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Aqueous Extraction of the Sulfated Polysaccharide Ulvan from the Green Alga *Ulva rigida*—Kinetics and Modeling

Ricardo Pezoa-Conte[1] · Allison Leyton[2,3] · Andrea Baccini[4] · María Cristina Ravanal[2,5] · Päivi Mäki-Arvela[1] · Henrik Grénman[1,6] · Chunlin Xu[7] · Stefan Willför[7] · María Elena Lienqueo[2] · Jyri-Pekka Mikkola[1,8]

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Abstract The incentives for utilizing a versatile range of renewable feedstocks in novel ways are continuously increasing. Sulfated polysaccharides from green algae, such as ulvan, are interesting due to the rare sugar constituents which can be utilized for new materials and chemicals in industry. However, before valorization fractionation needs to be performed in a controlled way. In the current work, the kinetics of the aqueous extraction of ulvan was studied in the temperature range 60–130 °C. The highest yield of 97.6 wt.% was attained after 2 h of extraction at 130 °C, and the extraction efficiency was observed to be heavily temperature dependent. Interestingly, two regimes of extraction kinetics were observed, presumably due to the different ulvan fractions contained within the cell wall of green algae. The experimental data was modeled with first-order kinetics, and an apparent activation energy of 53.8 kJ mol^{-1} was obtained for the process. The algal residue was processed using simultaneous saccharification and fermentation, and 0.48 g ethanol g^{-1} of sugars was obtained.

Keywords Green algae · Bio-ethanol · Extraction · Kinetics · Ulvan · Rhamnose

✉ Jyri-Pekka Mikkola
jpmikkol@abo.fi; jyri-pekka.mikkola@umu.se

1 Industrial Chemistry and Reaction Engineering, Johan Gadolin Process Chemistry Centre, Åbo Akademi University, Biskopsgatan 8, FI-20500 Åbo/Turku, Finland

2 Centre for Biotechnology and Bioengineering, University of Chile, Beauchef 851, Santiago, Chile

3 Unit of Bioprocess, Department of Chemistry Engineering, University of La Frontera, Francisco Salazar 01145, Temuco, Chile

4 Department of Industrial Engineering, University of Padova, Via F. Marzolo 9, 35131 Padova, Italy

5 Center for Bioinformatics and Integrative Biology, Facultad de Ciencias Biológicas, Universidad Andrés Bello, Avenida República 239, Santiago, Chile

6 Molecular Process and Materials Technology, Åbo Akademi University, Biskopsgatan 8, FI-20500 Åbo/Turku, Finland

7 Wood and Paper Chemistry, Johan Gadolin Process Chemistry Centre, Åbo Akademi University, Porthansgatan 3, FI-20500 Åbo/Turku, Finland

8 Technical Chemistry, Department of Chemistry, Chemical-Biological Centre, Umeå University, SE-901 87 Umeå, Sweden

Introduction

Our society is heavily dependent on fossil resources which has triggered an increase in the carbon dioxide levels in our atmosphere, and consequently contributed to an ongoing change in our climate. The deforestation caused by the use of rainforests for the production of crops, pulp, and conversion of forests to arable land to produce beef is particularly worrying, since forest are responsible for converting an important share of the total carbon emissions into oxygen and carbohydrates through photosynthesis. In 2014, the global production of pulp reached 150 billion of tons [1], while the production of bioethanol, one of the main bulk chemicals produced from biomass, reached 115 billion of liters [2]. In early 2016, Norway became the first country on Earth to commit to zero deforestation, which means banning the sales of products which contribute to deforestation.

The usage of lignocellulosic resources in the production of chemicals and fuels in the so-called biorefineries is slowly increasing, with very good examples located strongly in the Nordic countries [3]. Consequently, it is extremely important to explore different resources when aiming at the production of various chemicals. In this regard, algae are very promising since they contain different types of carbohydrates that can be used for the production of chemicals, materials, medicines,

and fuels. Moreover, the cell wall of algae almost completely lacks lignin, which eases the extraction of carbohydrates by a number of different methods [4]. Algae contain less glucose than terrestrial plants, but it contains different carbohydrates with interesting industrial applications such as alginate, carrageenan, fucoidan, agarose, and ulvan [5].

Hemicelluloses from green algae are formed from a number of different units of which l-rhamnose, d-glucuronic acid, L-iduronic acid, glucose, and xylose are the most abundant reported ones in the open literature for different *Ulva* species. Ulvan contains polysaccharides with sulfates, featured mainly as two types of disaccharides: ulvanobiuronic 3-sulfate type A [→4)-β-D-GlcpA-(1 → 4)-α-L-Rhap3-sulfate-(1→] and ulvanobiuronic 3-sulfate type B [→4)-α-L-IdopA-(1 → 4)-α-L-Rhap3-sulfate-(1→] [6–8]. Notwithstanding, among the different sulfate polysaccharides, ulvan is by far the least studied [9]. However, in the recent years, it has gained more attention because it is a rather abundant source of rare sugars for the subsequent synthesis of different chemicals, as well as due to its biological activity based on its resemblance to hyaluronic acid, heparin, and chondroitin structures [10]. Consequently, the research has focused more on the structure of ulvan and the use of different extraction solvents. Among these solvents, water and solutions containing different salts have been the alternatives of choice [11–13]. Balboa and co-workers used aqueous processing for the extraction of anti-oxidants where the presence of ulvan was detected [14]. In our previous work, we reported the extraction of ulvan from the green alga *Ulva rigida* using ionic liquids. In the experiments, 69 wt.% of the ulvan (calculated as the sum of rhamnose and glucuronic acid) was extracted after 6 h with the distillable tetramethylguanidine propionate [$TMGH^+$][$EtCO_2^-$] ionic liquid at 120 °C, and also 69 wt.% of the glucan was co-extracted. Interestingly, in this work, water appeared to be the best solvent in the selective extraction of ulvan among the different constituents in the cell wall [15].

The aquaculture of marine algae in Chilean shores has mainly focused on red and brown species aiming at the production of hydrocolloids, while no harvesting of green seaweeds is performed in the country [16–18]. With regard to Chlorophyceae, *U. rigida* is the most abundant seaweed growing in intertidal and rocky ecosystems along the Chilean shore, particularly in the central and northern part of the country [19, 20]. Vazquez and co-workers studied the growth of Chlorophyta species in intertidal environments, observing important seasonal variations with growth peaks during summer [19]. However, the literature is scarce upon the cultivation of green seaweeds [21]. The farming of Ulva species in artificially seeded ropes located offshore as well as the culture of Ulva species in cultured tank vessels has shown prominent results [22–25].

