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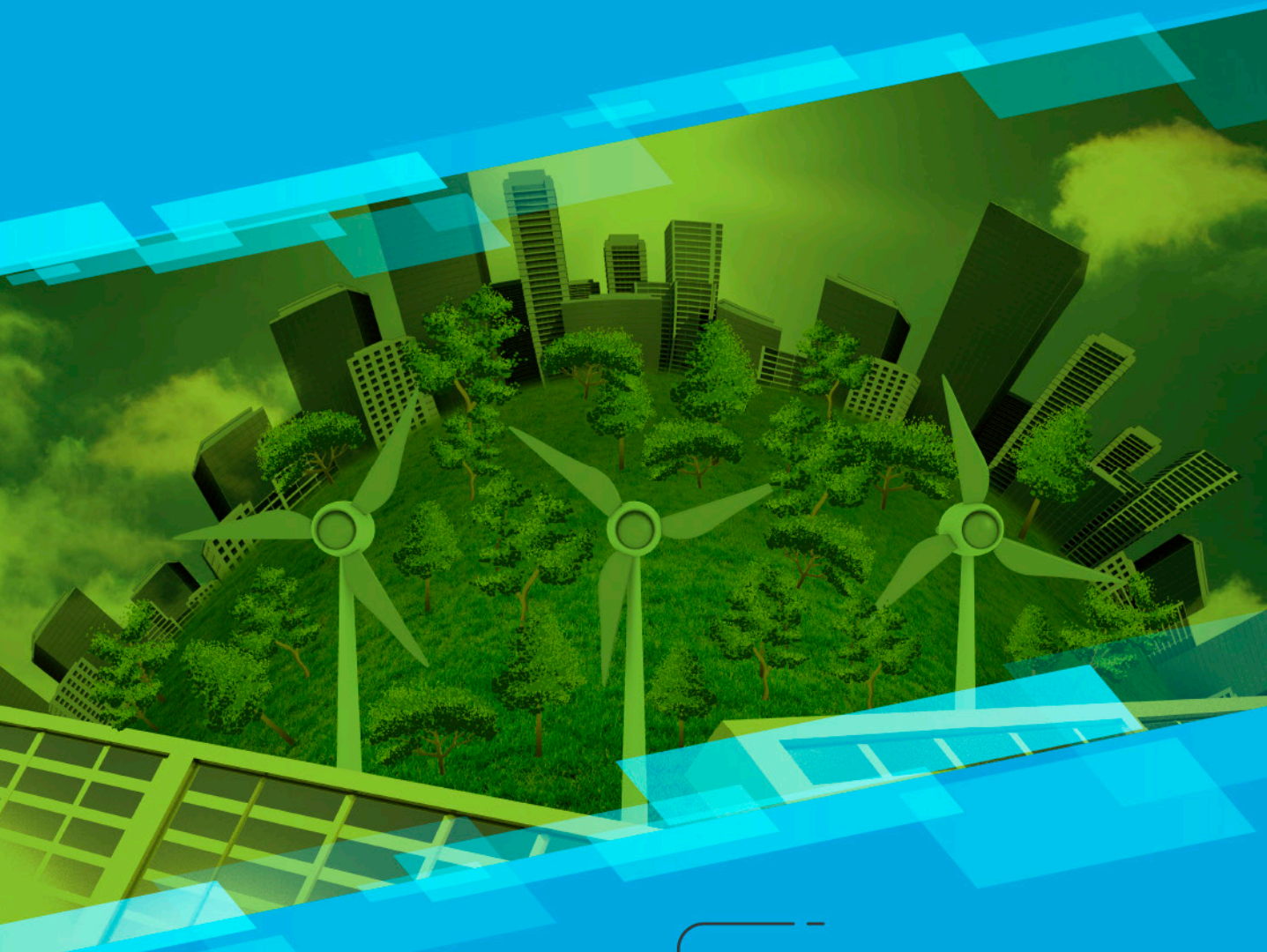

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Acid depolymerization of cell wall polysaccharides from Ulvan-rich extracts of green seaweeds

