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Chemical Composition and Carbohydrate Characterization of Beach-Cast Marine Macrophytes from the Mexican Caribbean: Implications for Potential Bioethanol Production

Freile-Pelegri n Yolanda¹ · Robledo Daniel¹ · Ch vez-Quintal Cresencia¹ · Erika V zquez-Delf n¹ · Pliego-Cort s Hugo² · Bedoux Gilles² · Bourgougnon Nathalie²

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Abstract

Marine macrophytes are considered promising biomass for bioethanol production. The increases in anthropogenic nutrients and climate change have caused unprecedented blooming of ‘sargasso’ across the Atlantic since 2011. This biomass reaches the Caribbean Sea, stranding in large amounts along shorelines, and creating a serious waste management problem. The knowledge of its chemical composition is important to assess whether this material could serve as feedstock for third-generation bioethanol. The beach-cast marine macrophytes collected on the Mexican Caribbean coast in December 2018 were composed of brown seaweeds and a seagrass (23.5 and 76.5% relative abundance, respectively) including *Sargassum fluitans*, *Sargassum natans* I, *Sargassum natans* VIII, *Turbinaria turbinata*, and the angiosperm *Syringodium filiforme*. For valorization purposes, glucans, non-glucans carbohydrates and lignin were determined. Besides its abundance, underutilization, and low-cost this whole biomass may have potential as a promising raw material for third-generation bioethanol because it contains easily fermentable glucose such as mannitol (36.3% in whole biomass and 56% in the *Sargassum* species) and cellulose (36.3% on average). Other specific carbohydrates such as alginate (20–31%) and fucoidan (9.1–8.2%) were present in smaller amounts but they can also be converted to fermentable sugars with the proper methodology. Some advantages and limitations for the potential production of third-generation bioethanol from this biomass are discussed.

Statement of Novelty

Abundant beach-cast marine macrophyte biomass from the Mexican Caribbean is believed to be a feedstock to produce bioethanol, allowing waste management and contributing to positive environmental, social, and economic impacts. Beach-cast marine macrophytes include holopelagic and benthic brown seaweeds and seagrass. This biomass is rich in easily fermentable carbohydrates, such as cellulose and mannitol. Other specific carbohydrates such as alginate and fucoidan are also present in suitable amounts and can also be converted to fermentable sugars with the proper methodology.

Keywords Mannitol · *Sargassum* Stranding · Seagrass · Seaweeds

Introduction

The rising cost of oil and extensive use of fossil fuels due to the increasing world population are the main causes of resource depletion. Although fossil fuels, when burned, produce large amounts of carbon dioxide and other greenhouse gases that contribute negatively to global warming and climate change, they remain the world’s primary source of energy [1]. In addition to environmental concerns, this type of nonrenewable energy is running out, driving efforts to produce biofuels from renewable feedstocks. Bioethanol

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produced from plant biomass which contains fermentable sugars such as cellulose, or complex carbohydrates is a promising renewable energy. Nonetheless, when biomass from terrestrial feedstock is used to produce ethanol debates arise over food *versus* fuel, land use and freshwater resources [2]. The feasibility of using lignocellulosic biomass is often limited by the low yield in addition to the high cost of the hydrolysis process for the conversion of recalcitrant lignocellulose into useful sugars due to the lignin content [1]. Thus, the separation of lignin content from lignocellulose has become an obstacle to be solved. An alternative is to use carbohydrate-rich macrophytes from the ocean, marine macroalgae and seagrasses as feedstock. Among them, marine macroalgae, also known as seaweed, have shown recognized advantages over terrestrial biomass due to their high growth rates, abundant and diverse carbohydrate content, and low or no lignin content [3, 4]. This biomass requires relatively mild processing conditions when compared to lignocellulosic biomass such as shorter reaction times, less severe acid conditions, and lower temperatures [5]. In this context, third-generation biofuel (TGB) based on marine macrophytes has been postulated as an excellent alternative to displace fossil fuels [6]. For the development of a sustainable TGB production process, abundant and cheaper feedstocks are required to avoid high processing costs associated with common raw materials used today [7]. In general, the average photosynthetic efficiency of marine biomass, seaweeds and seagrasses, is higher than that of terrestrial biomass [8], and many seaweed species show very high mass productivity [9]. Moreover, an increase in macronutrients in the marine environment favors the fast growth and spread of marine macrophytes worldwide. Thus, due to the relatively large increase in anthropogenic nutrients, mainly nitrogen and phosphorous supply from terrestrial runoff and atmospheric inputs, unprecedented blooming of marine macrophytes across the equatorial Atlantic has been observed in the last decade [10]. This biomass reached the Caribbean Sea in 2011, resulting in large amounts of a mixture of seaweed and seagrass, stranding along shorelines throughout the region, creating a serious waste problem but also providing some emerging opportunities [11]. In general, seaweeds, classified according to their pigmentation as green, red, and brown (Chlorophyta, Rhodophyta, and Phaeophyceae, respectively), are composed of minerals, proteins, and lipids as well as different glucans derived from glucose such as cellulose and non-glucans carbohydrates [12]. Whereas seagrasses, a small but diverse group of angiosperms, form submersed meadow communities that are among the most productive on earth, however, despite their use as raw materials for paper production due to their cellulose content [13], studies related to its potential as feedstock are limited.

As beach-cast marine macrophyte biomass could be useful for bioethanol purposes, their chemical and biological understanding as feedstocks is important from a technological point of view. Both the chemical composition and abundance of the different species of marine macrophytes found in the stranding biomass will allow us to assess the most appropriate uses, evaluate the theoretical yields, and explore the appropriate processes for obtaining TGB. In this study, the proximate and elemental chemical composition as well as the glucan and non-glucan carbohydrates of the beach-cast macrophytes biomass found on the Mexican Caribbean coast were evaluated. A further detailed analysis of the sugar composition of the species was also determined by high-performance anion exchange chromatography (HPAEC). The implication of this biomass as feedstock for third-generation bioethanol production is also discussed.

Materials and Methods

Collection of Beach-Cast Biomass and the Species Proportion

Beach-cast macrophytes biomass was collected on the Mexican Caribbean coast (20° 51' 04.3" N; 86° 52' 20.8" W, Puerto Morelos, Quintana Roo) in December 2018. Samples were obtained using a 100 m transect parallel to the shoreline over 10 quadrat plots (0.25 × 0.25 m) separated by 5 m from each other. The total biomass within each plot was collected and transported to the laboratory for taxonomic identification. Biomass was then sorted according to the species of seaweed and seagrass, washed with tap water to remove sand and salt, oven-dried at 60 °C, and ground in a mill for further chemical analysis. The relative abundance of each species was determined from a subsample of 1 kg of the total biomass collected.

