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Research paper

Chemical, structural, and ultrastructural analysis of waste from the carrageenan and sugar-bioethanol processes for future bioenergy generation

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ABSTRACT

Macroalgae and sugarcane biorefineries are designed to generate bioproducts for commercial use. Due to the high carbohydrate content of *Kappaphycus alvarezii* and sugarcane bagasse (SCB), biorefineries are aimed at generating such bioproducts as bioethanol, sucrose, carrageenan, and electricity production. Although there are several studies on SCB, the comparative study between residue carrageenan extraction and SCB has not yet been published. The samples were chemically characterized followed by structural, ultrastructural, and enzymatic hydrolysis analyzes. The content of protein in the wastes varied depending on the method used. Galactan (8.2%) and glucan (55.3%) were major polysaccharides in the residue carrageenan extraction, whereas in SCB, major polysaccharides were hemicellulose (25.2%) and glucan (38.2%). SCB was found to contain 24.5% of lignin, but the residue carrageenan extraction showed the presence of only 4.5% of insoluble aromatic compounds. ATR, NMR, and UV-Visible data on the residue carrageenan extraction revealed that the glucan fraction was similar to that in SCB. In contrast, the XRD analysis of SCB revealed a higher index of crystallinity than in the residue carrageenan extraction. The residue carrageenan extraction from the *K. alvarezii* does not show any type of recalcitrance against the enzymatic hydrolysis of cellulases, being hydrolyzed 100% in glucose. However, SCB showed a maximum conversion to glucose of 30%, requiring an additional pretreatment step. Thus, a biorefinery of *K. alvarezii* can be exploited not only to produce carrageenan but also to generate glucose for future bioenergy generation. An example would be the production of fourth generation bioethanol.

1. Introduction

The total production of sugarcane in Brazil in the 2015/2016 period was 656 million tons. The juice from sugarcane generated in the same period was sufficient to produce 38 million tons of sugar (sucrose) and 28 million of cubic meters of bioethanol (anhydrous plus hydrate) [1]. SCB is an abundant agricultural residue from the sugar-bioethanol process. The yield of SCB (dry bagasse) after extraction of sugarcane juice (wet sugarcane) is approximately 13% [2]. It is mainly used for production of steam/electricity and is a promising substrate for biorefineries like the second-generation ethanol production facilities [3]. Although SCB contains sufficient cellulose to serve as an excellent source of sugars for ethanol production, it is a recalcitrant lignocellulosic material that requires efficient pretreatment to ensure the conversion of cellulose to glucose by enzymes [4,5]. The recalcitrance of lignocellulosic materials is related to several factors, including the close association of cellulose with hemicellulose and lignin in the cell wall; this situation hinders the enzyme infiltration and action [6].

The utilizing of algae is a promising and economically viable alternative for the manufacture of such bioproducts as biofuels, foods,

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cosmetics, nutraceuticals, pharmaceuticals, and biofertilizers [7–10]. Besides, the cultivation of algae species promotes advantages such as absorbing carbon dioxide through the process of photosynthesis. This capture of carbon dioxide can help minimize the greenhouse effect. [11–14]. Moreover, algae produce more oxygen than consume in their breathing process in contrast to terrestrial plants. In addition, algal cultures show an average productivity of 22 $kgm^{-2}year^{-1}$ while terrestrial plants of 0.5–4.4 $kgm^{-2}year^{-1}$ [14–16]. Therefore, algae biomass can provide many environmental and economic benefits.

Marine macroalgae can be classified into three main groups: brown macroalgae (Phaeophyceae), green macroalgae (Chlorophyceae), and red macroalgae (Rhodophyceae) [17,18]. The composition of red macroalgae varies from species to species but generally includes cellulose, glucan, galactan, and minerals (measured as ash). Glucans consist of polymers containing the fundamental unit of glucose. Galactans consist entirely of galactose and 3,6-anhydrogalactose. The substitution pattern of the sulfate groups and the amount of 3,6-anhydrogalactose vary among different classes of these compounds, depending on ecological conditions. The cell wall of red seaweed is made of glucan and two kinds of long-chain structural polysaccharides that are valued for their gel-forming ability, i.e., agar and carrageenan [19]. Carrageenan can be classified as lambda (λ), kappa (κ), or iota (i) according to the-gel-forming ability and is used mainly for thickening of foods such as yogurt, ice cream, and pudding [17,18]. The difference between residue carrageenan extraction and SCB lignocellulose is that SCB cellulose is protected by hemicellulose and lignin; in addition, hemicellulose contains the highest amount of xylose and a lower amount arabinose and acetic acid [2].

Joint studies of chemical, structural, ultrastructural analyzes of red macroalgae and SCB are scarce. Nevertheless, there are studies of these separately, for example: chemical [19–24] and structural [25,26] analysis of the macroalgae *K. alvarezzi* and/or its fractions in the carrageenan extraction process, and the chemical [4] and [27–30] structural analysis of SCB.

Species *K. alvarezii* belongs to the class of red macroalgae and is mainly cultivation on the Philippines. The cultivation of seaweed *K. alvarezii* has been established as an important economic activity in 9 countries contributing approximately 6% of the total production of seaweed [31]. This species is widely used for the production of Kappa carrageenan hydrocolloid. Carrageenan represents an annual global market of over US$ 200 million [20]. Brazil produces small amounts of carrageenan, approximately 10 tons a year, from natural harvests of *Hypnea musciformis* (Wulfen) Lamouroux. Brazil [20], also produced in 2015–700 tons of macroalgae *K. alvarezii* [31]. This production is not sufficient to satisfy the national economic demand, which is ~1800 tons a year, necessitating importation of 1100 tons of *K. alvarezii* from the Philippines [20,21]. The basic chemical composition of *K. alvarezii* includes galactan, glucan, minerals, sulfate groups, and proteins [19,22,32]. In addition to the use for the production of carrageenan, *K. alvarezii* has been used for bioethanol production [19,23,24]. In addition, the residue carrageenan extraction is a glucan-rich substrate and hydrolysable with enzymes, serving to produce bioethanol [22].

In this paper, the objective of this study was to compare the chemical composition, structural, and ultrastructural properties of waste from the carrageenan and sugar-bioethanol processes for future manufacture of bioproducts, such as forth generation bioethanol.

2. Methods

2.1. Raw materials and marine biomass preparation

The macroalgae (seaweed) *K. alvarezii* brown strain was used as raw material of four different strains (the brown, red, green and G11). The brown strain were grown in the Atlantic Ocean in the experimental field base at Itaguá beach in Ubatuba, SP, Brazil (GPS coordinates 23°27′5,8″S; 45°02′49,3″W). The structure used to grow the seaweed strain consisted of a raft anchored in the bay [22,33,34]. Ten shoots of vegetative growth from each strain (approximately 70 g on wet basis) were pre-weighed and bound on a nylon line in a sub-assembly on the surface of the seawater, which provided a cultivation density of 6.7 plants per m^2. For cultivation, the brown strain remained in the structure for 30 days. After 30 days, the brown strain was weighed again. The wet weight and dry mass were determined using an average humidity of 35% (commercial value) [22,33,34]. The growth rate was calculated according to the equation (1): Growth rate (percentage on day^{-1}) = $[(w_t/w_0)1/t - 1]* 100$, where wt is the final wet mass (g); w_0 is the initial wet mass (g); and t is the cultivation time (30 days) [22,33,34]. The productivity was calculated according to the equation (2): Productivity (gm^2 day^{-1})(w/w, dry basis) = [(dwtf − dwti)/t ∗ (dwt/wwt)]/A, where dwtf is the final dry mass (g); dwti is the initial dry mass (g); t is the cultivation time (30 days); dwt = total dry mass; wwt is the total wet mass and A is the total area of cultivation. After collection, the biomass was dried at 25 °C [22,23,33–35].