Even though several authors have reported on the extraction of ulvan from different species among the *Ulva* genus, open literature lacks studies regarding the extraction kinetics of this polysaccharide, providing mainly approaches aiming bioenergy applications [26–29]. The kinetic studies on the extraction of polysaccharides from biomass have mainly focused on the extraction of hemicelluloses from terrestrial plants, often related to the pulp and paper or biorefinery industry with the aim of utilizing this fraction which is usually lost downstream in the processes. Having this goal in mind, Grénman and co-workers studied and modeled the hot water extraction of hemicelluloses from spruce [30]. They used a cascade reactor in order to study the reaction kinetics in the temperature range 150–170 °C. A totally non-cellulosic carbohydrate yield between 60 and 80 wt.% was obtained, mannose being the main sugar quantified in the extracts. However, degradation of sugars was observed since both hydroxymethylfurfural (HMF) and furfural were obtained in the extracts, presumably due to the decrease of the pH caused by the deacetylation of galactoglucomannan (GGM). The extraction of hemicelluloses from spruce was found to be highly dependent on the temperature, but not on the pH of the aqueous solvent. As a result of the kinetic modeling of the data, the authors suggested that the process was governed by first-order kinetics in the concentration of hemicelluloses in the solid phase, obtaining an activation energy of 135 kJ mol^{-1} [30]. Afterwards, Rissanen and co-workers also studied the hot water extraction of hemicelluloses from spruce at lower temperatures (120–170 °C) using the same cascade reactor system. The apparent activation energy reported was 120 kJ mol^{-1} [31, 32]. These values are in agreement with the ones reported in the open literature by other authors for the extraction of hemicelluloses from different terrestrial plants, those being in the range of 81–251 kJ mol^{-1} [30].

The total ulvan content in this work was defined as the sum of rhamnose, glucuronic acid, and iduronic acid units, which have been reported to be the most important constituents of ulvan. In this regard, glucose and xylose were not included since they are also constituents of cellulose and xyloglucan that occur in association to ulvan in the cell wall of green algae. However, xylose has also been reported to be part of ulvan in a minor extent in the so-called ulvanobiose 3-sulfate ([→4)-α-D-Xylp-(1 → 4)-α-L-Rhap3-sulfate-(1→]) and ulvanobiose 2-sulfate, 3 sulfate ([→4)-α-D-Xylp2-sulfate-(1 → 4)-α-L-Rhap3-sulfate-(1→]) units [8]. Additionally, glucuronan is a polysaccharide formed by glucuronic acid units that also occurs in association with ulvan in green algal cell wall, and is generally linked to proteins [33]. It is also important to mention that rhamnose is usually linked to a sulfate group on O_3, adding another important constituent to the ulvan backbone [11, 33, 34]. Consequently, the exact quantification of ulvan extracted in the experiments is challenging, since ulvan is a heteropolymer containing many different units. However, all the main sugar constituents of the cell wall are reported, including glucose and xylose.

This work aims to elaborate the understanding of the phenomena involved in the extraction of ulvan. Additionally, a mathematical modeling is presented for the extraction kinetics

and results which are in agreement to those reported for terrestrial plants were obtained. According to the best of our knowledge, this is the first time that the kinetics of the extraction of ulvan is reported, and consequently, these results widen the knowledge on utilizing this carbohydrate. In previous works, ulvan has been characterized, whereas the main focus in this work was to study extraction kinetics of ulvan aiming for potential industrial benefit from this fraction.

Experimental Section

Materials

The green algae *Ulva rigida* (C. Aghard 1823 Chlorophyta, Ulvaceae) were collected in 2013 from submerged marine rocks located in La Herradura de Guayacán Bay, in the City of Coquimbo, Región de Coquimbo in northern Chile. The algae were placed on plastic carpets and left to air dry for 48 h avoiding contact with sunlight. As the next step, the algae were further dried in an oven at 40 °C overnight and finally milled to 90% #30 mesh in a cross beater mill Retsch SK10. The algal samples were stored in a freezer and freeze-dried prior to analyses.

Chemicals were used as received: hexamethyldisilazane (HDMS; Fluka, ≥99.0%), chlorotrimethylsilane (TMCS; Aldrich, ≥99.0%), pyridine (Aldrich, ≥99.0%), L(+)-arabinose (Sigma, ≥99%), D-(+)-galacturonic acid (Fluka, ≥93%), D-(+)-galactose (Fluka, ≥99.5%), D-(+)-glucose anhydrous (Fluka, ≥98%), D-glucuronic acid (Sigma, ≥98%), L-iduronic acid (LC Scientific Inc.), D-(+)-mannose (Fluka, ≥99%), L-rhamnose monohydrate, D-(+)-xylose (SAFC, ≥99%), Etax Aa ethanol (Altia Oyj., ≥99.5%), sodium bicarbonate (Sigma-Aldrich, ≥99.7%), sodium carbonate (Merck, ≥99.9%), sodium nitrate (Merck, ≥99.5%), and trifluoroacetic acid (Sigma-Aldrich, ≥99%).

Washing of Algae

Washing of algae was studied in order to remove salts from it. The influence of washing time was performed as follows: 5.5 g of algae was placed in a glass reactor containing 110 mL of Milli-Q water, at room temperature. The mixtures were stirred at 650 rpm for 20 min and 16 h. Consequently, the liquid phase was filtered and both the liquid and the solid phases were stored in freezer for subsequent analyses.

Ulvan Extraction Experiments

An amount of 60 g of algae was disposed into a glass reactor containing 1.2 L of Milli-Q water and stirred at 650 rpm for 20 min. The washed algae were filtered and thoroughly washed with Milli-Q water to remove remaining salts. Then, the algae were freeze-dried overnight. Subsequently, 110 mL of Milli-Q water was placed in a 250-mL glass reactor and heated to the desired temperature (60, 70, 80, and 90 °C). The glass reactor was equipped with a thermometer and a cooling system. Once the set point temperature was reached, 5.5 g of washed algae was disposed into the reactor. Samples were withdrawn at 1, 5, 10, 30, 60, and 120 min of extraction and stored in freezer for subsequent analyses. The experiments carried out at 110 and 130 °C were performed in an autoclave, where 15 g of washed algae was disposed together with 100 mL of Milli-Q water. Subsequently, 200 mL of Milli-Q water was heated to 150 °C in a pre-heater coupled to the autoclave. The experiment was initiated (time zero) by letting the water contained in the pre-heater enter the autoclave by opening the valve located in the line connecting both vessels. In this set of experiments, samples were withdrawn after 1, 5, 10, 20, 30, 45, 60, and 120 min. The samples were stored in freezer prior to further characterization.

Exploratory Fermentation of the Processed Alga

Four grams of dry *U. rigida* was processed in a 1:10 ratio (wt) with deionized water and placed in an autoclave at 125 °C and 1.5 bar for 1 h without stirring. Subsequently, the processed alga was washed with 80 mL deionized water eight times, vacuum filtered, and freeze-dried overnight. The fermentation was carried using simultaneous saccharification and fermentation (SSF) of the alga. Of either fresh algae or processed algae, 0.75 g was added to 2 mL of medium (sodium acetate 500 mM, yeast extract 50 g/L, $MgSO_4x7H_2O$ 0.25 g/L, and $(NH4)_2HPO_4$ 5 g/L), 460 μL of cellulases (Sigma), 62 μL cellobiases (Sigma), 0.2 g yeast LALVIN EC1118 (Lallemand), and 27.48 mL of DI water in 50-mL flasks. The fermentation was performed at 40 °C in a G24 Environmental incubator shaker, New Brunswick Scientific Co., Inc., for 3 days.

Ash Content of Fresh and Processed Algae

The ash contents of fresh and processed algae were analyzed using thermogravimetric analysis (TGA), which was carried out as described in our previous work [15]. The quantification of the different ions was carried out by weighing the samples into Teflon bombs and subsequently dissolved in a solution of 5 mL HNO_3 (65%) + 1.2 mL H_2O_2 (30%) in a microwave oven (Anton Paar, Multiwave 3000) and diluted to 100 mL. The liquid samples were then analyzed via inductively coupled plasma-optical emission spectrometry (ICP-OES; PerkinElmer, Optima 5300 DV).