Chemical Composition of the Beach-Cast Marine Macrophytes

For each marine macrophyte species the moisture, ash, protein, and lipid content were determined based on dry weight. Moisture was measured by drying the samples in an oven at 60 °C for 24 h, while the ash content was determined by burning the dry samples in a furnace at 500 °C for 4 h. Determination of protein content was performed using the method described by Lowry et al. [14] with bovine serum albumin as a protein standard. Lipids were extracted with dichloromethane/methanol (7:3 v/v) for 24 h and determined by gravimetric method [15].

Elemental analysis of species was achieved by combustion of the dried samples. The carbon (C) and nitrogen

(N) content were determined by a Flash EA 1112 NC Soil Analyzer (Thermo Quest, USA) whereas hydrogen (H) and sulfur (S) content were achieved employing a Flash 2000 CHNS-O Analyzer. The oxygen (O) content was calculated by difference and corrected for ash. All measurements were performed in triplicate.

Extraction and Quantification of Cell Wall Polysaccharides and Lignin

Cellulose, fucoidan, and alginate as well as the lignin content were determined in dry samples. The cellulose content was obtained using the method described by Siddhanta et al. [16] modified according to Freile-Peegrín et al. [17]. Briefly, 1 g of defatted biomass (7:3 v/v dichloromethane:methanol for 24 h) was soaked in 50 mL of 30% active H₂O₂ solution at 100 °C for 1.5 h for bleaching. After filtration, the bleached macrophyte residue was treated with 50 mL of 1% NaOH solution at 60 °C for 0.5 h. The alkali-treated biomass was washed with distilled water until neutrally and then filtered. The solid residue was resuspended in 50 mL of 0.1 N HCl and heated up for 10 min. The resultant slurry was cooled at room temperature, washed with water to remove excess acid, filtered, and freeze-dried to obtain cellulose yields based on the initial biomass.

For the seaweeds found in the beach-cast biomass, fucoidan and alginate were sequentially extracted through microwave-assisted extraction (MAE) in a Microwave Accelerated Reaction System (MARS, CEM Company, USA) following the methodology described in Vázquez-Delfin et al. [18] and according to Chale-Dzul et al. for fucoidan [19] and Hernández-Carmona et al. for alginates [20]. Briefly, 1 g of defatted biomass was suspended in 25 mL of distilled water and fucoidan was extracted from the resulting slurry into a closed vessel system (OMNI XP 1500) at microwave power of 800 W, 120 psi, 200 °C, 5 min heat-up and 1 min extraction. Thereafter filtrated, and the solid fraction dried at 60 °C and stored for alginate extraction, whereas the resultant water fraction was treated with 1% CaCl₂ and maintained overnight at 4 °C to precipitate and remove any residual alginates by centrifugation at 5,000 rpm for 30 min. The water fraction was then dialyzed for 48 h using H₂O changes every 12 h to recover the crude fucoidan thereafter freeze-dried and stored until required.

For alginate extraction, the solid fraction recovered after the fucoidan extraction was treated as follows: the dry residual biomass was soaked in 20 mL of HCl pH 4 for 30 min, with stirring at room temperature; after filtering and washing with distilled water, 20 mL of 1% formaldehyde was added. Samples were decanted and washed with distilled water. The samples and 20 mL of Na₂CO₃ were placed into the closed vessel system and the extraction was carried out at 800 W,

90 °C, 5 min heating, and 10 min extraction. After extraction, a hot filtration was done using diatomaceous earth. The supernatant was precipitated in pure ethanol. Alginate was dried at 60 °C, milled in a mortar, and weighed. Fucoidan and alginate yields were expressed as a percentage of the initial dry weight of the alga (% dw). Uronic acid content [21, 22] in both alginate and fucoidan and sulfate content [23] in the fucoidan was also determined.

The lignin content of the macrophytes was obtained based on the van Soest procedure [24] employing the Fiber-Cap System specifically designed for fiber following the Weende and van Soest method. An acid detergent extraction step to remove protein, hemicelluloses, and other components from the cellulose was performed using 1.0 N H₂SO₄ with cetyltrimethylammonium bromide (CTAB), boiling for 1 h. For lignin determination, the produced acid detergent fiber (ADF) was subsequently treated with 72% H₂SO₄ for 3 h at room temperature, to isolate the acid detergent lignin (ADL).

Sugars Composition

The sugar composition of the beach-cast marine macrophytes was determined by high-performance anion-exchange chromatography (HPAEC) with pulsed amperometric detection (PAD, Thermo Dionex, France) based on the procedure described by Pliego-Cortés et al. [25]. Briefly, samples were subjected to acid hydrolysis for 48 h at 100 °C (10 mg dw) with 110 µL of HCl 1 N and 1 mL Milli-Q water in a flame-sealed glass ampule. The hydrolysates obtained were then neutralized with NaOH 1 M and then filtered for analysis. An aliquot of 0.1 mL was taken from the sample, and 0.9 mL of aqueous solution containing deoxyribose (internal standard) was added to a final concentration of 50 ppm. An analytical column CarboPac PA-1 column (4.6 × 250 mm) preceded by a CarboPac pre-column (Thermo Dionex, France) was used. The injection volume was set to 25 µL and the elution was carried out isocratically, keeping the mobile phase for 30 min with 82% A solution (Milli-Q water) and 18% solution B (NaOH 0.1 M), followed by a gradient from minutes 31 to 35 with 100% C solution (NaOH 0.1 M + NaAcO 1 M), and from minute 36 to 80 with solutions A and B (82/18%). The column temperature was fixed at 30 °C. Carbohydrates were detected by PAD with a detector composed of a silver standard electrode and a gold working electrode. Peaks were read using Chromeleon 6 software (Thermo Scientific, France). A mixture of commercially available monosaccharides was used as standard, composed of fucose, rhamnose, arabinose, glucosamine, galactose, glucose, mannose, xylose, fructose, glucoheptose and glucuronic acid, at concentration ranges from 1.95 to 125.0 ppm. Deoxyribose was used as an internal standard

at 50 ppm. Results were expressed as micrograms of monosaccharides per milligrams of dry weight ($\mu\text{g mg}^{-1}$ dw) and as individual content in percentage of each monosaccharide from the total content (% of total content).

Fourier Transform Infrared (FT-IR) Analysis of Alginate and Fucoidan

The FT-IR spectra were recorded on a PerkinElmer FT-IR/NIR spectrophotometer FRONTIER (USA), at room temperature. Commercial alginate and fucoidan (Sigma, México) were used as controls. A spectral scan of 4000 to 400 cm^{-1} and 40 scans for the sample were carried out.

Statistical Analysis

All experiments were performed in triplicate and expressed as means \pm standard deviation. Data were evaluated using a one-way ANOVA to assess significant differences among groups of samples, followed by Tukey or Games-Howell tests, employing Jamovi 1.0.7.0 statistical software. For monosaccharide analysis, Welch's ANOVA test for multiple comparisons between groups using IBM SPSS Statistics 21 software was performed, and the Games-Howell post hoc test was applied as data did not fit the assumption of homogeneity of variances.