2.2. Carrageenan processing of a sample

The brown strain of *K. alvarezii* was processed and the following fractions were obtained: treated KOH algae, semirefined carrageenan and residue carrageenan extraction (Fig. 1). Approximately 30 g (dry weight) of brown strain was soaked in 240 mL of 6% KOH solution (w/v) for 24 h at 25 °C ("cold" alkali transformation). The material was copiously washed with distilled water, sun bleached for 12 h, and dried

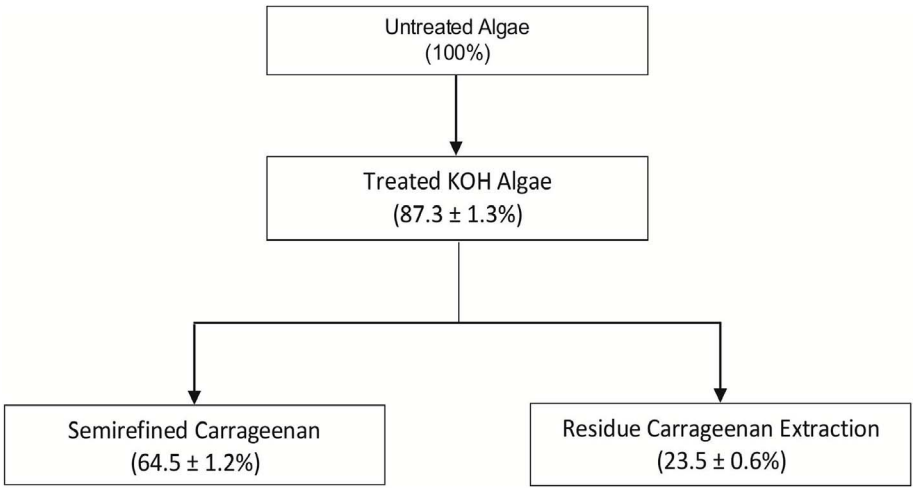

Fig. 1. Flowchart and yields (%, w/w) of obtaining fractions of Carrageenan and Residue Carrageenan Extraction.

at 60 °C until constant weight was achieved. The material was weighed, milled, and passed through a 0.84 mm screen. Approximately, 8g (dry weight) of the material obtained after alkaline transformation was extracted with 640 mL of distilled water in flasks and incubated at 65 °C for 2 h with rotary agitation at 120 rpm. The solution was then filtered using nylon tissue, and the extract was dried at 50 °C until constant weight was achieved (hereafter referred to as carrageenan semirefined). The material retained on the nylon tissue after filtering was recovered and dried to constant weight at 50 °C (hereafter referred to as residue carrageenan extraction). Both the semirefined carrageenan and the residue carrageenan extraction were weighed. The yield from the alkali treatment and extraction process was determined from the difference between the original (untreated material) and final weights (dry weight basis) [20,22,34].

2.3. Chemical composition of the fractions from K. alvarezzi and commercial carrageenan

The first chemical composition was the percentage of lipid (Table 3) determined by extraction with hexane using a Soxhlet apparatus in triplicate measurements [19,22].

The second chemical composition was the determination of galactan and glucan calculated from the sugars glucose and galactose and anhydrogalactose, respectively. The concentration of these sugars in the soluble fractions of the macroalgae and commercial carrageenan was determined by HPLC [22,36–38]. The milled samples were hydrolyzed with 72% (w/w) sulfuric acid at 30 °C for 1 h (3 mL of acid to 300 mg of sample) as described previously [34,35]. The acid hydrolysate was diluted with 79 mL of distilled water (4% (w/w) sulfuric acid), and the mixture was autoclaved at 121 °C for 1 h. The residual material was cooled and filtered through a porous glass filter (Scott number 3, Germany). The solids were dried to a constant weight at 105 °C and were assessed as the insoluble aromatics component. The filtrates were evaluated via HPLC analysis (using HPLC-Shimadzu, C- R7A model). Glucan and galactan contents were determined on a BIORAD AMINEX HPX-87H column with 0.005M sulfuric acid as eluent at a flow of 0.6 mL min^{-1} at 60 °C and detection by refractive index at 60 °C. The experiments were carried out in triplicate [22,36–38].

EA 1108 Fisons Instruments CHNS microanalyzer was used for elemental analysis of fractions from *K. alvarezzi* and commercial carrageenan. The certain elements were as follows: Carbon, hydrogen, nitrogen, sulfur and oxygen. Oxygen content was determined via the difference of components (percentage by mass) [22]. Two methods were utilized for determinate the sulfate content. The modified spectrophotometric was first method. About 0.05 g of the milled sample was weighed and placed in test flasks. One milliliter of 0.5 M HCl was added to each flask and the flasks were capped with aluminum foil and autoclaved at 120 °C for 1 h at 1 atm. At the end of the reaction, the sample was transferred into 15 mL Falcon tubes. Water was then added to each tube to achieve a volume of 10 mL followed by centrifugation at 7000 × g for 10 min. The supernatant (2 mL), 18 mL of distilled water, and 2 mL of 0.5 M HCl were added to the test flasks and stirred for a few seconds. Afterwards, 1 mL of $BaCl_2$ gelatin (Difco-Laboratories, Detroit, EUA) was added. The tubes were kept at 25 °C for 30 min with stirring, and absorbance readings were taken at 550 nm (Genesys 10S, Termo-Scientifc) [20,22,39,40]. The second method uses the mass of predetermined sulfur (elemental analysis) using equation (3).

$$S(\%) = \left(\frac{MS}{MmS}\right) x\ MmSu \quad (3)$$

where S (%) is sulfate groups (in percentage points), MS is the sulfur (%), MmS is the molar mass of sulfur (32 g mol^{-1}) and MmSu is the molar mass of sulfate (96 g mol^{-1}).

Other analyses of fractions from macroalgae and commercial carrageenan was quantified the protein content by three methods: The first method used the Kjeldahl digester to determine total nitrogen [22,41], but used the factor of 4.92 for calculate the protein content in triplicate measurements [42]. The second method was Lowry modified by Hartree [43]. For this purpose, we weighed ~200 mg of a sample into test tubes that were pretreated with 20 mL of 1 M NaOH at 70 °C for 4 h to dissolve proteins. Aliquots of 0.5 mL dissolved in the 1 M NaOH protein solution were added to 2.5 mL of a solution consisting of 2% sodium carbonate (w/v), 1% sodium tartrate and potassium (w/v) and 1% copper sulfate (w/v) (ratio of 100:1:1 (v/v), respectively). The mixture was homogenized maintaining it at rest for 15 min and then was added to 0.25 mL of 1N Folin-Ciocalteu reagent and stirred again. After 30 min, readings were taken at 660 nm. For quantitative protein determination, we constructed a calibration curve using bovine serum albumin (BSA) as a standard [19,43,44]. The three methods use the mass of predetermined nitrogen (elemental analysis). The protein content was determined using 4.92 as nitrogen conversion factor [42].

For quantification of the ash content of the samples, approximately 1 g of milled sample was weighed into a porcelain crucible and combusted in a muffle furnace at 575 ± 25 °C using a pre-programmed heating ramp. At the end of 3 h, the pots were kept in the oven until the temperature was about 105 °C. The crucibles were cooled and weighed. The experiments were performed in duplicate [22,45]. The metal content of the samples was also quantified by treating approximately 0.05 g of the milled sample with 1 mL of sulfuric acid and 2 mL of nitric acid in a glass digester at 150 °C until the solutions become clear. These solutions were allowed to swell in a 100 mL volumetric flask and analyzed using a spectrophotometer (ICP Optima Perkin Elmer Model 8000) [22]. The following metals were analyzed: manganese, calcium, sodium, copper, silicon, iron, and potassium. The experiments were performed in triplicate.