Carbohydrate Analysis

An equivalent of 100 μL of ulvan extract and 10 mg of solids were used for the total carbohydrate content quantification employing an acid methanolysis method and gas chromatography. Total glucan content was calculated using the acid hydrolysis method. The details of the carbohydrate analyses can be found in our previous works [15, 35].

Organic Elemental Analysis for the Quantification of Proteins

The quantification of proteins was calculated measuring the nitrogen content of solid samples through organic elemental analysis performed in a Flash 2000 Thermo Scientific equipment. The conversion value used to calculate the protein content in *U. rigida* samples was 5.12, reported by Shuuluka and co-workers [36]. Details of the protocol can be found in our previous work [15].

pH Determination

The pH of the extracts was analyzed right after sampling and quenching of the samples with running water. A pH meter pH 100L Phenomenal was used for the analysis performed at room temperature.

Sulfate Content

An amount of 600 mL of Milli-Q water was heated to 90 °C in a 1-L glass reactor. Once the set temperature was reached, 30 g of freeze-dried algae washed at room temperature for 20 min was added to the reactor. Samples of about 7 mL were withdrawn after 1, 5, 10, 30, 60, and 120 min and immediately stored in a freezer. Subsequently, the samples were quenched at room temperature and 5 mL of the samples was mixed with 10 mL of Etax Aa ethanol (Altia Oyj., ≥99.5%). The samples were stored for 72 h to allow for the precipitation of carbohydrates. Then, the samples were centrifuged, filtered, and washed with additional ethanol to remove the non-ulvan associated sulfate groups. Eventually, the precipitate was freeze-dried and stored in a freezer in sealed test tubes prior to characterization.

The sulfate content in the ulvan extracts was analyzed by following the same procedure as described by Ray and Lahaye [37]. About 8–12 mg of precipitate was placed into test tubes and 3 mL of 2 M trifluoroacetic acid was added and the sample was incubated at 100 °C for 3 h, in order to attain a maximum release of sulfate groups. Subsequently, the samples were centrifuged, diluted, and filtered through a 0.22-μm nylon syringe filter before injection to the HPLC. The quantification of sulfates was performed using a Metrohm ion chromatograph (881 Compact IC pro) coupled to a suppressor module. The column was a Metrosup A Supp 5–100 (100 × 4 mm) featuring polyvinyl alcohol resin with quaternary ammonium groups, operated at room temperature. The eluent was 1.3 mM sodium carbonate and 2.0 mM of sodium bicarbonate aqueous solution at a flow rate of 0.8 mL min^{-1}.

Molecular Size Distribution Determination

The molar mass distribution of the ulvan extracts was determined using high-performance size-exclusion chromatography (HPSEC) in online combination with a multi-angle laser light scattering (MALLS) instrument (miniDAWN, Wyatt Technology, Santa Barbara, USA) and a refractive index (RI) detector (Shimadzu Corporation, Japan). The system featured two columns in series, 2× Ultrahydrogel™ linear 7.8 × 300 mm (Waters, Milford, USA). Of the sodium nitrate ($NaNO_3$), 100 mM was used as the elution solvent, with a flow rate of 0.5 mL min^{-1}. The samples were first diluted to a concentration of around 3 mg mL^{-1}, and subsequently filtered through a 0.22-μm nylon syringe filter before injection. A sample of 100 μL was injected to the column. The refractive index utilized dn/dc for calculations was equal to 0.146 mL g^{-1}, used in accordance to other authors [38, 39]. Astra software (Wyatt Technology, Santa Barbara, USA) was used to analyze the data.

Determination of Glucose and Ethanol Concentration

The amount of glucose and ethanol were quantified by HPLC of samples filtered through a 0.22-μm pore size filter. The analyses were conducted with an Agilent 1100 Series HPLC System, Biorad Aminex HPX-87H column with 5 mM H_2SO_4 using a flow rate of 0.6 mL/min and column temperature of 45 °C. Standard solutions of glucose and ethanol were prepared (1 to 10 mg/mL).

Results and Discussion

Washing of the Algae

Table 1 presents the differences in the total carbohydrate content and the ash content between the fresh and the washed *U. rigida* biomass. The results show that the ash content in the fresh *U. rigida* biomass was 178 mg g^{-1} fresh dry alga (FA), while the ash content in the algae washed at room temperature for 20 min and 16 h was 64.8 and 59.0 mg g^{-1} FA, respectively. These values represent 63.6 and 66.9 wt.% of ash removal, respectively. Consequently, 16-h washing resulted in 1.05-fold more ash removal compared to 20-min washing. Evidently, the majority of these ashes were salts which were not chemically associated to the different constituents of the biomass cell wall. As a result, the slight difference in the ash

Table 1 Difference between the fresh and washed alga, in terms of different biomass constituents

Components	Units	Fresh biomass	Washed RT—20 min	Washed RT—16 h
Weight loss	wt.%	–	19	26
TCC	mg g^{-1} FA	461	426	370
Ulvan	mg g^{-1} FA	174	159	139
Glucan	mg g^{-1} FA	233	219	188
Others	mg g^{-1} FA	54	48	43
Proteins	mg g^{-1} FA	159	121	–
Lipids	mg g^{-1} FA	41	–	–
Ash	mg g^{-1} FA	178	65	59
Al	mg kg^{-1} FA	120 ± n.d.	90 ± 0.89	n.a.
B	mg kg^{-1} FA	70 ± n.d.	n.d ± n.d.	n.a.
Fe	mg kg^{-1} FA	390 ± n.d.	381 ± 6.9	n.a.
Si	mg kg^{-1} FA	500 ± n.d.	336 ± 6.6	n.a.
Sr	mg kg^{-1} FA	50 ± n.d.	43 ± 0.30	n.a.
Ca	g kg^{-1} FA	4.3 ± n.d.	3.6 ± 0.04	n.a.
K	g kg^{-1} FA	16 ± n.d.	1.7 ± 0.02	n.a.
Mg	g kg^{-1} FA	20 ± n.d.	9.7 ± 0.13	n.a.
Na	g kg^{-1} FA	26 ± n.d.	2.4 ± 0.03	n.a.
P	g kg^{-1} FA	1.9 ± n.d.	0.52 ± 0.01	n.a.
S	g kg^{-1} FA	34 ± n.d.	18 ± 0.10	n.a.

Lipid content was not analyzed to the sample used in these experiments and refers to different sample from the same specie used in previous work [15]