Results and Discussion

In the Caribbean beaches, the large accumulation of stranded marine macrophytes is a phenomenon that generates ecological impacts causing economic disruption to tourism, aquaculture, and traditional fisheries in coastal areas [11, 26]. One of the key recommendations of the Caribbean Sea Commission to address this problem is to support research for the utilization of this biomass, and one of the best options points to its use as feedstock for bioenergy [11]. However, due to their variable seasonal proportion and species composition, proposals for management strategies require baseline information on the macrophyte species present as well as on their chemical composition, which could determine their potential use as a source of renewable energy.

Species Proportion of the Beach-Cast Biomass

The beach-cast biomass collected in December 2018 at the Mexican Caribbean coast included both holopelagic (floating) and benthic (bottom-attached) brown seaweeds, and a benthic seagrass (Fig. 1). The seagrass *Syringodium filiforme* Kützting showed the highest relative abundance ($76.5 \pm 9.0\%$) of the beach-cast biomass, followed by the holopelagic species of *Sargassum* ($\sim 13.4\%$), while other benthic brown seaweeds including *Turbinaria turbinata* (Linnaeus) Kuntz ($4.9 \pm 8.3\%$), as well as a small amount of benthic *Sargassum* ($5.2 \pm 6.3\%$) composed of a variety of species. Although some previous studies have reported the



Fig. 1 Proportion of the different marine macrophytes found in the beach-cast biomass collected at Puerto Morelos, Quintana Roo, Mexico and their relative abundances

dominance of holopelagic *Sargassum* in the strandings of the Mexican Caribbean beaches [18, 27], it is well known that the composition of the beach-cast biomass can vary considerably according to the season and location. Thus, Vázquez-Delfin et al. [28, 29] reported that in the cold north wind season of 2022, the seagrass *Syringodium filiforme* was the dominant species, reaching a mean relative abundance of up to 65.6% (October) and 58.8% (December) of the total fresh biomass. These abundances are consistent with our results since the fresh beach-cast macrophytes biomass was also collected in December.

Within the holopelagic species of *Sargassum* (Fig. 1), *S. fluitans* (Linnaeus) Gaillon, and *S. natans* I (Børgesen) Børgesen were more abundant ($6.8 \pm 3\%$, $4.8 \pm 1\%$, respectively) than *S. natans* VIII ($1.8 \pm 1\%$). These results confirm those found in previous research on the most recent macrophyte massive influxes in the Caribbean [11, 18, 27], where holopelagic *Sargassum* is the main component (78.1–99.6%), with *S. fluitans* and *S. natans* I as the majority species. As for the benthic *Sargassum* species, Robledo et al. [26] and Vázquez-Delfin et al. [18, 28, 29] also described the presence of benthic macrophytes as minor component accounting for six benthic *Sargassum* species (*S. acinarium*, *S. buxifolium*, *S. platycarpum*, *S. polyceratium* var. *ovatum*, *S. pteuropleuron*, and *S. ramifolium*).

Chemical Characterization of the Beach-Cast Marine Macrophytes

All chemical analyses were performed on the three holopelagic species of *Sargassum* (*S. fluitans*, *S. natans* I, and *S. natans* VIII) as well as on the main benthic macrophytes found, the macroalga *Turbinaria turbinata* and the seagrass *Syringodium filiforme*.

Proximal Composition

Data from the proximate composition of the species is shown in Table 1. In general, the results obtained for moisture, ash, protein, and lipids were in agreement with data previously reported for marine macrophytes [30–32] but somewhat different from those reported for terrestrial plants. The water content (~72–83%) was much higher when compared to those reported for terrestrial crops which

are usually between 14 and 31% [33 and references therein]. The ash content was between 15.1 and 23.8% dw, in contrast to the typical wood ash content (0.5–2% dw) [33]. The protein content was around 6–12.4% dw, with a very low lipid content (<4% dw). The high percentage of ashes and water found in marine macrophytes studied could be seen as a disadvantage in obtaining biofuels. However, it should be noted that, in addition to the abundant availability of this biomass, the economy of bioethanol production could be sustained by the simultaneous use of different biomass components other than sugars. Among these, the protein content present in high concentrations in algae could be a candidate for different uses in a bioethanol refinery context [34]. In this regard, Del Río et al. [35] reported that the high protein content in *Sargassum muticum* (10.5% dw) allowed the fermentation step to be carried out without the addition of any nutrient as the first step since the production of bioethanol is based on the hydrolysis of polysaccharides followed by fermentation of the liberated monomeric sugars. Among the pretreatment processes, hydrothermal pretreatment (also called autohydrolysis or hot water pretreatment) is an environmentally friendly pretreatment process, compared with chemical pretreatment, because it uses only water for a reaction medium, without additional chemicals. This could represent a decrease in the industrial cost for TGB production since the initial pretreatment, dewatering, for the removal of water from the marine macrophytes biomass by mechanical methods is not desirable since it may result in a significant loss of fermentable content such as laminarin and mannitol which are easily fermentable [4].

Elemental Analysis

Elemental analysis of marine macrophytes is presented in Table 2. In general, C, N, H, S, and O in *S. fluitans*, *S. natans* I, *S. natans* VIII and *T. turbinata* from the Mexican Caribbean were in the range of values reported for other brown seaweeds, including other species of *Sargassum* [34, 36, 37]. Except for the H content, in the seagrass *S. filiforme* all elemental components, as well as elemental ratios, were significantly different compared to the brown seaweed. As expected, the highest S content was obtained in the seaweeds due to the sulfated polysaccharide (fucoidan) that is usually present in brown algae [12].

Table 1 Proximate composition (% dw) of the beach-cast marine macrophytes: holopelagic *Sargassum* species (*S. Fluitans*, *S. natans* I and *S. Natans* VIII); benthic macroalga *Turbinaria turbinata*; benthic seagrass *Syringodium filiforme*. Values are mean \pm standard deviation. Different superscript letters in the row indicate statistically significant differences ($p < 0.05$)

	<i>S. fluitans</i>	<i>S. natans</i> I	<i>S. natans</i> VIII	<i>T. turbinata</i>	<i>S. filiformis</i>
Moisture	83.5 ± 1.5^a	82.0 ± 0.9^a	83.6 ± 1.6^a	72.0 ± 1.1^b	77.4 ± 2.1^c
Ash	23.8 ± 1.0^a	23.5 ± 2.3^a	23.3 ± 1.9^a	15.1 ± 0.2^b	19.2 ± 1.1^c
Protein	10.4 ± 1.9^a	12.4 ± 1.8^a	11.2 ± 2.5^a	6.2 ± 2.0^b	11.0 ± 2.5^a
Lipids	1.0 ± 0.1^a	0.6 ± 0.0^b	0.7 ± 0.1^b	4.0 ± 0.1^c	2.2 ± 0.0^d

Table 2 Elemental analysis (% dw) of the beach-cast marine macrophytes: holopelagic *Sargassum* species (*S. Fluitans*, *S. natans* I and *S. Natans* VIII); benthic macroalga *Turbinaria turbinata*; benthic seagrass *Syringodium filiforme*. Values are mean \pm standard deviation. Different super-script letters in the row indicate statistically significant differences ($p < 0.05$)