2.4. Enzymatic hydrolysis of residue carrageenan extraction and SCB

Enzymatic hydrolysis was performed using commercial enzyme preparations (Cellic CTec II, Novozymes, Denmark) at a dosage of 10 FPU per gram of sample (dry weight basis), corresponding to 200 IU of β-glucosidase. The total cellulases and β-glucosidase activity determined using the Celic CTec II extract were 92 FPU.mL^{-1} and 1800 UI.mL^{-1}, respectively. Each hydrolysis experiment was conducted in 50 mL Falcon tubes containing 200 mg of milled sample (dry weight basis) and 10 mL of 50 mM sodium-acetate buffer (pH 4.8) in addition to the enzyme solution. The flasks were incubated at 45 °C with rotary agitation at 120 rpm. The reaction was stopped at defined periods from 4 to 72 h by heating the flask to 100 °C for 5 min, followed by centrifugation of the material at 7000 × g for 10 min [22]. The determination of glucose was performed by HPLC, as described in the section on chemical composition.

2.5. Structural and ultrastructural analysis of the samples

For these analyses, the samples were air dried, milled, and screened through a 0.84-mm screen. Then, these samples were dried in an air circulation oven at 50 °C for 12 h, homogenized and stored. The morphological features of the samples were evaluated by SEM analysis using a JEOL JSM-7500F microscope. Milled and dried and stored samples were placed an aliquot of a dilute suspension in isopropanol in a vessel and this was stirred; the samples were then fixed on aluminum substrates coated with 1–10 mm carbon deposition for 60 s (2 Kb and 9.5 A); the microscope was run at an accelerating voltage of 2 kV for examination of the morphology of each sample. All images of the surface of the samples were captured at magnification of 25000 × , except for sugarcane bagasse: 20000 × .

XRD of the milled and dried samples were obtained at 25 °C within an angle range of 5°–70° 2θ (Bragg angle) and a scan speed of 2° min^{-1}. The equipment was a Siemens Kristalloflex diffractometer, running at the power output of 40 kV with a current of 30 mA and the Kα Cu

radiation (λ = 1.5406 Å). The crystallinity index (CrI) of the samples was calculated from an X-ray diffractogram by the deconvolution method (curve fitting). For peak fitting, we used the PeaKFit software, version 4.12, assuming Gaussian functions for each peak [31]. After that, the (CrI) was calculated using equation (4) [46]:

$$CrI\,(\%) = \left(\frac{A_c}{A_c + A_A}\right) \text{x } 100\% \quad (4)$$

where A_C is the area of the crystalline and A_A is amorphous regions. The plots of the spectra were generated in the OriginPro 8 software.

ATR of the dried and milled samples were examined between 4000 and 400 cm^{-1} at 25 °C with 2 cm^{-1} resolution, 32 scans per spectrum. The ATR method used in a FTIR-VERTEX 70 / BRUKER spectrophotometer with a source: HeNe laser (emits radiation in the mid-infrared region); Detector: DLaTGS. The graphics were built in OriginPro 8.

Molecular absorption spectrometry of the milled and dried samples was conducted in the UV and visible regions on a Varian Cary 5000 UV-Vis-NIR spectrophotometer, with a Cary Praying Mantis diffuse reflectance accessory for dried powder samples. The samples were analyzed at selected wavelengths from 200 to 350 and 350–700 nm using MgO as a reference substance. The spectra were normalized between 0 and 1 and graphics were built, both in OriginPro 8.

CP/MAS ^{13}C NMR spectra of milled and dried samples in the solid state were run at 25 °C on a Bruker Ascend 400WD spectrometer. The spectrometer was operated at 400 MHz in a static magnetic field of 9.04 T. The samples were compacted in a 4 mm zirconia rotor. The contact time used for all samples was 5.0 ms. The scan range was 0–200 ppm, and the speed of rotation was 10 kHz. The graphics were built in OriginPro 8.

2.6. Statistical analysis

Analyzes of variability were performed in the chemical composition section. The standard deviations were included; in the case of Table 2 also the Tukey test was performed in GraphPad Instat Software. Table 1 contains some data that were not performed experimental replication because they are data obtained in an elemental analysis that uses an analytical scale with accuracy of five decimal places. In the section of structural analysis (infrared, NMR and UV-visible), experiments were not performed experimental replication. In this case, these analyzes were used in qualitative terms to corroborate with the chemical composition. Only exception was the X-ray diffractograms (XRD experiments), which in spite of not performed experimental replication the technique was used in semi-quantitative terms for analysis of the CrI.

Table 2
Protein content by Lowry, Kjeldahl and Elemental analysis methods in "untreated material" biomass and soluble fraction after acid hydrolysis of brown strain from *K. alvarezii.*

Analysis method of protein	Proteins in biomass from *K. alvarezii* (%)	Protein in hydrolysate from *K. alvarezii* (%)
Lowry	4.4 ± 0.1[a1]	4.7 ± 0.1[a1]
Kjeldahl	3.0 ± 0.3[a2]	2.2 ± 0.4[a2]
Elementary Analysis	8.9 ± 0.7[b]	na

Contents present in percentage (grams of protein/100 grams of original material in dry basis).
Cultivation for May 2014. na = not analyzed.
All reported data are the average values followed by their standard deviations. In different columns, the values with the same superscript letters do not differ among themselves at significance level of 0.05 (Tukey test, Software GraphPad Instat). In same columns, the values with the same superscript numbers do not differ among themselves at significance level of 0.05 (Tukey test, Software GraphPad Instat).

3. Results and discussion

3.1. Chemical composition of fractions from K. alvarezii and sugarcane bagasse

The growth rate and productivity of untreated *K. alvarezii* (brown strain) were evaluated on the basis of an experimental field test in Ubatuba, São Paulo (SP), Brazil. The productivity and growth rate were 50.9 $gm^{-2}day^{-1}$ (corresponding to 18.3 $kgm^{-2}year^{-1}$) and 6.3% day^{-1}, respectively. The data presented are similar to those reported in the literature, and the values are characteristic of *K. alvarezii* crops in the Ubatuba-SP region [20,22,34].

The composition profile (carbon, nitrogen, hydrogen, sulfur, and oxygen) in different fractions from the macroalgae show in the Fig. 2. Nitrogen levels were higher in the untreated sample (66.6% more N_2) than in the other samples from *K. alvarezii*. Carbon levels were lower in the semirefined and commercial carrageenan (11.8% and 29.4%, respectively) than in the other samples from *K. alvarezii*, which is proportional to the glucan content as shown in Table 3. Hydrogen levels were lower in the commercial and semirefined carrageenan (32% and 14%, respectively) than in the other samples from *K. alvarezii*. Sulfur levels were lower in the untreated algae, treated KOH algae, and residue carrageenan extraction samples (34.6%, 42.3%, and 61.5%, respectively) compared to carrageenan samples, which is proportional to the ashes percent as shown in Table 3. Oxygen levels were higher in commercial carrageenan (16.7%) than in the other samples from *K. alvarezii.*

As shown in Fig. 2, the composition profile (carbon, nitrogen, hydrogen, and oxygen) of SCB was 45.4%, 0.8%, 5.5%, and 48.2%, respectively. The SCB showed higher levels of carbon (23%) and lower levels of oxygen (15%) than did the residue carrageenan extraction

Table 1
Protein content by Lowry, Kjeldahl and Elementary analysis methods. Content sulfate groups by Spectrophotometric and Elemental analysis methods of different fractions of brown strain from *K. Alvarezii.*

Samples	Protein analysis			Sulfate groups analysis	
	Lowry (%)	Kjeldahl (%)	Elemental analysis (%)	Spectrophotometric (%)	Elemental analysis (%)
Untreated Algae	4.7 ± 0.1	3.0 ± 0.3	8.0 ± 0.7	9.4 ± 0.4	10.3 ± 0.7
Treated KOH Algae	1.4 ± 0.02	0.4 ± 0.01	3.0	10.8 ± 0.3	9.3
Residue Carrageenan Extraction	1.2 ± 0.1	0.2 ± 0.01	3.2	8.1 ± 0.2	5.9
Semirefined Carrageenan	1.6 ± 0.1	0.2 ± 0.01	2.7	13.8 ± 0.2	15.9
Commercial Carrageenan	1.3 ± 0.02	0.1 ± 0.01	2.5	12.8 ± 0.1	15.8
Sugarcane Bagasse	22.0 ± 3	0.7 ± 0.1	4.0	nd	nd

Contents present in percentage (grams of protein/100 grams of original material in dry basis).
Cultivation for May 2014. nd = not detected.
Partial data reported are the average values followed by their standard deviations.