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Introduction

There is an emerging interest for marine biomass resources due to its unique and complex structures which serve as renewable sources for chemicals and polymers into a wide variety of applications. Seaweed polysaccharides (fucoidan, carrageenan, alginate, agar-agar, ulvan, etc) are bioactive compounds with therapeutic applications (i.e. antiviral activity, immuno-inflammatory or antitumoral among others). Ulvan is a structural sulfated polysaccharide from the cell walls of *Ulva spp* green seaweed *(Chlorophyta)* that contains mainly two types of disaccharides, ulvanobiuronic 3-sulfate type A and ulvanobiouronic 3-sulfate type B; containing L-rhamnose, D-glucose and D-xylose as sugars and D-glucuronic and L-iduronic as uronic acids (Tako et al., 2015) and represents about 8-29% of *Ulva spp* total dry weight. Ulvan can form thermoreversible gels, exfoliated complexes with clays, and its demonstrated biological properties are valuable form agricultural, food and pharmaceutical applications (Cunha & Grenha, 2016). However, there is not a clear consensus regarding adequate ulvan composition or analytical procedures. Therefore, the exact chemical structure and composition as well as the molecular weight of ulvan is not well-known (Pankiewicz et al., 2016). These parameters directly affect the biological activity and the physic-chemical properties of the different ulvans isolated from *Ulva spp* seaweed cell-wall which is crucial for meeting the required specifications of each final application (tissue engineering, skin care, pre-biotics, etc) of the different potential markets (cosmetics, nutraceutics, pharmaceutics). In order to study the chemical composition of ulvan, different depolymerization methods can be used. Most widely used in the literature for ulvan-rich extracts depolymerization is methanolysis (Costa et al., 2012; Pezoa-Conte et al., 2015). Nevertheless, in a previous study, authors studied three different methods for depolymerization of Ulvan-rich extracts from *Ulva rigida* (methanolysis, mild acid hydrolysis -hydrochloric acid- and strong acid hydrolysis- sulfuric acid) and the highest sugar conversion was observed under mild acid hydrolysis (Macías et al., 2019). For this reason, the main goal of this research is to study the acid depolymerization of the cell-wall polysaccharides of *Ulva rigida* with HCL under different conditions and the quantitative analysis of the neutral and acidic monomers.

Materials and Methods

Ulva rigida provided by Investalga Ahti S. L. company was three times washed with ultrapure water, oven-dried and milled. Then, extraction of the sulfated-polysaccharides was done by hot water extraction. After that, ulvan-rich extracts were separated from the spent green seaweed, filtered, centrifuged (30 min, 5000 rpm) and supernatant was oven-dried at 105ºC until constant weight.

HCL at three different concentrations (0.5 M, 1 M, 2 M) and three different temperatures (80ºC, 100ºC and 120ºC) were used in glass test tube digester. Depolymerization time was also studied. A total of 17 times ranging from 1 min to 24 h were carried out in triplicated. 10 mg of the dried extracts were weighted and mixed in a vortex shaker with 2 mL of HCL solution. All experiments were done in triplicate. Once the experiment is finished, 3 mL of ultrapure water and 200 µL of the pyridine were added at each tube to stop the reaction. Then sample vials of 1.5 mL are prepared using syringe filters of 0.22 µm.

Quantification of the depolymerized ulvan-rich extracts and the *Ulva rigida* was carried out by HPLC-IR with the column Shodex SH 1011 (300x8 mm, 6µm) under the method conditions found in the literature (Llano, Quijorna, Andrés & Coz, 2017). MWD was determined by HPLC-SEC-DAD using the column CHO-9231 Polysep-GFC-P 6000 (300x7.8mm). Sulfates were determined by using the sodium rhodizonate colorimetric method. Ash after 12 h at 575ºC and 900ºC in a muffle furnace were also measured.

Results

The chemical characterization of the cell-wall compounds of the *U.rigida* green seaweed was done. Cell-wall polysaccharides (CWPs) constituted between 38-54% w/w of the dry Ulva sp. The major CWPs fraction constituting 18-29% w/w of the green seaweeds is ulvan. Other CWPs are insoluble cellulose, glucuronan and xyloglucan. Molecular Weight Distribution of the extracts was also determining giving values of $3{\cdot}10^6$ Da which is similar from ulvan extracts provided in the literature ranging from $2.8{\cdot}10^5$ Da up to $1.6{\cdot}10^6$ Da (Champenois, 2009).

In order to study the chemical composition of ulvan-rich extracts, samples were depolymerized with HCl at three acid concentrations (0.5 M, 1 M and 2 M) and three temperatures (80ºC, 100ºC and 120ºC). Graph bars of all conditions assayed are shown in Figure 1, together with a plot graph of the best neutral and acid sugars conversion.

Maximum sugar conversion of Ulvan-rich extracts was achieved after 6 h of depolymerization via acid hydrolysis with HCl 2 M at 100ºC Hot water extraction method resulted in high yield of 54.0% and ulvan extraction efficiency of 96.6%.