FA fresh dry algae, *n.d.* not determined, *n.a.* not analyzed, *TCC* total carbohydrate content, determined as the sum of ulvan (rhamnose, glucuronic acid, and iduronic acid), glucan (glucose), and others (arabinose, galactose, galacturonic acid, mannose, and xylose)

removal might be explained only for the fraction of metals or sulfate groups that are chemically associated to carbohydrates or proteins. In this regard, the ICP-OES analyses conducted to the fresh and the washed algae evidenced major removal of salts. The Na content was reduced from 26 to 2.4 g/kg FA after 20-min washing, while the Mg and K contents were reduced from 20 to 9.7 and 16 to 1.7 g/kg, respectively. The P and S contents were also substantially reduced from 1.9 to 0.52 and 34 to 18 g/kg, respectively. Al and Si showed major variations in the range of 25–33 wt.%, while Fe showed a minor removal, accounting for 2 wt.%. In turn, the total mass losses were more noticeable, since 19 wt.% of the mass was dissolved in the 20-min washing compared to 26.0 wt.% dissolved in the 16-h washing. These results are in agreement to those obtained for the total carbohydrate content (TCC) analyses, in which TCC decreased from 461 to 426 mg g^{-1} FA in the 20-min washing, resulting in a 7.6 wt.% carbohydrate loss. In turn, the 16-h washing resulted in 20 wt.% carbohydrate loss, 2.6-fold higher than that obtained with 20-min washing. The ulvan fraction appeared to be more easily dissolved than the glucan fraction, which is understandable since ulvan is more water soluble compared to cellulose and starch. The total content of ulvan decreased from 174 mg g^{-1} fresh dry alga in fresh *U. rigida* to 159 mg g^{-1} FA in 20-min washing and to 139 mg g^{-1} FA in 16-h washing, representing 8.6 wt.% loss in 20-min washing and 20 wt.% loss in 16-h washing. Glucan losses were less prominent than those quantified for ulvan, and they only accounted for 1.8 wt.% in 20-min washing and 15.9 wt.% in 16-h washing. Regarding the proteins, the content was reduced from 158 to 121 mg g^{-1} FA, representing a 23 wt.% loss. The lipid content was analyzed in a previous work for *U. rigida* biomass, accounting for 41 mg g^{-1} FA, demonstrating that it is not a major constituent of the alga. In order to study the kinetics of the extraction of ulvan, 20-min washing was utilized since it removed enough ash elements, without a substantial loss of the carbohydrates.

Reproducibility

Two set of experiments were performed at 80 and 90 °C in order to evaluate the reproducibility of the extraction results. Table 2 displays the average of the extraction yields, as well as the standard error in the experiments. The results show that the reproducibility was good, especially for more concentrated samples. The samples with low ulvan concentration displayed more variation (samples withdrawn after 1, 5, and 10 min). This observation is logical because the method for the quantification of sugars used in this work is less precise when using

Table 2 Results of the reproducibility in extraction of *Ulva rigida* at 80 and 90 °C

Time (min)	80 °C	90 °C
1	9.93 ± 8.49	33.3 ± 6.92
5	12.2 ± 6.73	36.9 ± 5.72
10	18.3 ± 4.92	40.8 ± 1.56
30	23.4 ± 4.59	49.4 ± 0.53
60	30.9 ± 4.77	55.5 ± 4.14
120	45.2 ± 6.05	65.2 ± 1.78

Extraction yield of ulvan at different temperatures, units in wt.%. Values indicate the average ± standard error of two independent experiments

low-concentration samples due to the sensitivity. Nevertheless, the standard errors calculated for the samples were all below 7.0%.

pH of the Extracts

The structure of ulvan includes carboxylic and sulfate groups, which can affect the pH of the solution when extracting carbohydrates from algal biomass. Additionally, the high ash content observed in algae consists also of different cations associated to these groups [15, 33], which consequently also play a role in the pH when extracting polysaccharides. However, the results obtained from the analysis of the extracts showed a low variation of the pH in the conditions evaluated in this work. The results showed that the maximum pH was 7.25 and occurred after 5 min of extraction at 110 °C. In turn, the lowest pH value was 6.82 after 120 min of extraction at 90 °C. Since the maximum pH was measured systematically in the beginning of the reaction (1–10 min) at all the temperatures, this high pH might be attributed merely to the extraction of polysaccharides. In contrast, the lowest pH was observed at the very end of the extraction for all the experiments in this work, which consequently might be attributed to a partial collapse of the ulvan structure and/or hydrolysis of the backbone. This slight decrease in the pH was consequent with the decrease of the molecular weight observed in the extracts and the increase of the sulfate content, as discussed later.

Ulvan Extraction Kinetics

The composition of the ulvan extract at different sampling times and reaction temperatures is shown in Fig. 1. Rhamnose and glucuronic acid were the most prominent sugars extracted during the whole experiments. Accordingly, ulvan accounted for 77.7 wt.% of the sugars in the extract after 1 min of extraction at 60 °C as shown in Fig. 1a. After 2 h of extraction at 60 °C, the content of ulvan in the extract was 83.7 wt.% (Fig. 1b), slightly higher than the concentration

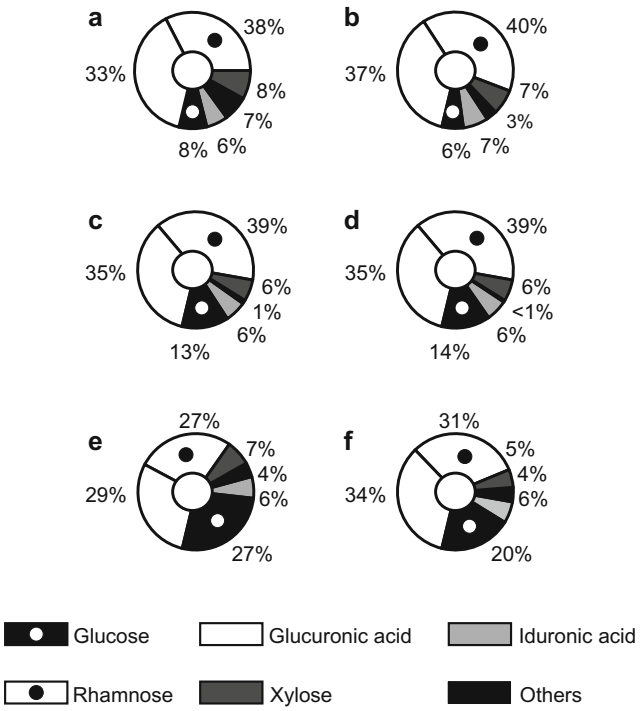

Fig. 1 Monomer distribution of the ulvan extract obtained at 60–110 °C at different sampling times. Figures show the sugar composition of the extract at **a** 60 °C—1 min, **b** 60 °C—120 min, **c** 90 °C—1 min, **d** 90 °C—120 min, **e** 130 °C—1 min, and **f** 130 °C—120 min. Other sugars comprise the content of arabinose, galactose, galacturonic acid, fructose, and mannose

measured in the beginning of the reaction. Consequently, there was an increment of the ulvan content in the extract during the extraction time, since glucan content remained below 10.0 wt.%. When the extraction was performed at 90 °C, after 1 min, the ulvan comprised 80.7 wt.% of the sugars in the extract, while after 2 h, the ulvan content decreased slightly to 79.9 wt.% of the total. This pattern was observed in all the samples in this study, confirming that ulvan was the main carbohydrate extracted. However, when the temperature was increased to 110–130 °C, the total ulvan content in the extract accounted for only 62.0 wt.% after 1 min and 70.7 wt.% after 2 h due to the increase on the extraction of xylose and glucose, which were considered as impurities in the ulvan extract. In fact, the glucose content increased from 6.45 wt.% after 120 min of reaction at 60 °C to 26.7 wt.% after 5 min of reaction at 130 °C, representing a >4-fold increase. Other authors have reported the presence of minor quantities of glucose in ulvan extracts, suggesting that it is present either within the ulvan backbone linked to rhamnose and xylose units [→4)-Rha-sulfate-(1 → 3)-Glc-(1 → 4)-Xyl] [40] or due to the co-extraction of other units such as amorphous cellulose or xyloglucans [33]. In turn, xylose has been also suggested to be a part of the ulvan backbone linked to glucuronic acid, rhamnose, and glucose residues [→4)-D-GlcA-(1 → 3)-D-Xyl-(1 → 4)-L-Rha-sulfate-(1 → 3)-D-Glc-(1 → 4)-D-Xyl]

[40], and recently identified forming ulvanobiose units [8]. However, xylose is present in xyloglucans which occur in association to ulvan in green algal cell walls [33].