Table 3
Yield and chemical composition of different fractions of brown strain from *K. Alvarezii* and chemical composition of commercial carrageenan and sugarcane bagasse.

		Components of samples (% on pulp basic, w/w)						
Samples	Yield of sample (g/100g of material) (%)	[b]Galactan [a]Hemicellulose (%)	Glucan (%)	Ashes (%)	Proteins (%)	[b]Aromatics [a]Lignin (%)	Sulfate groups (%)	Sum (%)
Untreated Algae	–	37.2 ± 0.5	13.6 ± 0.4	15.5 ± 0.1	4.7 ± 0.1	2.6 ± 0.1	9.4 ± 0.4	83.8
Treated KOH Algae	87.3 ± 1.3	35.1 ± 0.7	14.4 ± 0.5	20.2 ± 0.1	1.4 ± 0.02	2.0 ± 0.2	10.8 ± 0.3	83.9
Residue Carrageenan Extraction	23.5 ± 0.6	8.2 ± 0.2	55.3 ± 0.8	13.7 ± 0.1	1.2 ± 0.1	4.5 ± 0.2	8.1 ± 0.2	91.0
Semirefined Carrageenan	64.5 ± 1.2	44.3 ± 1.1	nd	23.5 ± 0.1	1.6 ± 0.1	1.0 ± 0.1	13.8 ± 0.2	84.2
Commercial Carrageenan	-	32.5 ± 0.8	0.4 ± 0.1	34.8 ± 0.1	1.3 ± 0.02	0.7 ± 0.1	12.8 ± 0.1	82.5
Sugarcane Bagasse	-	25.2 ± 0.4	38.2 ± 0.2	2.5 ± 0.2	0.7 ± 0.1	24.5 ± 0.5	nd	93.9
		Components of samples (% of original material, w/w)						
Samples	Yield of sample (g/100g of material) (%)	Galactan (%)	Glucan (%)	Ashes (%)	Proteins (%)	Insoluble aromatics (%)	Sulfate Groups (%)	Sum (%)
Untreated Algae	–	37.2 ± 0.5	13.6 ± 0.4	15.5 ± 0.1	4.7 ± 0.1	2.6 ± 0.1	9.4 ± 0.4	83.0
Treated KOH Algae	87.3 ± 1.3	30.6 ± 0.7	12.6 ± 0.5	17.6 ± 0.1	1.2 ± 0.02	1.7 ± 0.2	9.4 ± 0.3	73.1
Residue Carrageenan Extraction	23.5 ± 0.6	1.9 ± 0.2	13.0 ± 0.8	3.2 ± 0.1	0.3 ± 0.1	1.0 ± 0.2	1.9 ± 0.2	21.3
Semirefined Carrageenan	64.5 ± 1.2	28.6 ± 1.1	nd	15.1 ± 0.1	1.0 ± 0.1	0.6 ± 0.1	8.9 ± 0.2	54.2

Contents present in percentage (grams/100 grams of basic and original material in dry basis).
Protein content using Lorwy method (different fractions from K. alvarezii and commercial carrageenan) and Kjeldahl method (sugarcane bagasse).
Cultivation for May 2014, commercial carrageena = Sigma and nd = not detected. All reported data are the average values followed by their standard deviations. Pulp basic (data representing the biomass without considering a mass balance) and original material (data corrected considering the yield of the process, i.e., performing a mass balance).
[a] Sugarcane bagasse (refers to hemicelulose and total lignin). Extractives content = 2.2% (w/w).
[b] Different fractions from K. alvarezii and commercial carrageenan (refers to galactan and insoluble aromatics). Lipids content in untreated sampl = 0.8% (w/w). The other fractions showed negligible levels of lipids.

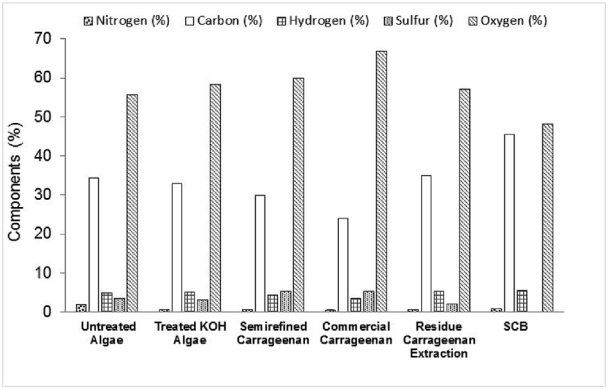

Fig. 2. The composition profile (percentage, w/w) of carbon, nitrogen, hydrogen, sulfur and oxygen in different fractions of macroalgae *k. alvarezii* and SCB.

from the semirefined carrageenan process. In contrast, sulfur was not detected in SCB, whereas the residue from *K. alvare*zii showed a ~2%. The carbon and sulfur levels have the same proportion with the percent of glucans and sulfur in Table 3.

The protein levels were assessed by three methods in various fractions from *K. alvarezii*. The protein content (percentage w/w) in the untreated algae, treated KOH algae, residue carrageenan extraction, semirefined carrageenan, commercial carrageenan, and SCB samples were 4.7%, 1.4%, 1.2%, 1.6%, 1.3%, and 22% (Lowry method); 3%, 0.4%, 0.4%, 0.2%, 0.1%, and 0.7% (Kjeldahl method); and 8.9%, 3%, 3.2%, 2.7%, 2.5%, and 4% (elemental analysis method), respectively (Table 1). The elemental analysis method showed significantly higher content of protein in the untreated algae, treated KOH algae, residue carrageenan extraction, semirefined, and commercial carrageenan samples (47.2%, 53.3%, 62.5%, 40.7%, and 48%, respectively) compared with the Lowry method (Table 1). This difference can be attributed to the presence of inorganic compounds in the biomass of *K. alvarezii.* Nevertheless, SCB yielded protein content of 22% (Lowry method), 0.7% (Kjeldahl method), and 4% (elemental analysis method; Table 1). The high value given by the Lowry method in sugarcane bagasse can be inferred to interference by sugars and phenolic compounds, which could cause a false positive result [44]. The elemental analysis method also showed a significantly higher content of protein in the untreated algae, treated KOH algae, residue carrageenan extraction, semirefined, and commercial carrageenan samples (66.3%, 86.7%, 87.5%, 92.6%, and 96%, respectively) compared with the Kjeldahl method (Table 1). The low content of proteins obtained by the Kjeldahl method can be attributed to possible inefficiency of extraction of the organic phase because macroalgae proteins are associated with cell membranes that pose a difficulty for extraction [42]. Thus, the Lowry method can be considered more accurate for the samples from *K. alvarezii* because there is no indication of interference. Besides, this method does not detect inorganic nitrogen, as can happen with elemental analysis and Kjeldahl methods. Nonetheless, SCB showed a large amount of proteins when we used the Lowry method, indicating interference [44].

