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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

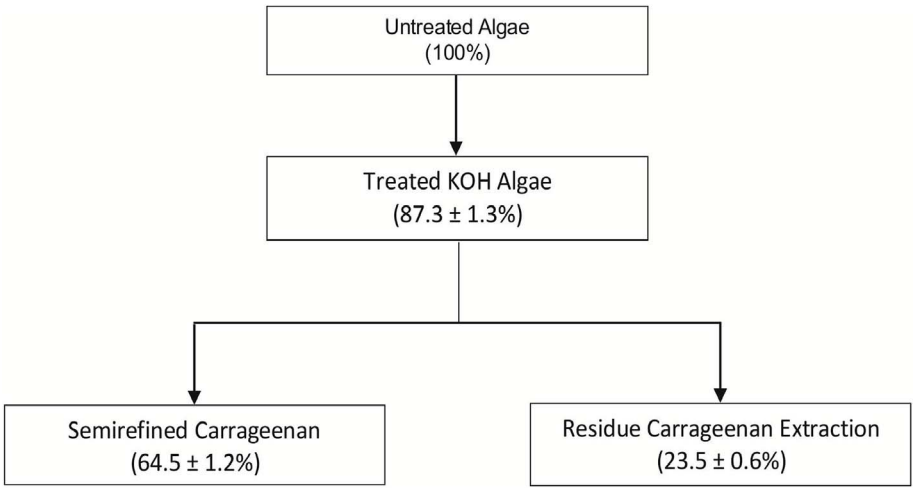

Fig. 1. Flowchart and yields (%, w/w) of obtaining fractions of Carrageenan and Residue Carrageenan Extraction.