	<i>S. fluitans</i>	<i>S. natans</i> I	<i>S. natans</i> VIII	<i>T. turbinata</i>	<i>S. filiformis</i>
C	31.0 \pm 0.6 ^a	31.0 \pm 2.6 ^a	31.8 \pm 1.0 ^a	39.8 \pm 0.2 ^b	39.9 \pm 0.6 ^b
N	1.2 \pm 0.1 ^a	1.1 \pm 0.1 ^a	1.1 \pm 0.1 ^a	1.4 \pm 0.0 ^b	2.1 \pm 0.1 ^c
H	4.5 \pm 0.3 ^a	4.2 \pm 0.2 ^a	4.4 \pm 0.1 ^a	5.1 \pm 0.3 ^b	4.6 \pm 0.1 ^a
S	1.5 \pm 0.1 ^{ab}	1.3 \pm 0.1 ^{ab}	1.3 \pm 0.1 ^{ab}	1.2 \pm 0.0 ^b	0.5 \pm 0.0 ^c
O	38	38.9	38.1	37.4	33.7
Elemental ratios					
C: N	25.8	28.2	28.9	28.4	19
C: O	0.81	0.8	0.83	1.06	1.18
C: H	6.89	7.38	7.23	7.8	8.67

The higher C: N ratios in seaweeds (25.8–28.9) compared to those in *S. filiforme* (19) are worth mentioning. This ratio is an indicator of the nutritional status of marine macrophytes and can vary depending on the availability of nutrients. Thus, the average C: N ratio of *Sargassum* reported for oceanic waters is 47 in contrast to 27 which corresponds to neritic or nutrient-rich waters [38], which coincides with the C: N values obtained in the present study for the brown seaweeds. In this sense, as a first step in the production of bioethanol from biomass, simple carbon compounds such as soluble sugars, organic acids, etc. must be degraded, and, therefore, the C: N ratio plays a vital role in its production [39, 40]. Following the recommendations of De Bertoldi et al. [41] to optimize the development of a biological degradation process, the C: N ratio should be from 20 to 30. Biomass with an excess of degradable substrate represented by a C: N ratio > 30 , slows the process, likewise a C: N ratio < 20 results in nitrogen losses that also slow down the process [42]. In general, the C: N ratios obtained in the present study, particularly for the seaweeds, are within the range of the optimal values which favor the rapid and adequate decomposition of the biomass. As for the C: O and C: H ratios, values obtained in the present study are similar to those reported for *Sargassum* species [37, 43]. It is interesting to note that the slightly lower C: O values in seaweed compared to seagrass are probably associated with their higher oxygen content related to the carboxylic acid groups present in their alginate and fucoidan contents (Table 2).

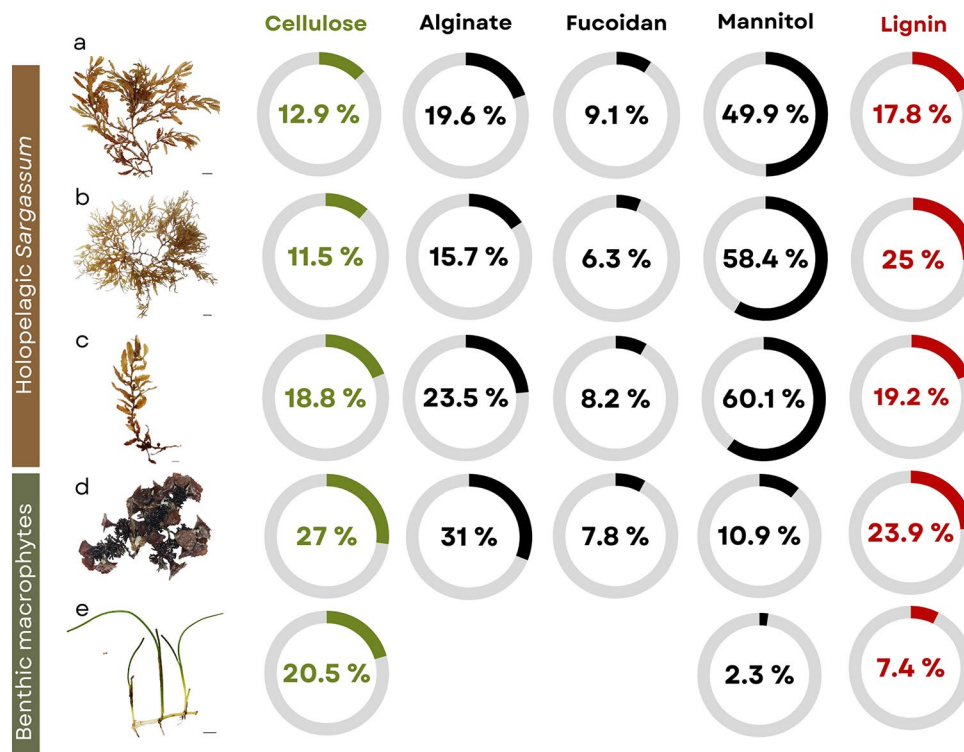
Glucan and Non-Glucan Carbohydrates

For bioethanol production purposes, beach-cast marine macrophyte glucan (cellulose) and non-glucan (alginate, fucoidan, and mannitol) carbohydrates, as well as lignin content, are of particular interest (Fig. 2). It should be noted that laminaran content has not been considered in the present study since it is found as a very minor component ($< 1\%$ dw) in the species studied [44].

The cellulose content found in analyzed macrophytes ranged between 11.5 and 27% dw. In general, brown seaweeds have been reported to contain around 10–13% cellulose [16, 45, 46]. However, the cellulose content in the studied macrophytes was almost double, with a maximum obtained for *Turbinaria turbinata* with $27.0 \pm 3.7\%$ dw, followed by $18.8 \pm 1.6\%$ dw in *Sargassum natans* VIII, and *Syringodium filiforme* ($18.8 \pm 3.0\%$ dw), which is of interest for potential TGB production since cellulose in lignocellulosic biomass represents 15–30% of the dry weight [1]. For the other *Sargassum* species (*S. fluitans*, *S. natans* I) no significant differences in their cellulose content were found ($p > 0.05$) with an average of 12.2% dw. In the seagrass *Syringodium filiforme* the cellulose content is in the range with values reported for other seagrass species [30].