The sulfate levels were assessed by two methods in the fractions from *K. alvarezii*. The proportion of sulfate groups in the untreated algae, treated KOH algae, residue carrageenan extraction, semirefined carrageenan, and commercial carrageenan samples were 9.4%, 10.8%, 8.1%, 13.8%, and 12.8% (spectrophotometric method) and 10.3%, 9.3%, 5.9%, 15.9%, and 15.8% (elemental analysis method; Table 1). Both methods tested on the samples of *K. alvarezii* biomass showed similar results. Thus, the spectrophotometric method was assumed to be suitable for the quantification of sulfate groups in various fractions from *K. alvarezii*. On the other hand, both tested methods did not detect sulfate groups in sugarcane bagasse (Table 1).

The proteins were analyzed in solids (untreated algae from *K. alvarezii*) and its respective acid hydrolysate (filtered after the acid hydrolysis step performed for quantification of insoluble aromatic compounds and monomeric sugars; Table 2). The data showed protein

Table 4
Yield and metals composition of different fractions of brown strain from *K. Alvarezii*.

		Components of samples ($mg.g^{-1}$ on pulp basic)		
Samples	Yield of sample (g/100g of material) (%)	Potassium ($mg.g^{-1}$)	Calcium ($mg.g^{-1}$)	Sodium ($mg.g^{-1}$)
Untreated Algae	–	33.5 ± 0.03	2.4 ± 0.02	4.8 ± 0.03
Treated KOH Algae	87.3 ± 1.3	44.1 ± 0.02	3.3 ± 0.01	1.3 ± 0.06
Residue Carrageenan Extraction	23.5 ± 0.6	20.6 ± 0.06	3.5 ± 0.03	2.1 ± 0.02
Semirefined Carrageenan	64.5 ± 1.2	50.3 ± 0.05	2.9 ± 0.04	1.0 ± 0.05
Commercial Carrageenan	-	72.8 ± 0.3	25.8 ± 0.2	8.6 ± 0.1
Sugarcane Bagasse	-	13.6 ± 0.02	4.8 ± 0.02	nd
		Components of samples ($mg.g^{-1}$ of original material)		
Samples	Yield of sample (g/100g of material) (%)	Potassium ($mg.g^{-1}$)	Calcium ($mg.g^{-1}$)	Sodium ($mg.g^{-1}$)
Untreated Algae	–	33.5 ± 0.03	2.7 ± 0.02	4.8 ± 0.03
Treated KOH Algae	87.3 ± 1.3	38.5 ± 0.02	2.9 ± 0.01	1.1 ± 0.06
Residue Carrageenan Extraction	23.5 ± 0.6	4.8 ± 0.06	0.8 ± 06	0.5 ± 0.02
Semirefined Carrageenan	64.5 ± 1.2	32.4 ± 0.05	1.9 ± 0.01	0.6 ± 0.05

Contents present in mass (milligrams/grams of basic and original material in dry basis).
Cultivation for May 2014 and commercial carrageenan = Sigma. All reported data are the average values followed by their standard deviations. Pulp basic (data representing the biomass without considering a mass balance) and original material (data corrected considering the yield of the process, i.e., performing a mass balance). nd = not detected.

content of 4.4% and 3% in untreated biomass according to Lowry and Kjeldahl methods, respectively (Table 2). The acid hydrolyzate (filtered) showed the protein content of 4.7% and 2.2% in the untreated sample for Lowry and Kjeldahl methods, respectively (Table 2). The data indicate that there was no significant difference in the protein content between the untreated biomass and its respective acid hydrolyzate, suggesting that all proteins were solubilized in the filtrate (Table 2). Thus, the fraction retained after acid hydrolysis (solids retained in the filter from the acid hydrolysate) can be considered insoluble aromatic components.

The yield of biomass from treated KOH algae was 87.3% (Table 3). The main components detected in the biomass from the K. *alvarezii* were galactan, glucan, ash, sulfate groups, proteins, insoluble aromatics, and lipids (Table 3). The percentages (w/w) of galactan, glucan, ash, sulfate groups, proteins, insoluble aromatics, and lipids in the untreated algae sample were 37.2, 13.6, 15.5, 9.4, 4.7, 2.6, and 0.8, respectively (Table 3). The values for these components were similar in the untreated and treated KOH algae, with the exception of protein content (which was reduced by ~ 70.2%), ash (which showed an increase of ~30%), and lipids (which were not detected in the case of treated with 6% KOH algae). The dissolution of proteins is common in an alkaline medium, while the increase in ash content reflects adsorption of potassium on the material upon treatment with 6% KOH (w/v) [43] (Table 3). The cumulative data are in the range of 83%–91%. For direct comparison of the chemical composition between the macroalgal material treated with 6% KOH and untreated algae, a mass balance was required. The content of various components in the treated with 6% KOH algae were reduced by 17.7% (galactan), 74.4% (protein), and 34.6% (insoluble aromatics). In contrast, glucan, ash, and sulfate contents were not reduced in the sample treated with 6% KOH algae (Table 3).

The yields of extracted semirefined carrageenan and the residue carrageenan extraction obtained from the treated with 6% KOH (w/v) algae were 64.5% and 23.5%, respectively (Table 3). The concentrations of galactan, glucan, ash, protein, insoluble aromatics, and sulfate groups in the residue were 8.2%, 55.3%, 13.7%, 1.2%, 4.5%, and 8.1%, respectively (Table 3). The galactan content in the residue was lower and glucan content was higher in comparison with the samples treated with 6% KOH (w/v) algae (Table 3). The galactan, ash, protein, insoluble aromatics, and sulfate contents of semirefined carrageenan were 44.3%, 23.5%, 1.6%, 1%, and 13.8%, respectively (Table 3). The glucan was not detected in this fraction of semirefined carrageenan. The composition profile of the obtained semirefined carrageenan was similar to that of commercial carrageenan; the main components detected were galactan, ash, and sulfate groups (Table 3). For direct comparison of the chemical composition among the residue carrageenan extraction, semirefined carrageenan, and the macroalgal material treated with 6% KOH (w/v), a mass balance was needed. The content of various components in the residue carrageenan extraction and semirefined carrageenan were reduced by 93.8% and 6.5% (galactan), 81.8% and 14.2% (ash), 75% and 16.7% (protein), 41.2% and 64.7% (insoluble aromatics), and by 79.8% and 5.3% (sulfate groups), respectively. By contrast, glucan content was not reduced in the residue carrageenan extraction and not detected in semirefined carrageenan (Table 3). The chemical composition of the samples under study was similar to that reported in the literature [22]. After completing the process of isolation of semirefined carrageenan (treatment with 6% KOH (w/v) solution and subsequent extraction with hot water), the yields of semirefined carrageenan and residue carrageenan extraction were ~57% and 20.0%, respectively. The overall yields of semirefined carrageenan are similar to those reported in the literature [22,34,47]. The main components of SCB (waste from the processing of sugarcane juice) are lignin, cellulose, hemicellulose, ash, and extractives [2]. The average chemical composition of the commercial SCB was 24.5% lignin, 25.2% hemicellulose, 38.2% glucan, 2.5% ash, 0.7% protein, and 2.2% extractives. The cumulative data are 93.9% (Table 3) [2].

The metal content of the samples treated with 6% KOH (w/v), extracted with hot water, and SCB was assessed. The three main metals that we evaluated were potassium, calcium, and sodium (Table 4). In addition to these metals, traces of manganese, iron, and silicon (metalloid) were detected. The chemical composition of the original treated with 6% KOH (w/v) algae indicated an increase in potassium (~30%) and calcium content (~37.5%) and a reduction in sodium content (~73%; Table 4). The potassium, calcium, and sodium contents in the residue carrageenan extraction and semirefined carrageenan were 20.6 and 50.3 $mg.g^{-1}$, 3.5 and 2.9 $mg.g^{-1}$, and 2.1 and 1.0 $mg.g^{-1}$, respectively (Table 4). The potassium and calcium contents of the commercial carrageenan and SCB were 72.8 and 13.6 $mg.g^{-1}$ and 25.8 and 4.8 $mg.g^{-1}$, respectively. In contrast, sodium content of commercial carrageenan was 8.6 $mg.g^{-1}$ and not detected in sugarcane bagasse (Table 4). The composition (metal profile) of our semirefined carrageenan was different from that of commercial carrageenan (Table 4).