Figure 2 depicts the extraction kinetics of ulvan within the temperature range of 60–130 °C. Not surprisingly, the extraction yield of ulvan increased at higher temperatures, which is logical in terms of the energy required to extract higher molecular weight fractions. The maximum yield of ulvan was 97.6 wt.% after 2 h of treatment at 130 °C. In the case of rhamnose, the yield was 99.8 wt.% after 2 h of extraction at 130 °C, while after 2 h of extraction at 90 °C, the yield was 74.8 wt.%. These results are consistent with those reported in the open literature for the extraction of ulvan from different algae among the *Ulva* genera. Robic and co-workers extracted ulvan from *U. rotundata* obtaining a 59.2 wt.% yield based on the total content of rhamnose. The process included brining of the biomass with acetic acid (100 g L^{-1}), citric acid (10 g L^{-1}), and sodium chloride (50 g L^{-1}) for 7 weeks at 4 °C and subsequently extraction with 0.05 M sodium oxalate at 85 °C for 2 h and re-extraction with water for 3 h at room temperature [39]. In turn, Yaich and co-workers reported a yield of 89.9 wt.% for rhamnose after 2 h of extraction of *U. lactuca* with a water solution containing HCl (pH 1.5) at 90 °C [41]. In our previous work involving *U. rigida* fractionation with ionic liquids, the use of tetramethylguanidine propionate was demonstrated to extract roughly 70 wt.% of the carbohydrates in 6 h at 120 °C. Moreover, the use of Milli-Q water as a solvent in an autoclave at 125 °C for 1 h demonstrated to extract 87.5 wt.% of the ulvan, and when 0.18 M sulfuric acid (H_2SO_4) was added under the same conditions, a 99 wt.% yield of ulvan extraction was achieved [15]. The extraction of glucuronic and iduronic acids behaved rather similarly compared to rhamnose, reaching a yield of 96.9 and 92.1 wt.%, respectively, at 130 °C after 2 h of extraction. When the temperature was lower, the extraction yield was also reduced for both sugar acids as shown in Fig. 2c, d.

The extraction of ulvan at 60 °C was observed to be very slow, accounting for 19.7 wt.% of total ulvan from the fresh biomass after 2 h of extraction. The increase of the temperature to 70 °C improved the extraction yield to 49.2 wt.%, but more interestingly, it induced the extraction of higher molecular weight ulvan fractions as discussed later. Successively, the largest increase observed in the extraction of ulvan was attained when the temperature was increased to 90 °C, enhancing the extraction of ulvan 1.44-fold compared to that at 80 °C and 3.31-fold compared to the one at 60 °C. Accordingly, within the range of 60–80 °C, the extraction yield was not higher than 10.5 wt.% of ulvan after 1 min of reaction. In turn, when the temperature was increased to 90 °C already, 33.2 wt.% of the ulvan was extracted after 1 min of reaction, suggesting that there is a fraction that was very easily extractable during the very first contact of the fibers with

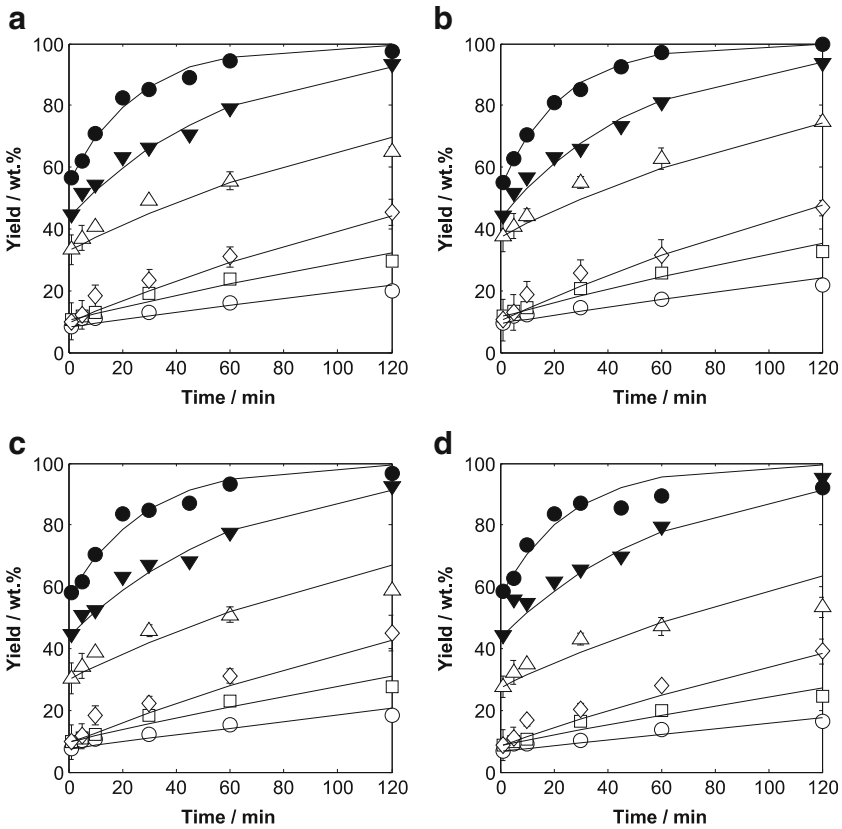

Fig. 2 Extraction kinetics of ulvan extracted at 60–110 °C for 2 h. **a** Ulvan. **b** Rhamnose. **c** Glucuronic acid. **d** Iduronic acid. The experimental data is represented by the following symbols: *white circle* 60 °C, *white square* 70 °C, *white diamond* 80 °C, *white triangle* 90 °C, *black inverted triangle* 110 °C, and *black circle* 130 °C. The results for 80 and 90 °C experiments are displayed as the average of two parallel experiments, whereas *bars* exhibit the standard error of the data. The *lines* represent the modeling results

water at this temperature, and particularly at temperatures higher than 90 °C. After the initial rapid extraction within 1 min, the reaction rate appeared to be slower, suggesting that there might be higher molecular weight fractions that take longer time to be extracted compared to extraction of shorter fractions. In the work of Costa and co-workers, ulvan was extracted from *U. lactuca* after subsequent treatments within the temperature range of 75–100 °C, showing three different fractions: two macromolecular fractions centered at 775 and 150 kDa and a third fraction centered at 1.5 kDa [42]. Consequently, it is very likely that these different fractions have different extraction kinetics as was also observed in this work. The washed algae might have contained some ulvan that was already extracted during the washing step and remained within the fibers even after washing. However, the difference in ulvan extraction yields between 90 and 80 °C after 1 min of extraction was 3.4-fold higher at 90 °C, and even higher compared to 60 °C (4.0-fold higher). Moreover, the extraction experiments carried out at 60–90 °C were performed with the very same batch of the washed algae; consequently, the starting material was the same for all the extraction experiments. This observation suggests that there was a significant advantage in using temperatures of 90 °C and higher temperatures compared to lower temperatures (60–80 °C). Figure 3a depicts the extraction yields after 1 min. The extraction at temperatures between 60 and 80 °C obtained relatively similar values, but in the temperature range of 90–130 °C, an increasing linear trend was observed. Robic and co-workers demonstrated that ulvan aggregate in bead-like structures, and further suggested that different cations such as calcium or borate allow these beads to agglomerate. Within the cell wall, these structures may be further aggregated into a gel-like structure linked by fiber-like material, such as ulvan, proteins, or glucuronan [38]. This gel-like structure occurring in the cell wall may eventually collapse at a certain threshold temperature and explain the difference observed between the extraction at 80 and 90 °C. However, this hypothesis should be closer evaluated with additional experiments in future work. Henceforth, the kinetic modeling of this data was performed only for the time frame after 1 min of ulvan extraction, since it was clear that different kinetic behavior was observed in the initial stages at the threshold value 90 °C and over.