In brown seaweeds, a double-layered cell wall structure has been described. The inner layer is composed of cellulose that imparts rigidity, and the outer layer comprises an amorphous encrusting matrix of alginate and fucoidan which contribute to cell wall strength and flexibility [12]. The presence of alginate and fucoidan (non-glucan carbohydrates) in the beach-cast seaweed biomass collected was confirmed by the FT-IR spectra (Fig. 3). The absorption bands were assigned based on published data on alginate [47] and fucoidan [48]. Both alginate and fucoidan FT-IR spectra of all species showed a similar pattern following the respective standards. For alginates and fucoidan, a broad band between 3260 and 3400 cm^{-1} corresponding to the O-H stretching vibrations were evident (Fig. 3A and B). Alginate is a linear polyuronic acid consisting of mannuronic acid and guluronic acid, at this spectra (Fig. 3A), signals at around 2932 cm^{-1} corresponded to C-H vibration. The absorption bands around 1614 and 1410 cm^{-1} are attributed to stretching vibrations of asymmetric and symmetric bands of carboxylate anions, respectively. The signals at 948, 893, and 814 cm^{-1} specific to the guluronic and mannuronic acids were observed. Fucoidans are complex polysaccharides that, besides fucose and sulfate, also contain other monosaccharides (mannose, galactose, glucose, xylose, etc.) and uronic

Fig. 2 Cellulose, alginate, fucoidan, mannitol and lignin content of the species contained in the beach-cast biomass collected in Puerto Morelos, Quintana Roo, Mexico: *Sargassum fluitans*, *S. natans* I, *S. natans* VIII, *Turbinaria turbinata* and *Syringodium filiforme*



acid. In the fucoidan spectra (Fig. 3B) a band at the region of 2940 cm^{-1} was observed which indicated the presence of C-H stretching vibrations of the pyranoid ring and C6 groups of fucose and galactose units. The main information for the position of sulfate groups is contained in the ranges of wavenumber $1500\text{--}700\text{ cm}^{-1}$. Absorption bands were observed at 1240 cm^{-1} , which is common to all sulfate ester vibrations; the band around 825 cm^{-1} corresponds to sulfate groups in the equatorial positions C2 and C3 of fucose residues. Bands at 1620 and 1420 cm^{-1} correspond to $\text{C}=\text{O}$.

from *Sargassum fluitans*, *S. natans* I, *S. natans* VIII and *Turbinaria turbinata*.

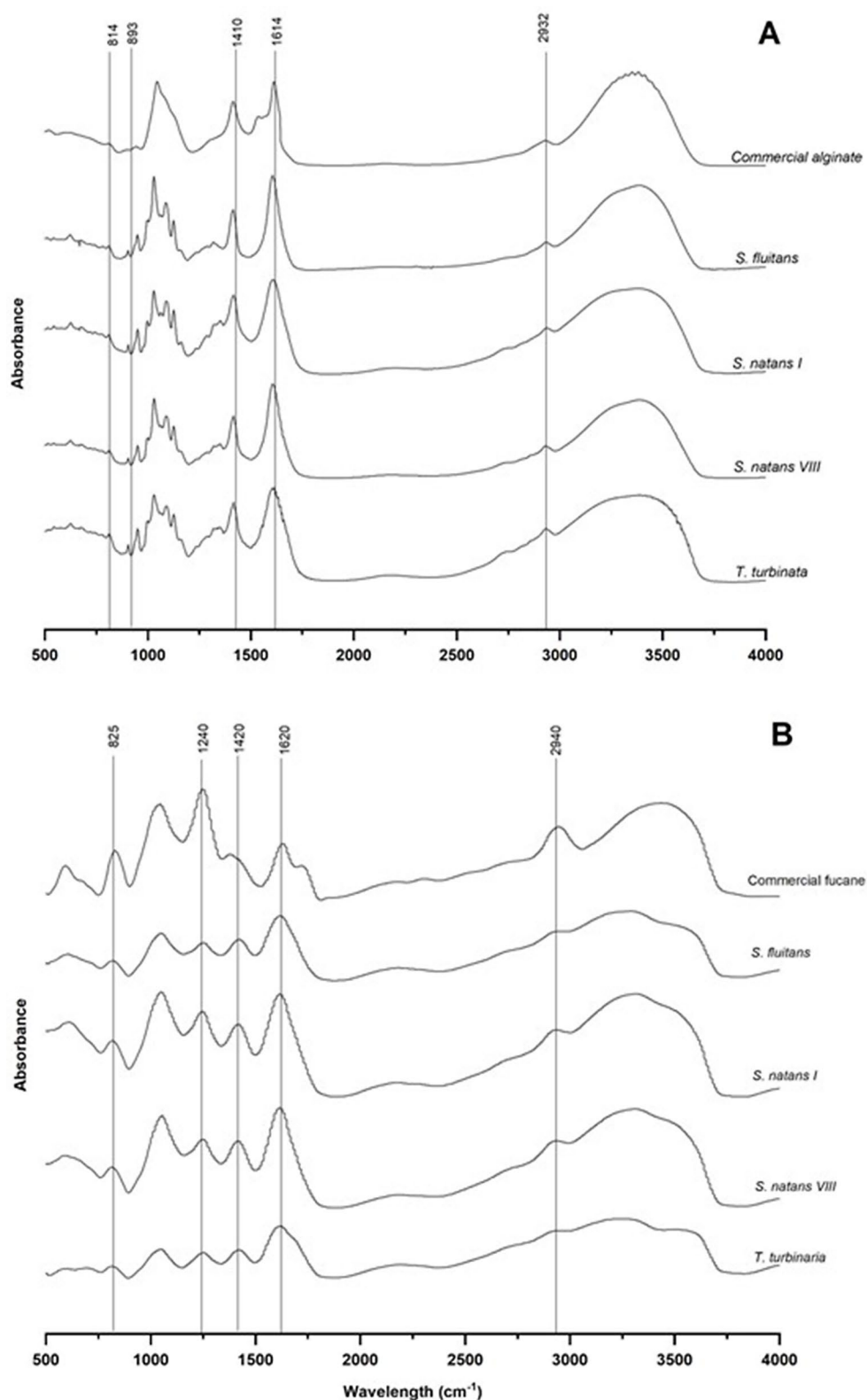
The alginate content in brown seaweed varied between 15.7 and 31% (Fig. 2) and agrees with earlier reports [44]. The highest alginate content was found in *T. turbinata* ($31.0 \pm 2.2\%$) followed by *S. natans* VIII ($23.6 \pm 0.6\%$) and no significant differences ($p > 0.05$) in the other *Sargassum* species were found with an average content of 17.7%. Fucoidan was found in minor proportion in all seaweed species ranging from 6.3 to 9.1% (Fig. 2), with the lowest content in *S. natans* I, and no significant differences ($p > 0.05$) between *S. fluitans* and *S. natans* VIII. Uronic acid and sulfate content found in alginate and fucoidan is shown in Table 3. For all species, uronic acid content ranged between 20.1 and 23.2% in alginate, and between 4.6 and 6.8% in fucoidan. The sulfate content in alginate was on average $\sim 1.3\%$ except for *T. turbinata* (2.7%) and approximately 11.1% in fucoidan,

except for *S. fluitans* (5.5%). These values were in the range of previous reports for other tropical brown seaweeds [49].