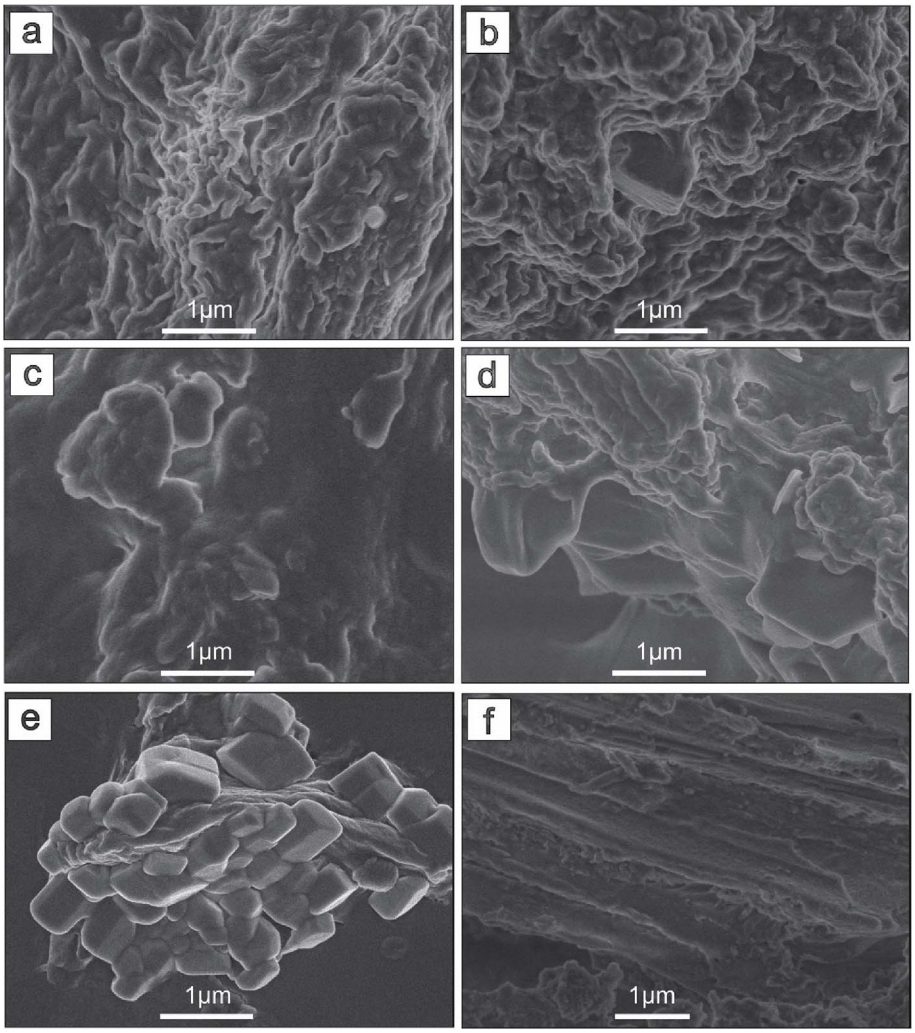

Fig. 3. SEM of different fractions from *K. alvarezii* and sugarcane bagasse. (a) Untreated Algae, (b) Treated KOH Algae, (c) Residue Carrageenan Extraction, (d) Semirefined carrageenan, (e) Commercial carrageenan, and (f) SCB.

For a direct comparison of the chemical composition among the residue carrageenan extraction, semirefined carrageenan, and the macroalgal material treated with 6% KOH (w/v), a mass balance was required. According to the mass balance, metal contents of the residue carrageenan extraction and semirefined carrageenan were reduced as follows: potassium (87.5% and 15.8%), calcium (72.4% and 34.5%), and sodium (54.5% and 45.4%), respectively (Table 4). The chemical composition (in terms of metals) of the different fractions from *K. alvarezii* being studied is similar to that reported in the literature [22].

3.2. Structural and ultrastructural analysis of fractions from K. alvarezii and sugarcane bagasse

Fig. 3a–e shows the different fractions of *K. alvarezii* from several steps of the carrageenan process, whereas Fig. 1f shows SCB. Fig. 1a (untreated algae) and 1b (treated KOH algae) show an irregular shape. Fig. 1c (residue carrageenan extraction) and 1d (semirefined carrageenan) also indicate irregular shapes but also show parallelepiped shapes, whereas Fig. 2e (commercial carrageenan) shows the main parallelepiped shape. By contrast, Fig. 3f (SCB) shows the characteristic cell wall of this plant [27]. We observed variations in the topographic surface of different fractions of *K. alvarezii* and lignocellulose structure of SCB.

Fig. 4 shows the XRD spectra of milled and dried samples. The calculations of the CrI from commercial carrageenan, semirefined carrageenan, untreated algae, treated KOH algae, residue carrageenan extraction and SCB were 4.8%, 6.9%, 4.1%, 2.1%, 13.6%, and 32.4%,

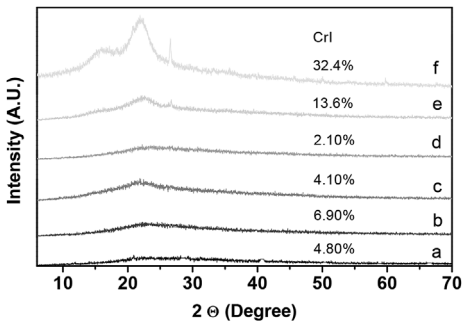

Fig. 4. XRD spectra of different fractions from *K. alvarezii* and sugarcane bagasse. (a) Commercial Carrageenan, (b) Semirefined carrageenan, (c) Untreated Algae, (d) Treated KOH Algae, (e) Residue Carrageenan Extraction, and (f) SCB.

respectively. The commercial carrageenan, semirefined carrageenan, and untreated algae showed an average CrI near 5%. The average data presented are similar to those reported in the literature for a carrageenan film with glycerol [48]. The residue carrageenan extraction has a high CrI due to the greater glucan content (Table 3), but this index is less than the CrI of SCB [27].

Fig. 5 presents the infrared spectra (ATR) of the milled and dried samples. The absorption band at 1639 cm^{-1} corresponds to the absorption pattern of angular deformation of the bonds H–O–H of water in the spectrum of SCB (Fig. 5f) [28]; also, the bands (#1 and #2) 1651 and 1637 cm^{-1} of the samples (Fig. 5a–e) can be attributed to water's

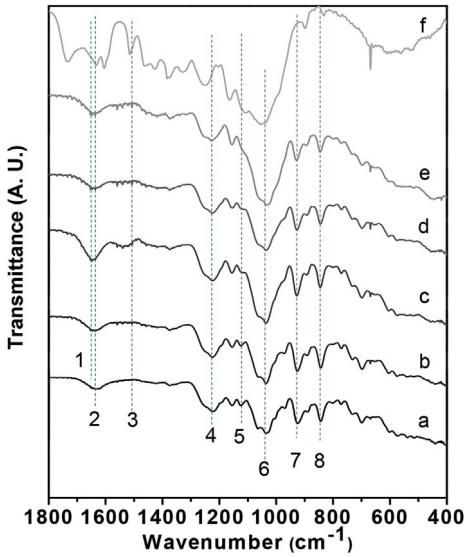

Fig. 5. ATR spectra of different fractions from *K. alvarezii* and SCB. (a) Commercial Carrageenan, (b) Semirefined carrageenan, (c) Untreated Algae, (d) Treated KOH Algae, (e) Residue Carrageenan Extraction, and (f) SCB.