Figure 3b–d depicts the correlation of different monomers in the extraction. The results show high correlation between rhamnose, glucuronic, and iduronic acids, all components in the ulvan structure, obtaining a correlation value R^2 of 0.97 for linear trends. It is evident that these three units were extracted at a similar rate in the temperature range of 60–110 °C evaluated in this work. Interestingly, xylose yields show also relatively high correlation with the extraction yields of rhamnose, which can be explained by its minor occurrence in the structure of ulvan. For glucose, it was observed that the extraction correlation was lower, suggesting that glucose was extracted at a different rate. This result is logical since the detection of glucose in the extracts was

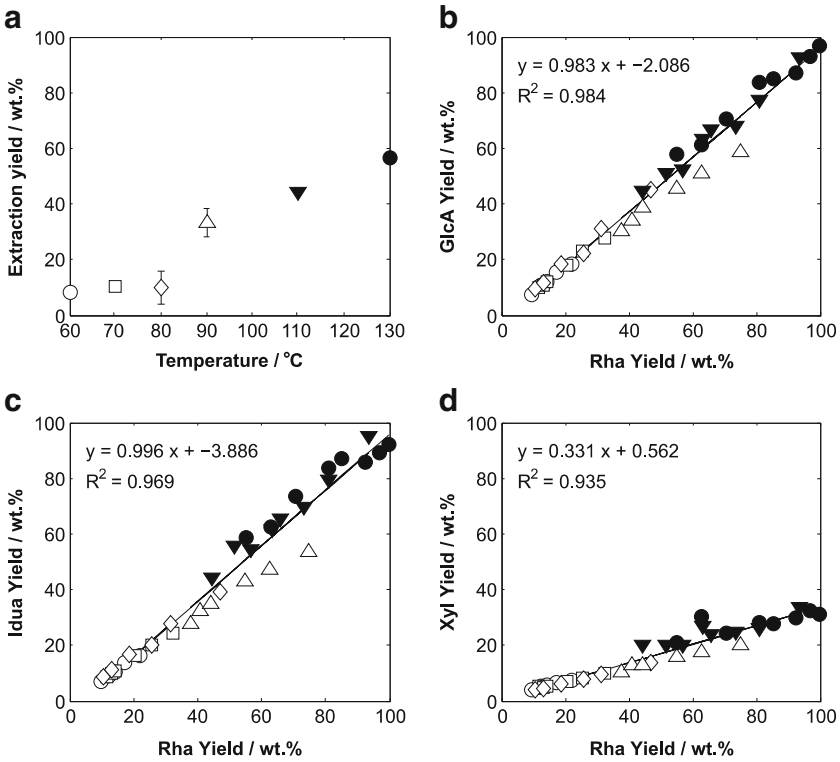

Fig. 3 Extraction of ulvan after 1 min and correlation of extraction yields for different sugars. **a** Extraction of ulvan at time 1 min vs. temperature of extraction. **b**–**d** Correlation of extraction yields in different sugars at different temperatures. **b** Glucuronic acid/rhamnose. **c** Iduronic acid/rhamnose. **d** Xylose/rhamnose. The experimental data is represented by the following symbols: *white circle* 60 °C, *white square* 70 °C, *white diamond* 80 °C, *white triangle* 90 °C, *black inverted triangle* 110 °C, and *black circle* 130 °C

presumably due to the extraction of molecules such as xyloglucans or even some amorphous cellulose, and not particularly from ulvan.

Molecular Weight Distribution of the Extracted Ulvan

Figure 4a shows the molecular weight distribution of the extracted samples as a function of the reaction time and temperature. The range of the molecular weight observed in the samples was between 237 and 709 kDa, slightly lower than the values reported by other authors for different *Ulva* species (530–3600 kDa) [33]. Interestingly, these results are in accordance with what was observed by Costa and co-workers in the ulvan extracts from *U. lactuca*, as they identified two broad macromolecular fractions centered at 775 and 150 kDa and one shorter fraction centered at 1.5 kDa [42]. According to the results of the extraction experiments performed in this work, it was clear that at 60 °C, no high molecular weight fractions were obtained. In turn, at 70 °C, it was evident that high molecular weight fractions were extracted, since the molecular weight was in the range of 550–650 kDa, confirming that higher temperatures allowed the extraction of larger fractions, as also observed by other authors [33]. The molecular weight remained relatively stable through the time frame of the extraction at 70 °C, since the decrease of the molecular weight at this temperature was relatively low as observed in Fig. 4a. Successively, when the temperature was increased to 80 °C, a molecular weight of 709 kDa was attained after 1 min of extraction, though it heavily decreased to 307 kDa after 2 h of extraction. When the extraction was performed at 90 °C, the molecular weight was 694 kDa at 1 min, and after 2 h of extraction, it also decreased heavily to a final value of 287 kDa. Similarly, when the extraction was performed at 110 °C, the molecular size was 448 kDa at 1 min of extraction and it decreased to 284 kDa after 2 h of extraction. Interestingly, the decrease on the molecular weight of the extract appeared to achieve a threshold at around 290 kDa. Consequently, though the temperature increased the extraction yield of ulvan as