Conclusions

Depolymerization of ulvan-rich extracts obtained after hot water extraction of *U.rigida* green seaweed was carried out by using HCL at different concentrations and temperatures. Neutral and acid sugars formation and degradation from 1 min to 24 h was determined to find the maximum conversion of the cell-wall polysaccharides extracted from the *U.rigida*. The highest sugars concentration (57.2%) was observed after 6 h of acid hydrolysis with 2 M HCl at 100ºC. Sugar conversion by acid hydrolysis was higher in comparison with methanolysis of the same *U.rigida* extracts where maximum sugars concentration of 34.7 % w/w was reached after 2 h of methanolysis (Macías et al., 2019).

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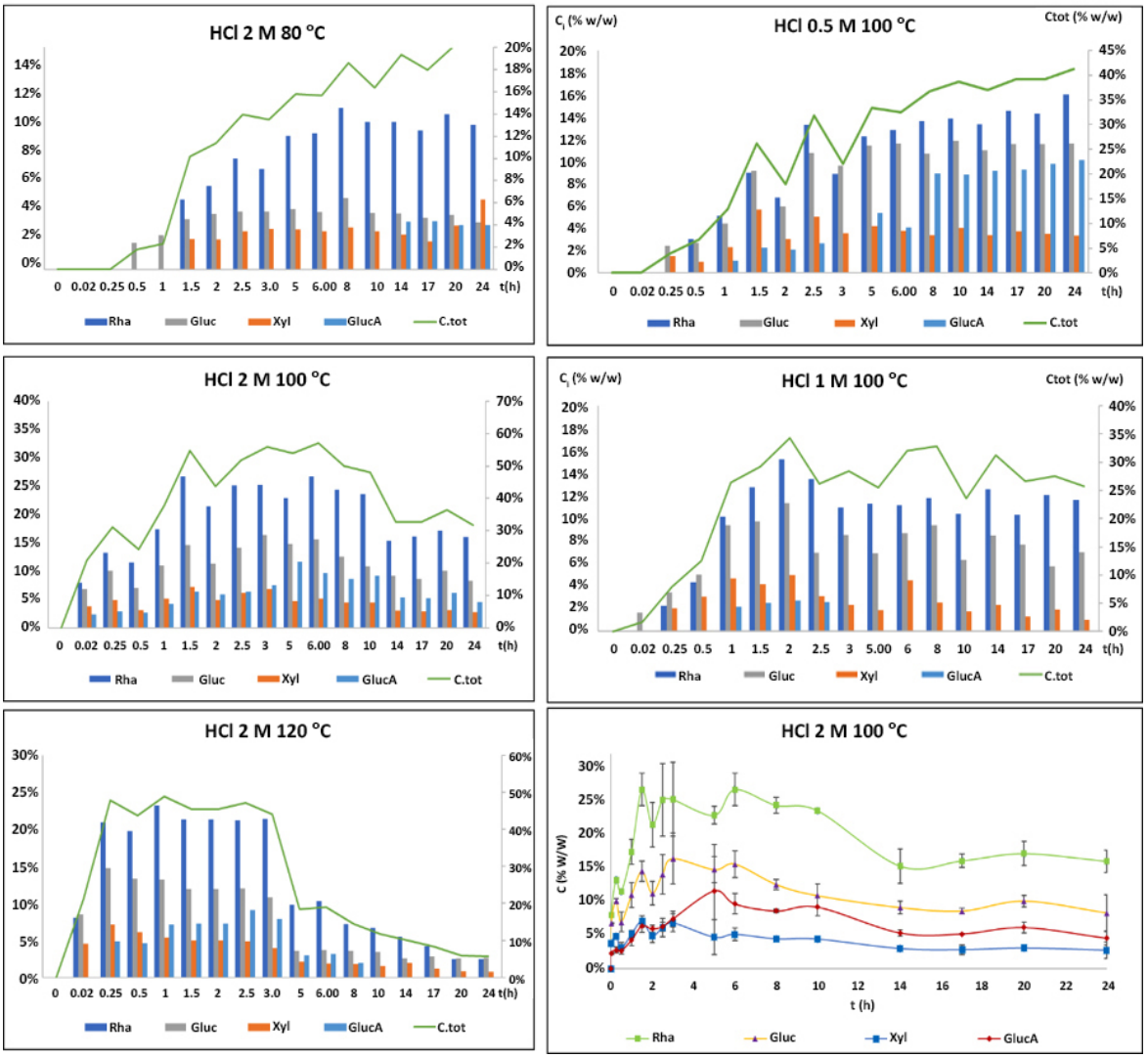

Figure 1. Schematic structure of the family of polysaccharides presented in Ulva sp. Source: own elaboration.

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