The mannitol content was highly variable in the beach-cast marine macrophytes studied and ranged from 2.3 to 60.1% (Fig. 2). The highest content in seaweed occurred in the three *Sargassum* species (49.9–60.1%) followed by *T. turbinata* ($10.9 \pm 0.3\%$) whereas the lower content was observed in the seagrass ($2.3 \pm 0.1\%$). Mannitol, a sugar alcohol derived from D-mannose is the first accumulation product of photosynthesis in brown algae and can constitute up to 30% of the dry weight [50]. Therefore, changes in mannitol concentrations are indicative of photosynthetic activity variation, which are probably much higher in the floating *Sargassum* species compared to benthic macrophytes, due to their permanent exposition to high irradiation conditions [51, 52]. Since the calorific value of mannitol is higher than that of glucose (3025 kJ/mol versus 2805 kJ/mol), carbon distribution and different redox states, it has been revealed that mannitol was more favorable than glucose for ethanol production [53].

The main raw material for ethanol production, glucose, is obtained from the hydrolysis of glucans (i.e. cellulose), and through enzymatic fermentation, bioethanol is subsequently produced. For marine macrophytes, to achieve high concentrations of bioethanol the conversion of carbohydrates other than glucans is mandatory. The non-glucan carbohydrates in the seaweed, besides mannitol, were alginate and fucoidan, polysaccharides that were on average $\sim 22\%$ and $\sim 8\%$

Fig. 3 Fourier transform infrared (FT-IR) spectra of alginate (A) and fucoidan (B)



dw, respectively. Therefore, the initial step to the bioethanol conversion of these beach-cast macrophytes needs to involve a hydrolysis pretreatment to loosen and depolymerize cell wall linkages to proceed with the saccharification

process. On the other hand, for mannitol, which dissolves easily, neither saccharification nor pretreatment is necessary. Although mannitol is rarely fermentable, bacteria recently isolated, can convert mannitol to produce ethanol [53]. In

Table 3 Uronic acid (% dw) and sulfate content (% dw) in alginate and fucoidan extracts from the holopelagic *Sargassum* species (*S. Fluitans*, *S. natans* I and *S. Natans* VIII); benthic macroalga *Turbinaria turbinata*. Values are mean \pm standard deviation. Different superscript letters in the row indicate statistically significant differences ($p < 0.05$)

	S. fluitans	S. natans I	S. natans VIII	T. turbinata
Alginate				
Uronic acid	21.7 \pm 1.59	23.22 \pm 1.04	20.96 \pm 0.34	22.40 \pm 1.89
Sulfate	1.61 \pm 0.15 ^b	0.98 \pm 0.03 ^c	1.22 \pm 0.08 ^{bc}	2.72 \pm 0.09 ^a
Fucoidan				
Uronic acid	6.57 \pm 0.41	4.60 \pm 0.61	6.68 \pm 0.54	6.80 \pm 0.35
Sulfate	5.51 \pm 0.45 ^b	11.09 \pm 0.92 ^a	9.62 \pm 0.56 ^a	12.54 \pm 0.68 ^a

general, the high proportion of mannitol in the beach-cast marine macrophytes from the Mexican Caribbean, mainly found in *Sargassum* species, makes this biomass a promising feedstock for bioethanol production.

Monosaccharides Profile

The monosaccharides found in the beach-cast marine macrophytes are shown in Table 4. The main common monosaccharides were glucose, fucose, mannose, and galactose; minor amounts of xylose, fructose, glucoheptose, and arabinose were also detected. Glucuronic acid was markedly present only in the brown seaweed, due to alginate and fucoidan content, with the highest content in *Turbinaria turbinata*, linked to the higher content of these polysaccharides compared to *Sargassum* species. It is interesting to note that in *Sargassum natans* I small amount of glucosamine was observed most probably related to its fucoidan composition. Curiously, fucoidans containing this amino sugar have been

reported to possess multiple bioactivities [54]. Some differences between the holopelagic and benthic seaweed were also observed: the highest number of monosaccharides was found in benthic *T. turbinata*; in particular, glucose (23.9%) almost twice that found in holopelagic *Sargassum* species (9.8% on average), probably associated to its higher cellulose content as a reflection of self-adaptation to withstand hydrodynamic forces, being bottom-attached, in contrast to floating *Sargassum* species. On the other hand, as expected, glucose was the dominant monomeric carbohydrate in the angiosperm *Syringodium filiforme* (~58%) analogous to land plants, cellulose is a major component of their cell walls [12, 55]. Furthermore, sucrose, a disaccharide composed of glucose and fructose, has also been reported as a dominant soluble storage carbohydrate in seagrasses [56]. Rhamnose was recorded only in *S. filiforme* and coincides with previous reports on the carbohydrate composition in this species [57].

Lignin Content

The lignin content in all macrophytes was also determined with the lowest content for *Syringodium filiforme* (7.4 \pm 1.1%) in the range of other seagrass species (5–11%) [13]. In brown seaweeds, the lignin content was two to three times higher than the seagrass. *Sargassum fluitans* and *Sargassum natans* VIII showed lignin content of 17.8 \pm 0.6% and 19.2 \pm 1.1%, respectively ($p > 0.05$), with the highest content in *S. natans* I (25.1 \pm 0.6%) and *Turbinaria turbinata* (23.9 \pm 0.2%) ($p > 0.05$). In general, seaweeds are known to contain no lignin at all or have a low content of lignin-like compounds in response to environmental conditions [3, 4]. However, recently the presence of lignin has been confirmed in *T. turbinata* [58] and for the genus *Sargassum* [59] reaching values of 25–29% dw which is

Table 4 Monosaccharide profiles of the beach-cast marine macrophytes: holopelagic *Sargassum* species (*S. Fluitans*, *S. natans* I and *S. Natans* VIII); benthic macroalga *Turbinaria turbinata*; benthic seagrass *Syringodium filiforme*. Values are mean \pm standard deviation. Different superscript letters in the row indicate statistically significant differences ($p < 0.05$)