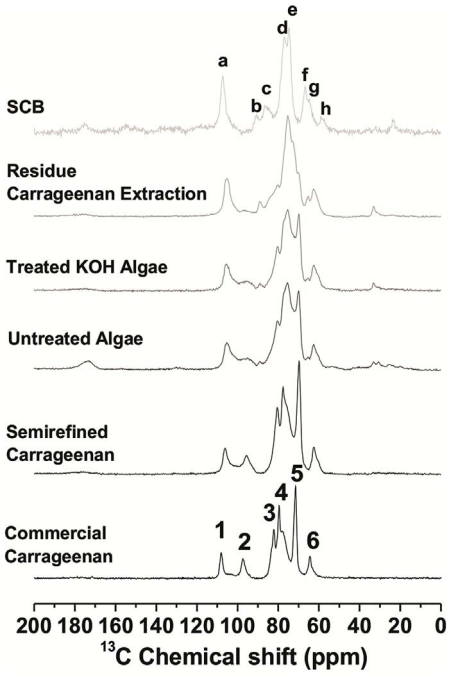

Fig. 6. CP/MAS ^{13}C NMR spectra of solid state of different fractions from *K. alvarezii* and SCB.

absorption pattern [49]. The untreated algae, treated KOH algae, and residue carrageenan extraction samples show a small aromatic band (#3, 1510 cm^{-1}), which is consistent with the data on chemical composition of these samples, that is low concentration of insoluble aromatic compounds (Table 3). Similarly, the band at 1516 cm^{-1} in the SCB spectrum was assigned to C=C stretches of the aromatic rings of lignin (Fig. 5f) [50]. By contrast, commercial carrageenan (Fig. 5a) and semirefined carrageenan (Fig. 5b) did not show the band of insoluble aromatics; this finding is also consistent with the data on chemical composition showing values lower than 1% for insoluble aromatics (Table 3).

The wavenumbers between 1300 and 800 cm^{-1} correspond to the fingerprint region. Samples (Fig. 5a–e) showed a band of sulfate groups (#4, 1225 cm^{-1}) [25,26,49,51]. On the other hand, the residue sample showed a slightly smaller band, which is in agreement with the data on chemical composition showing lower values for sulfate groups in the residue sample when compared with the other samples (Fig. 5a–d; Table 3). The sugarcane bagasse (Fig. 5f) did not show the band of sulfate groups (#4, 1225 cm^{-1}) corroborating the chemical composition data (Fig. 2 and Table 3). The band located at ~1252 cm^{-1} (in the SCB spectrum) corresponds to stretches of group C-O which are often observed in such groups as ether, ester, and phenol moieties of lignin and/or hemicellulose [52]. The obtained fractions of *K. alvarezii* and SCB (Fig. 5a–f) showed two bands with glycosidic-bond characteristics (#5 and #6, 1124 and 1037 cm^{-1}, respectively) [25,53]. The band located at 1166 cm^{-1} in the SCB spectrum (Fig. 5f) was assigned to C-O stretches of the cellulose structures, hemicellulose, and lignin and/or C–O–C stretching in the cellulose and hemicellulose structure [28,29,52]. The absorption of hydroxyl groups corresponds to the band located at 1050 cm^{-1} of the SCB spectrum and is related to vibration of the OH group of lignin or a fold the C-OH group of hemicellulose [28,52]. Bands #7 and #8 (927 and 847 cm^{-1}, respectively) were highly intense in the various fractions from *K. alvarezii* because these bands are typical of the galactose molecule, in agreement with the data on chemical composition of the samples under study (Table 3) [26,49,51,53–55]. On the other hand, bands #7 and #8 (927 and 847 cm^{-1}, respectively) were not detected in SCB (Fig. 5f), thus corroborating the chemical composition (Fig. 4).

The analysis of different fractions from *K. alvarezii* (Fig. 5a–e) revealed that band #4 (1225 cm^{-1}) is found in κ-, ι-, λ-, μ-, ν-, θ-, and ξ-carrageenans; band #7 (927 cm^{-1}) and 1066 cm^{-1} correspond to κ- and β-carrageenans; band #8 (847 cm^{-1}) is found in κ-, ι-, μ-, and ν-carrageenans; and the band located at 802 cm^{-1} corresponds to ι- or θ-carrageenan [26,55]. Thus, these data indicate that the semirefined carrageenan consists mainly of κ-carrageenan in terms of structure [25].

Carrageenans are linear-chain galactans formed by repeating disaccharide units of 4-linked α-D-galactopyranose (D) or 4-linked 3,6-anhydro-α-D-galactopyranose (DA) and 3-linked β-D-galactopyranose (G) joined via glycosidic bonds [25,26,56,57]. The units of the sulfates in galactans are represented by 3-linked β-D-galactopyranose-4-sulfate (G4S) or 3-linked β-D-galactopyranose-2-sulfate (G2S) [54]. The NMR spectra of semirefined and commercial carrageenan contain numbered peaks or chemical shift positions (ppm) #1 through #6; peaks #1–6 represent resonance of carbons involved in the glycosidic bonds of carrageenan: 1G4S; 1DA; 3DA, 3G4S, 4DA; 5DA, 5G4S, 4G4S; 2DA, 2G4S, and 6DA; 6G4S, respectively (Fig. 6) [58]. The NMR spectra of semirefined and commercial carrageenan are similar to the spectrum of κ-carrageenan reported in the literature [55]. The NMR spectra of semirefined and commercial carrageenan corroborate the ATR analysis, showing the presence of κ-carrageenan (Fig. 5a and b).

Chemical shifts in the SCB spectrum between 107.5 and 64.8 ppm represent the types of carbon from the cellulose and hemicellulose in lignocellulosic structure of the biomass [30]. The spectra of SCB contain numbered peaks or chemical shift positions (ppm) a through h; peaks a–h represent resonance of carbons involved in the glycosidic and lignin linkages: C-1 cellulose; C-4 crystalline cellulose; C-4 cellulose and hemicellulose amorphous; C-2, C-3, and C-5 cellulose and hemicellulose; C-2, C-3, and C-5 cellulose; C-6 crystalline cellulose; C-6 amorphous cellulose and methoxy aryl groups of lignin, respectively [30].

The untreated and treated KOH algae samples showed NMR spectra similar to those of the carrageenans samples. Nevertheless, these samples have resonance of carbons that overlaps with that of C-1 cellulose (SCB) and 1G4S; 1DA; and 3DA (carrageenans). The corresponding carbon resonance type of 3G4S links in carrageenans did not appear in the spectra of untreated and treated KOH algae samples. The carbon resonance-related links 3–6 of the carrageenan samples were displaced when compared to untreated and treated KOH algae samples, indicating

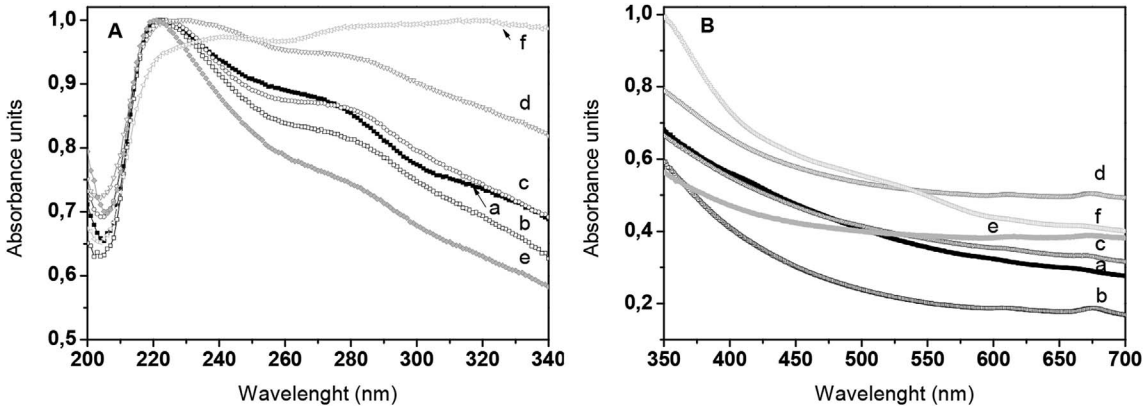

Fig. 7. UV (A) and Visible (B) spectra of different fractions from *K. alvarezii* and SCB. (a) Commercial Carrageenan, (b) Semirefined carrageenan, (c) Untreated Algae, (d) Treated KOH Algae, (e) Residue Carrageenan Extraction, and (f) SCB.

the overlay links: C-4 cellulose and amorphous hemicellulose; C-2, C-3, and C-5 cellulose and hemicellulose; C-2, C-3, and C-5 cellulose; and C-6 crystalline cellulose. These data corroborate the chemical composition of untreated and treated KOH algae samples, where glucans and galactans are present (Table 3).