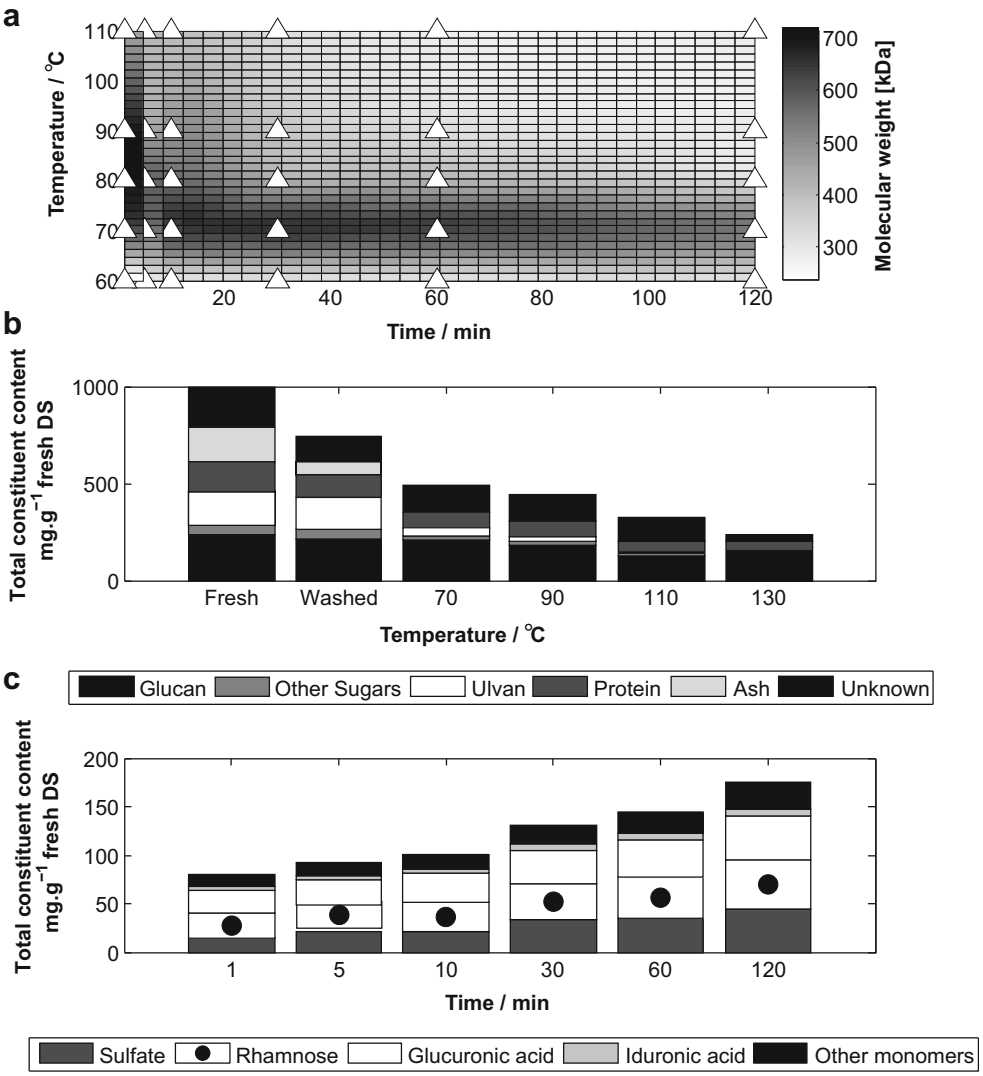

Fig. 4 **a** Molecular weight distribution of the extracts in function of extraction time and temperature. *white triangle* Experimental data. The profile was built using MATLAB software cubic interpolation of the experimental data. **b** Mass, glucan, and ulvan content of solid residue recovered after extraction per gram of washed algae. **c** Sugar and sulfate composition of the ulvan precipitated extracted at 90 °C per gram of fresh alga

described previously and it also allowed extraction of larger fractions, it obviously reduced the molecular weight of the extracted molecules at temperatures above 80 °C. The ulvan backbone has been reported to be very long as mentioned earlier; however, it also tends to form aggregates between the fibers due to its necklace structure, similar to the one that DNA features, as reported by Robic and co-workers [38]. Accordingly, rather than partial hydrolysis of ulvan producing shorter molecules, it might be also possible that the decrease of the molecular weight is due to a collapse of this necklace type of ultrastructure of ulvan, resulting in a decrease on the overall molecular weight.

Spent Algae

The mass and the composition of the residue left after the extraction of ulvan is depictured in Fig. 4b. Evidently, at more elevated temperature, more algae were dissolved into the liquid phase. The residue obtained from the algae processed at 70 °C accounted for 494 mg g^{-1} FA, equivalent to 33.2 wt.% alga dissolution after washing. Accordingly, the residue obtained from the algae processed at 110 °C accounted for 321 mg g^{-1} FA, equivalent to 56.6 wt.% alga dissolution after washing. Consequently, the temperature had an evident role in the dissolution of algal constituents. The ulvan content was reduced from 159 mg g^{-1} FA in the washed algae to 46 mg g^{-1} FA in the residue obtained from algae processed at 90 °C and to 5.0 mg g^{-1} FA in the residue obtained from algae processed at 110 °C. These results evidenced the removal of ulvan from the solid phase to the liquid phase, as observed in the results of the liquid phase. In turn, the glucan content in the residue was increased from 219 mg g^{-1} DS in the washed alga to 405–417 mg g^{-1} DS in the residue obtained from algae processed at 70–110 °C. The biggest increment was attained in the alga processed at 130 °C, where the glucan content increased to 612 mg g^{-1} DS. Consequently, the glucan content in the spent algae was increased, evidencing that glucans were solubilized at a lower rate compared to ulvan. With regard to the protein content, the protein content was slightly increased in the alga from 16.4 wt.% in the washed alga to 21.3 wt.% in the alga processed at 130 °C.

Sulfate Content on the Extracts

Figure 4c depicts the sulfate and sugar contents in the extracts. The sulfate content increased from 14.9 mg g^{-1} of fresh alga after 1 min of extraction to 44.4 mg g^{-1} of fresh alga after 120 min. Accordingly, the sulfate content in the extracts was observed to increase by 41.3 wt.% after 120 min of extraction, compared to the beginning of the extraction after 1 min. Consequently, it was evident that sulfate was co-extracted with rhamnose and glucuronic acid as the main constituents of ulvan. When the sulfate-to-rhamnose molar ratio was compared, an increase from 0.92 mol SO_4^{-2} mol^{-1} Rha after 1 min of extraction to 1.38 mol SO_4^{-2} mol^{-1} Rha after 120 min of extraction was observed. Lahaye and Ray demonstrated the presence of sulfate groups linked to O_3-rhamnose [34]. Additionally, xylose, which has also been reported to be a part of the ulvan backbone, features also sulfate groups linked to O_2 [33, 34, 43]. Unfortunately, in this research, the analytical methods utilized did not allow differentiating between non-sulfated and sulfated rhamnose in the ulvan backbone. The method for the quantification of carbohydrates comprises a methanolysis step in the presence of hydrochloric acid, where the hydroxyl groups of the ulvan backbone are methylated while cleaving the glycosidic bonds. As a consequence of this treatment, the sulfate groups from sulfated carbohydrates are lost, as observed by different authors [6, 44, 45]. Since the sulfate-to-rhamnose ratio increased during the time frame of the extraction, it might also be an indication that less sulfated fractions were extracted first. Accordingly, it might be reasonable to suggest that more sulfated ulvan fractions might be more associated to different cations, and consequently conforming more cross-linking within different fibers requiring longer extraction times, as was observed from the molecular size distribution results. However, more research should be carried out to study the particular composition of sulfates within the different ulvan fractions to confirm this hypothesis.

Exploratory Fermentation of the Processed Alga

The remaining algae obtained after the extraction of ulvan were fermented in a SSF process. Fresh *U. rigida* biomass was also fermented as a reference. The results are presented in Fig. 5. The fermentation of fresh *U. rigida* resulted in a maximum ethanol concentration of 1.3 g L^{-1}, equivalent to a yield of 0.09 g ethanol g^{-1} sugar (0.04 g ethanol g^{-1} DS). Conversely, the fermentation of processed *U. rigida* resulted in a maximum ethanol concentration of 6.2 g L^{-1}, equivalent to a yield of 0.48 g ethanol g^{-1} sugar (0.23 g ethanol g^{-1} DS). These values resulted in 5-fold increase in the fermentation yield comparing the processed alga with the fresh alga. While the fresh biomass contained 174 mg ulvan g^{-1} DS, the processed alga contained only 18 mg ulvan g^{-1} DS. Consequently, a combination of salt removal after washing and the decrease of the ulvan content after extraction resulted in a boost of the fermentation yield. In fact, cellulases are known to be inhibited by salts, but also by the presence of ulvan, suggesting ulvan taking a defensive role against the attack of marine microorganisms [46, 47]. These results show that the algal residue left after the extraction of ulvan may be efficiently used for the further production of biofuels (e.g., bioethanol).