Monosaccharide content (% of total)	S. fluitans	S. natans I	S. natans VIII	T. turbinata	S. filiforme
Fucose	16.41 \pm 1.72 ^a	13.91 \pm 0.69 ^a	12.56 \pm 0.54 ^a	22.96 \pm 0.91 ^b	3.47 \pm 0.06 ^c
Rhamnose	—	—	—	—	8.76 \pm 0.36
Arabinose	0.56 \pm 0.02 ^a	0.39 \pm 0.06 ^a	0.58 \pm 0.16 ^a	—	2.56 \pm 0.12 ^b
Glucosamine	—	0.48 \pm 0.01	—	—	—
Galactose	4.22 \pm 0.43 ^a	4.57 \pm 0.35 ^a	3.37 \pm 0.38 ^a	12.85 \pm 0.33 ^b	8.68 \pm 0.63 ^c
Glucose	12.52 \pm 1.99 ^a	8.33 \pm 0.38 ^a	8.74 \pm 0.10 ^a	23.92 \pm 1.13 ^b	57.89 \pm 1.41 ^c
Mannose	8.26 \pm 0.59 ^a	6.65 \pm 0.48 ^a	7.20 \pm 0.38 ^a	12.49 \pm 0.39 ^b	2.41 \pm 0.24 ^c
Xylose	0.70 \pm 0.19 ^a	0.51 \pm 0.11 ^a	0.60 \pm 0.11 ^a	0.37 \pm 0.07 ^a	8.66 \pm 0.94 ^b
Fructose	1.50 \pm 0.26 ^{a,b}	0.82 \pm 0.11 ^a	0.71 \pm 0.07 ^a	2.29 \pm 0.10 ^b	2.72 \pm 0.11 ^c
Glucoheptose	1.23 \pm 0.26 ^{a,b}	0.67 \pm 0.05 ^a	0.54 \pm 0.06 ^a	1.99 \pm 0.05 ^b	2.55 \pm 0.09 ^c
Glucuronic acid	4.88 \pm 0.51 ^a	5.39 \pm 0.55 ^a	5.82 \pm 0.19 ^a	12.17 \pm 0.22 ^b	—
Total content ($\mu\text{g mg}^{-1}\text{dw}$)	77.81 \pm 9.52	100.95 \pm 7.10	116.02 \pm 16.72	56.38 \pm 2.11	60.34 \pm 1.12

consistent with our results. These values are lower than those reported for woody biomass (30–60% dw) but comparable to grasses (10–30% dw) [1]. Although less complex than most vascular plants, brown seaweeds are morphologically more complex than other macroalgae groups [60]. Different phylogenetic studies have shown that brown algae are not related to the green or red lineages, revealing evolutionary processes that resulted not only in multicellularity and cell differentiation but also in the uniqueness of their cell wall composition. This evidence a horizontal transfer of genes responsible for the biosynthesis of some of the components of its cell wall from other organisms [61]. Lignin in vascular plants provides structural support and protects against microbial attack and desiccation. In the same way, lignin synthesized in brown seaweeds could be used for similar strategies. Since lignin is a complex phenolic polymer that naturally differs in composition between different biomass materials, the knowledge of its macromolecular structure is the key to designing suitable processes within biomass biorefineries.

Implications of the Use of Beach-Cast Marine Macrophytes in Bioethanol Production

Based on the above results, some advantages and limitations can be considered when converting marine beach-cast macrophytes into TGB, which are discussed below:

Seaweeds and seagrasses live in an environment with strongly changing abiotic conditions such as temperature, irradiation, nutrients, and salinity. These factors influence both their growth and their carbohydrate content. Through evolution, the metabolism of these plants has adapted to withstand these changes, acclimating their photosynthetic mechanisms to maximally absorb incident photons and use the absorbed energy with high efficiency, which results in increased carbohydrate accumulation [60, 61]. Thus, marine macrophytes usually have a greater photosynthetic capacity and faster growth rates compared to terrestrial plants [62] showing yields per unit area four times higher than those obtained by highly productive crop plants such as sugar cane [8]. Furthermore, one of the obstacles in commercial TGB production is being able to obtain a large amount of carbohydrate-rich biomass at a low price. Thus, high production of profitable biomass is a key success factor for its commercialization since there is a significant economic difference between the use of marine macrophytes that grow naturally or those that are cultivated with an estimated cost between 21 and 112 dollars per metric ton dry [63]. Concerning the stranded biomass in the Mexican Caribbean, the potential production of bioethanol from these marine macrophytes represents an interesting prospect because it could be generated from abundant locally available and

underutilized biomass that does not compete with any agricultural resource. However, one of the main limitations to producing bioethanol from this biomass consistently is its availability throughout the year, as well as the seasonality in the proportion of species, thus affecting their sugar composition [28, 29]. It should also be noted that there is likely to be a disconnection between the location of the resource and the conversion facility, so transporting large volumes of biomass represents an additional cost to consider.

On the other hand, there are some methodological challenges in obtaining TGB from beach-cast marine macrophytes. The production of bioethanol requires the transformation of polysaccharides into simple sugars, and the process consists of pretreatment, hydrolysis, either by acid or by enzymatic means, and fermentation. The ability to achieve conversion rates $> 80\%$, together with low energy consumption and high yields, under an environmentally friendly approach, make the enzymatic process more attractive for bioethanol production [64]. In particular for seaweeds, recent technology includes eco-friendly procedures that combine enzymatic hydrolysis with fermentations, showing positive results and cost reduction in *Sargassum* species [65]. Enzymatic hydrolysis seems a promising route compared to acid hydrolysis since a higher yield of bioethanol from seaweeds (0.909 g g^{-1}) has been reported compared to that obtained by acid hydrolysis (0.390 g g^{-1}) [64]. Although marine macrophytes produce high amounts of carbohydrates of a heterogeneous nature [13, 55] potentially suitable for enzymatic fermentation, it must be considered that the addition of specific and appropriate enzymes is required to obtain the different types of sugars. From our results with the stranded biomass from the Mexican Caribbean, a valuable cellulose content is present in the whole biomass (18% on average) as well as a notable mannitol content in all marine macrophytes (36.3% on average) higher in *Sargassum* species (56% on average). Although both carbohydrates are easily hydrolyzable and fermentable with high ethanol yields [3, 53] the conversion of other carbohydrates such as alginate and fucoidan is also necessary to produce profitable bioethanol from these beach-cast marine macrophytes. Because ethanologenic microorganisms do not use alginate or alginate degradation products as substrates, it is difficult to produce ethanol from alginate. However, the development of new metabolically modified microbes is making it possible to expand TGB production. In this context, Takeda et al. [66] produced ethanol from alginate using *Sphingomonas* sp strain A1, an alginate-assimilating bacterium, expressing both pyruvate decarboxylase and alcohol dehydrogenase. In another study, *Escherichia coli* was genetically engineered to assimilate alginate and produce ethanol. This engineered *E. coli* strain BAL1611 converted alginate from the brown seaweed *Laminaria*

japonica into ethanol [67]. As for the fucoidan, the enzyme fucoidanase, obtained from marine invertebrates, marine bacteria, and marine fungi can hydrolyze this sulfated polysaccharide [68]. In general, fucoidanase exhibits low activities, therefore, it is essential to continue studies screening of different microorganisms capable of producing highly active fucoidanase, as well as to optimize its production and purification. In this regard, the production of fucoidanase by *Dendryphiella arenaria*, an obligate marine fungus associated with macroalgae, has recently been reported as a promising cost-effective, and environmentally friendly process, and its crude enzymes have been successfully used in the hydrolysis of fucoidan from the brown seaweeds *Cystoseira trinodis* [69] and *Sargassum latifolium* [68].

Additionally, for high water content biomass, such as marine macrophytes, high-pressure technology pretreatment, hydrothermal processing also known as autohydrolysis, has gained increasing attention in recent years. Water present in the feedstock functions as a solvent, reagent, and catalyst in a cascade of organic reactions, and one of the main products obtained includes water-soluble products that can be subjected to fermentation for bioethanol production. The technology for the direct use of this biomass in TGB production is also considered a cost-effective and environmentally friendly process [70] that has been successfully applied to *Sargassum* species with high enzymatic susceptibility and high content of hexoses such as glucose, galactose, and mannose to obtain ethanol via fermentation [35, 65].