The residue carrageenan extraction showed a spectrum similar to that of the SCB sample. In this case, there was a smaller overlap with carbon resonance arising from the galactan chains, corroborating the chemical composition of the residue carrageenan extraction where glucans dominate over galactans (Table 3). The NMR spectrum of the residue sample shows evidence that its glucans may be partially or totally cellulose molecules; this finding also confirms the increase in the crystallinity index of the residue compared to the untreated and treated KOH algae samples (Fig. 4).

Fig. 7A and B shows the UV-Vis spectra of the samples under study. The UV spectra of the commercial and semirefined carrageenan samples show the maximum absorption peaks at 223 and 222 cm^{-1}, respectively (Fig. 7Aa,b). The UV spectra of commercial and semirefined carrageenan also have broad shoulders between 300 and 340 nm. These data are in agreement with a report on characteristics of κ-carrageenan [57]. These results are therefore in agreement with the ATR and NMR analyses. The maximum absorbance peaks in UV spectra of the untreated, treated KOH algae, and residue samples are located at 224, 228, and 221 nm, respectively (Fig. 7Ac,d,e). The UV spectrum of the SCB sample (Fig. 7Af) shows a characteristic band of lignin at 230 nm and a characteristic band of phenolic acid at 315 nm [59,60] in line with ATR and chemical composition analyses (Table 3 and Fig. 5).

The visible spectra of commercial and semirefined carrageenan (Fig. 7Ba,b) are in agreement with those reported in the literature [61,62]. The untreated algae, treated KOH algae, SCB, and residue carrageenan extraction samples show absorbance in visible spectra (Fig. 7Bc-f) that is characteristic of quinone-type chromophores [63,64] (aromatic compounds), thus supporting their aromatic or lignin content (Table 3).

3.3. The potential for utilization of waste from sugar-bioethanol and carrageenan processes

Compared to SCB, the residue carrageenan extraction contains increased content of glucans: at ~24% (Table 3). Furthermore, the residue carrageenan extraction does not show the presence of lignin but only a small amount of aromatic compounds in the biomass (Table 3). Fig. 8 shows that the conversion of glucan to glucose residues carrageenan extraction derived from a carrageenan process is 100%, without any recalcitrance in the material [22]. Nonetheless, studies show that the conversion of glucan to glucose in SCB from a different strain without any pretreatment is 20% on average [2]. The main factors that promoted a greater conversion of the residue extraction carrageenan

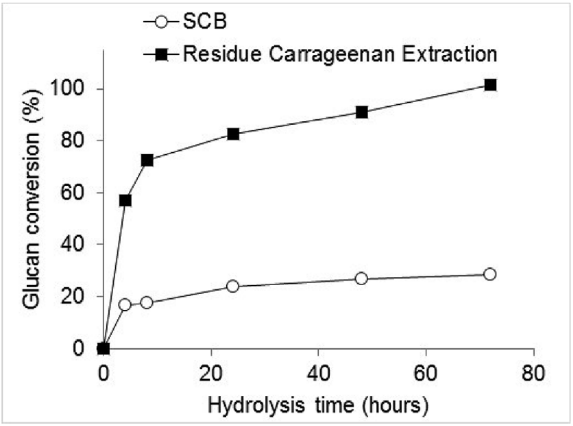

Fig. 8. Conversion glucan to glucose over time by direct hydrolysis enzymatic of Residue Carrageenan Extraction (Square filled) and SCB (ball empty).

when compared to the SCB was due to the high content of lignin (Table 3) and high crystallinity present in the SCB-cellulose (Fig. 4).

The residue yield (dry plant) obtained from macroalgae *K. alvarezii* during the processing of carrageenan (wet plant) is approximately 15%, at average moisture content of 35% (commercial value) [33,34]. Thus, the yield of the residue carrageenan extraction from *K. alvarezii* biomass during the carrageenan processing is similar to the yield obtained from SCB (approximately 13%) in the sugar-bioethanol process, considering the humidity of similar plants [2]. Besides, the average productivity of SCB in Brazil is 7.5 $kg{\cdot}m^{-2}{\cdot}year^{-1}$ [2] but for macroalgae *K. alvarezii* is 18.3 $kg{\cdot}m^{-2}{\cdot}year^{-1}$. The macroalgae *K. alvarezii* has a high growth rate and reaches maturity in approximately a month and can develop throughout the year [22]. By contrast, sugarcane has a lower growth rate and reaches maturity only after a year of cultivation [3].

4. Conclusions

The present analysis of various fractions from *K. alvarezii* suggests that carbohydrates represent the main component in *K. alvarezii* biomass fractions. The main carbohydrate polymers are galactan and glucan. Other major components are ash (mainly comprised of calcium, potassium, and sodium), insoluble aromatic compounds, proteins, and sulfate (groups on polymers). Semirefined carrageenan and its residue were obtained successfully. Similarly, SCB shows hemicellulose and glucan as the main carbohydrate polymers. Another major component is lignin.

These results highlight the usability of this byproduct of carrageenan and sugar-bioethanol processes as a monomeric sugar for the eventual production of bioethanol. The glucan residue carrageenan extraction from *K. alvarezii* is converted to glucose at the 100% rate in

the enzymatic hydrolysis with cellulases, without any pretreatment, whereas in SCB, glucan is converted to glucose at 20% on average without any prior pretreatment, thus requiring an additional pretreatment step. Furthermore, the residue carrageenan extraction from *K. alvarezii* contains a larger amount of glucan than SCB does.

Ultimately, we offer a novel approach to the biorefining of *K. alvarezii* for production of not only carrageenan but also other bioproducts. Such insights can advance this field toward better strategies for the manufacture of biofuels and other bioproducts. An example would be the production of fourth generation bioethanol, since biofuels produced directed from macroalgae are considered third generation.

List of abbreviations

CrI: Crystallinity index; UV-VIS: Molecular absorption spectrometry; ATR: Attenuated total reflectance; CP/MAS C^{13} NMR: Solid-state cross-polarization/magic angle spinning nuclear magnetic resonance; XRD: X-ray diffraction spectra; SEM: Surface image scanning electron microscopy; HPLC: High-performance liquid chromatography; UV: Ultraviolet; VIS: Visible; ESI: Electrospray ionization; SP: São Paulo; SD: Standard deviation; FPU: Filter paper units; UI: international units; SCB: Sugar-cane bagasse.

Ethical approval and consent to participate

Not applicable.

Consent for publication

All authors read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

IUMR, JPMD, and ATM performed chemical, structure, and ultrastructure analyses of samples and data interpretation. VCG provided the experimental algae strains, performed field trials, determined the agronomic productivity data, data interpretation and reviewed the manuscript. LEO, RM, and FM participated in the design of the study, data interpretation and reviewed the manuscript. All authors read and approved the final manuscript.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.biombioe.2017.10.008.

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