 1249 cm^{-1} are relative to the group ester sulfate, and S=O, 1155 and 1036 cm^{-1} are possibly related to the C–O–H, C–O, and C–C groups of polysaccharides. At 927 cm^{-1} it refers to the C–O–C group of 3,6 anhydro-α-L-galactopyranose band at 886 cm^{-1} characteristic of CO–SO_3 agar.[31,32]

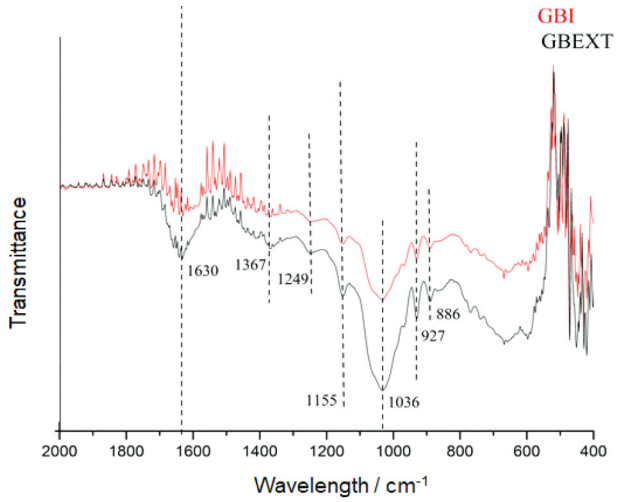

Figure 4. Infrared (ATR) spectra of *in natura* biomass (GBI) and residual biomass (GBEXT) from *Crassiphycus birdiae*.

The diffractograms show poor crystallinity in the algal cell wall, but regions indicative of amorphous substances were observed in both samples as shown in Figures 5 and 6. In a study by Long *et al.*,[33] the polysaccharide from *Gracilaria lemaneiformis* (Bory) Greville was observed in 2θ two broad peaks at 19 and 25.2°. These regions can be observed in both samples of seaweed in the present study.

In the DSC thermogram of the *C. birdiae in natura* sample, it was possible to observe two distinct regions, one endothermic (20-220 °C) with energy of 177.35 J g^{-1}, a process of possible dehydration, evaporation of volatile compounds, devolatilization, and a sample with a maximum peak at 90.5 °C, and the other exothermic (230-285 °C) with energy of –39.94 J g^{-1} regarding the degradation of polysaccharide (agar) with a maximum peak of 261.8 °C, as shown in Figure 7.

The residual biomass thermogram presented an endothermic region (40-130 °C) with a maximum peak

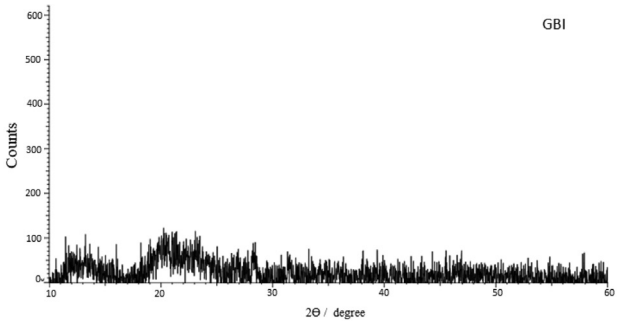

Figure 5. The diffractogram GBI is referent to seaweed biomass *in natura*.

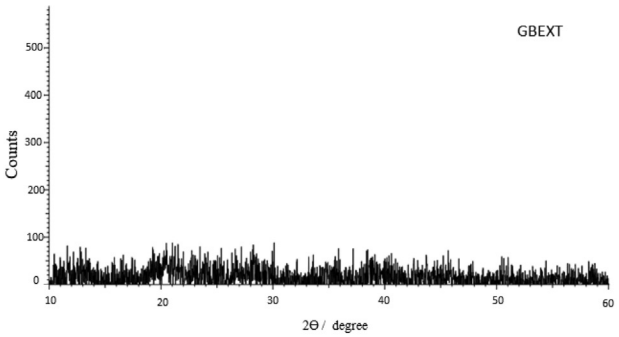

Figure 6. The diffractogram GBEXT to seaweed biomass after extraction.

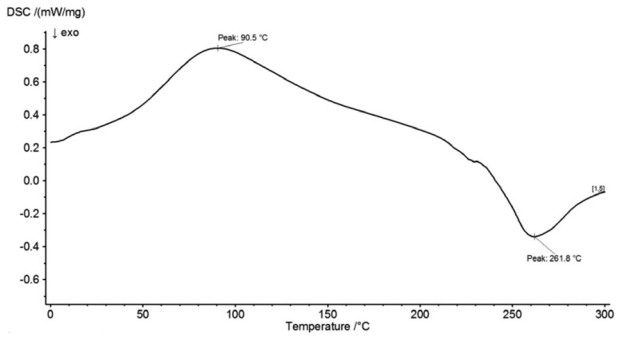

Figure 7. DSC thermogram of *Crassiphycus birdiae in natura* (GBI).

at 92.1 °C with energy of 50.52 J g^{-1} referent a process of possible dehydration and devolatilization. This endothermic temperature range appears to be lower than that *in natura* biomass, suggesting the influence of extraction with dichloromethane in this region. The other region is exothermic (245-270 °C) with a maximum peak of 260.6 °C with –7.881 J g^{-1} referent the degradation of polysaccharide (agar), as shown in Figure 8.

The samples analyzed in this work reached a temperature of 300 °C, because samples above this temperature would not be readable by the equipment used. The maximum peak in the two samples did not vary significantly. The higher mass loss of the macroalgae samples is associated with the exothermic region of the DSC thermogram, due to the decomposition of agar (polysaccharide of the algae cell wall), as has also been reported by Li *et al.*[34]

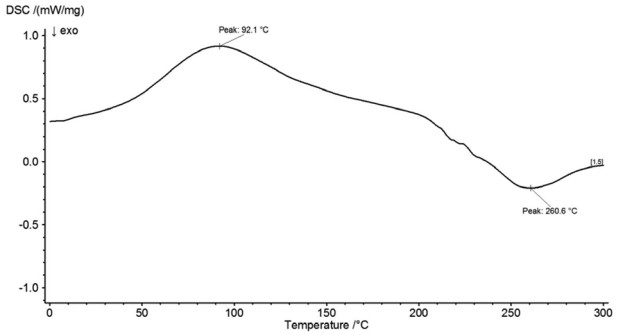

Figure 8. DSC thermogram of residual biomass from *Crassiphycus birdiae* (GBEXT).

Conclusions

In the SEM images from *Crasiphycus birdiae in natura*, it was possible to observe dense biomass. The micrographs of another sample revealed an increase in porosity morphology, with the chemical elements carbon, oxygen, sodium, magnesium, silicon, sulfur, chlorine, potassium, calcium, aluminum, and iron detected. Through micrographs, it was possible to observe the diatom; the chemical elements detected were C, O, K, and Si.

Infrared spectrum of the biomass showed bands characteristic of the polysaccharide agar, and while the X-ray diffraction presented poor crystallinity, it was possible to observe an amorphous region that indicated a polysaccharide presence in the seaweed cell wall.

A thermogram of DSC showed endothermic regions from dehydration and evaporation of the volatile compounds and exothermic regions (agar degradation) in both samples, but the GBEXT sample suggested a reduction in the evaporation region of the volatile compounds because this sample is the residual biomass after extraction with dichloromethane.

The characterization of the macroalgae residual biomass from *Crassiphycus birdiae* was important for showing how promising it can be as a matrix to obtain bioproducts, taking into account the residual valorization of the biomass (sustainability).

Acknowledgments

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