In summary, although hydrolysis and fermentation processes still present many challenges to be solved, new technologies developed for the production of TGB from marine macrophytes are promising. Improved pretreatment methods and suitable enzyme cocktails that can degrade a wide variety of polysaccharides into fermentable sugars could increase bioethanol production at economically viable concentrations. A variety of modified microorganisms appear to be appropriate for improved yields in TGB production, however, high production cost limits scale-up from the laboratory to the processing of large quantities of biomass. In this regard, a biorefinery approach could be used for this biomass if different value-added products are delivered [8, 71]. The utilization of carbohydrates and polysaccharides fractionated at the fermentation and hydrolysis process generates many organic wastes like proteins, and lipids, thereby increasing the economic value of macroalgae biomass. This could be maximized under a biorefinery approach, and techno-economic analyses to design viable processes [71]. Moreover, some biorefinery processes generate a cellulose-enriched residue, which can be hydrolyzed and fermented to bioethanol more efficiently by conventional yeast strains. In addition to the production of TGB, brown seaweed of

the genus *Sargassum* and *Turbinaria* both present in the stranded biomass of the Mexican Caribbean could be used in a biorefinery context to extract alginate, fucoidan, as well as biochemicals of pharmaceutical importance [26, 72, 73].

Ecological and Economic Context: Limitations and Challenges

Most of the Caribbean areas affected by beach cast marine macrophytes are touristic destinations, therefore, both the impacts and the need for solutions represent a great challenge. In this context ecological and socioeconomic impacts of these events for the Caribbean region and Western Atlantic coasts have been previously reviewed by Robledo et al. [26]. Impacts include both economic, such as tourism, fisheries, recreational activities, and ecological, such as perturbation of marine species (corals), beach erosion and decomposition of biomass. Moreover, the frequency and magnitude of beach cast events may limit the usage of these biomasses and the development of a green industry which may positively impact the socioeconomic aspects of local communities, as well as having some environmental benefits [29]. Therefore, in order to establish a bioethanol industry based on marine beach cast biomass long-term monitoring using citizen science could be used [28]. This may provide an opportunity to develop environmental education in the region to strengthen conservation, increase awareness, develop sustainable actions for resource management and lay foundations for the establishment of a long-term monitoring program for strandings in the region [28].

On the other hand, management of beach cast biomass to produce bioethanol may constitute an economical challenge as specific equipment and infrastructure are needed to collect, transport and process the biomass. These may enter in conflict with economic activities in coastal touristic areas, with hotels and natural reserves, as well as logistics such as accessibility to the beaches for collection. The management of beach cast events represent a challenge to governments, research institutions, local communities, tourist industry and other private sectors. The right balance between the valorization of natural resources, technological development and responding to the need for coastal management in the affected areas is fundamental to the development of strategic management.

Estimation of the Production Capacity of TGB from Beach-cast Macrophytes from the Mexican Caribbean

The potential for ethanol production from marine macrophytes can be roughly estimated theoretically from their carbohydrate contents (40–60% dry weight) [3, 30] and

their ethanol conversion rates (89–90%) [64, 67, 74]. Based on data from a review conducted by Kraan [8], fermentation of 1 g of sugar from seaweeds can produce 0.4 g of ethanol, which yields 0.22 kg or 0.27 L of ethanol from 1 kg of dry-weight biomass, corresponding to approximately 0.05 L of ethanol per kg wet weight. A similar result has been reported for seagrasses with 0.047 mL of ethanol per g of wet weight [75]. For brown seaweed, including *Sargassum*, yields have been also reported within this range [74, 76, 77]. In previous studies, most of the ethanol produced derives from mannitol due to the unusually high content of this sugar in these seaweeds. In the present study, the beach-cast macrophytes from the Mexican Caribbean have an advantageous potential as raw material since they are also rich in mannitol. For a rough estimate of the TGB production capacity from these potential feedstocks, it would be necessary to consider the spatio-temporal variation of the biomass. For a calculation of the same collection site of the present study (20° 51' 04.3" N; 86° 52' 20.8" W, Puerto Morelos, Quintana Roo), Vázquez-Delfín et al. (2024) [29] reported a maximum of 74.5 kg m⁻² of fresh weight with a volume of 1330.7 m³ per km of beach of stranded biomass during the summer (May 2019) which corresponds to 238.7 t of fresh biomass per km of beach. Considering that the coastal extension of the collection site where strandings occur is 17.7 km, the total amount of stranded biomass could be estimated as 4225 t of fresh biomass. Based on the bioethanol yields reported above, the ethanol production in the study site can be theoretically calculated to generate a yield of 211,250 L of ethanol. Likewise, to estimate bioethanol production throughout the Mexican Caribbean, precise information on the abundance of macrophytes in the area can be used, which is reported in the *Sargassum* Monitoring and Forecast Bulletin in the Caribbean Sea (Oceanographic Institute of the Gulf and Sea Caribbean-IOGMC) [78]. This is a periodic report on the monitoring and forecast of *Sargassum* which moves from the Western Central Atlantic to the Mexican Caribbean coasts. For 2018 and 2019 alone, data showed a maximum of 54,198 and 50,935 t of fresh biomass stranded on Caribbean beaches during the summer (July–August), which could translate into a production of 2,710,000 and 2,547,000 L of ethanol, respectively.

Conclusions

Beach-cast marine macrophytes biomass reaching the Mexican Caribbean coast generates serious environmental and social problems but can be regarded as a promising alternative source for TGB production due to their abundance, underutilization, and low-cost. This feedstock is also rich in polysaccharides composed of glucose, and other fermentable

carbohydrates, in addition to the elevated amounts of mannitol in *Sargassum* and *Turbinaria* species which may increase ethanol yields. Specific carbohydrates such as alginate and fucoidan are present in adequate amounts and can also be converted to fermentable sugars with the proper methodology, thus increasing bioethanol yield. Nevertheless, the technologies for large-scale production are underdeveloped and the main bottleneck is to achieve species-specific and appropriate methodologies for the complete hydrolysis of complex polysaccharides to obtain fermentable sugars. Further studies and exhaustive research on hydrolysis and fermentation processes are required to improve yield and reduce costs associated with its production. To compensate for the high cost of production, these macrophytes could be a promising feedstock in the context of biorefineries for, in addition to TGB production, obtaining high-added value compounds. However, techno-economic analysis are still required for the correct implementation of a biorefinery process to obtain these compounds for a commercial future. It is worth noting that the unpredictability of the marine macrophyte strandings requires long-term monitoring to evaluate the seasonal and interannual variation of the abundance and species composition of the beach-cast macrophytes.

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Data Availability All data generated or analyzed during this study are included in this published article.

Declarations

Competing Interests The authors declare no competing interests.

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