Modeling of the Ulvan Extraction Kinetics

A mathematical model for describing the extraction kinetics of ulvan from *U. rigida* algae was developed based on the

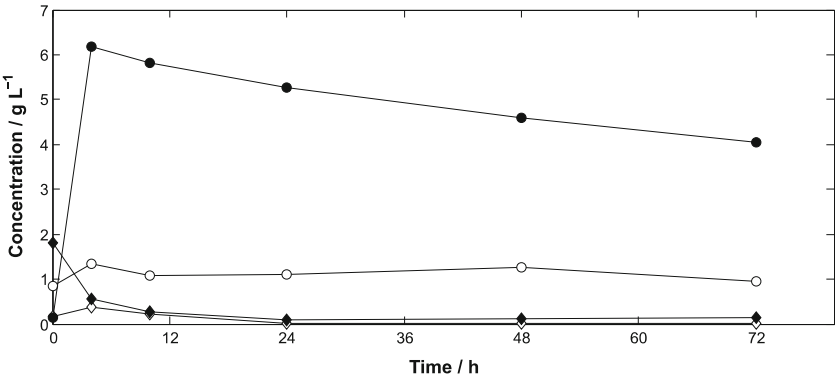

Fig. 5 Results of the fermentation of fresh and processed *U. rigida* biomass. *black and white circle* Ethanol and *black and white diamond* glucose. *Filled symbols* represent the fermentation of processed alga, while *empty symbols* represent the fermentation of fresh alga

observation made from the experimental data, for the temperature interval evaluated in this work (60–130 °C). The model is based on first-order kinetics in the concentration of ulvan in the solid (Eq. (1)) and it takes into account the temperature of the experiment. The model is based on the assumptions that no degradation of the sugars occurred and that the extraction was irreversible. Consequently, the mass balance of the sugars between the liquid and the solid phase can be described by Eq. (2). The pH of the mixture was not included into the model since it remained relatively constant throughout the experiments. The modeling was made for each of the main constituents of ulvan, namely, rhamnose, glucuronic, and iduronic acids. Glucose and xylose were excluded since they are not exclusively a part of ulvan. In order to make test plots for first-order kinetics, Eq. (1) can be integrated and Eq. (3) is obtained.

$$\frac{dc_{i,S}}{dt} = -k_{T,i} \cdot {c_{i,S}}^{\alpha} \tag{1}$$

$$c_{i,0} = c_{i,S} + c_{i,L} \tag{2}$$

$$ln\left(\frac{c_{i,S}^{t^*}}{c_{i,S}}\right) = k_{T,i} \cdot \left(t - t^*\right) \tag{3}$$

In this equations, c stands for the concentration of component i in the solid (s) or liquid (L) phase at time t and t^* is defined as the beginning of the extraction (1 min). Additionally, k stands for the rate constant of component i at temperature (T) and α stands for the reaction order.

The experimental data showed good agreement to first-order kinetics in the extraction time evaluated in this work. This result is in accordance with previous findings for alginate extraction from brown algae [48], and with results obtained in the extraction of hemicelluloses from forestry biomass [30, 31, 49, 50].

Consequently, the reaction order of the concentration in the solid phase was fixed to one. A modified Arrhenius expression featuring a reference temperature (T_{ref}) (95 °C) was utilized (Eq. (4)), in order to suppress the correlation between the activation energy and the pre-exponential factor [31]. No mass transfer limitations were considered explicitly in the model, but combined into the kinetic constant. External mass transfer limitations were avoided by the intense agitation applied in the experiments (650 rpm) and the relatively low solid to liquid ratio (1:20). The overall model can be expressed as shown in Eq. (5). The parameter $k_{0,i}$ stands for the pre-exponential factor and $E_{a,i}$ stands for the activation energy for component i, while R stands for the universal gas constant.

$$k_{T,i} = k_{0,i} \cdot e^{-\frac{E_{a,i}}{R} \cdot \left(\frac{1}{T} - \frac{1}{T_{ref}}\right)} \tag{4}$$

$$\frac{\mathrm{d}c_{\mathrm{i,S}}}{\mathrm{dt}} = -k_{0,i} \cdot e^{-\frac{E_{a,i}}{R} \cdot \left(\frac{1}{T} - \frac{1}{T_{ref}}\right)} \cdot {\mathrm{c_{i,S}}}^{\alpha} \tag{5}$$

The model was implemented with the software MATLAB b2010. The system of ordinary differential equations was solved using the function "ode45" provided by the software. The kinetic parameters were estimated by subsequent iterations of the objective function FOB defined as the normal function between the estimated values proposed by the model and the experimental data (Eqs. (6) and (7)), using the "fminsearch" function provided by the software. The parameters for rhamnose, glucuronic acid, and iduronic acid were estimated all together in the same objective function (Eq. (6)), while ulvan was treated separately. The FOB used for the monomers considered the experimental data as well as the model expressed in terms of conversion (X) of the different units (i), temperatures (T), and sampling time (t). The concentrations of ulvan were calculated as the sum of the values for rhamnose, glucuronic acid, and iduronic acid. The kinetic parameters for ulvan were estimated separately from the estimation performed for the monomers; thus, a different FOB function was utilized to find the optimized parameters (Eq. (7)).

$$FOB_{monomers} = \sqrt{\sum_{\mathrm{i=1}}^{\mathrm{i_{tot}}} \sum_{\mathrm{j=1}}^{\mathrm{j_{end}}} \sum_{\mathrm{t=1}}^{\mathrm{t_{end}}} \left(\mathrm{X}_{\mathrm{i,j,t}}^{model} - \mathrm{X}_{\mathrm{i,j,t}}^{experimental}\right)^2} \tag{6}$$

$$FOB_{ulvan} = \sqrt{\sum_{\mathrm{j=1}}^{\mathrm{j_{end}}} \sum_{\mathrm{t=1}}^{\mathrm{t_{end}}} \left(\mathrm{X}_{ulvan,\mathrm{j,t}}^{model} - \mathrm{X}_{ulvan,\mathrm{j,t}}^{experimental}\right)^2} \tag{7}$$

The fit of the mathematical model to the experimental data for the extraction of ulvan are displayed in Fig. 2. A good fit of the model to the experimental data was obtained. The parameter values obtained in the estimation are introduced in Table 3. The

Table 3 Kinetic parameters estimated for the extraction of ulvan from *Ulva rigida*

Sugar/carbohydrate	Pre-exponential factor k_0 ($min^{-1} \times 10^{-3}$)	Activation energy E_a (kJ mol^{-1})	Coefficient of determination R^2
Rhamnose	9.47	53.8	0.972
Glucuronic acid	7.93	52.9	0.933
Iduronic acid	7.43	57.6	0.890
Ulvan	8.51	53.8	0.959

Pre-exponential factor was calculated at the reference temperature 95 °C

R^2 values for rhamnose, glucuronic acid, and ulvan obtained values over 0.933; however, the fit of the model to the results for iduronic acid were not as good as for rhamnose and glucuronic acids. The activation energy obtained for the extraction of ulvan was 53.8 kJ mol^{-1} and the activation energies estimated for rhamnose and glucuronic acid were rather similar 53.8 and 52.9 kJ mol^{-1}, respectively. Iduronic acid exhibited slightly higher activation energy of 57.6 kJ mol^{-1}. One explaining factor could be that iduronic acid occurs in the ulvan structure in the dimer ulvanobiuronic 3-sulfate type B, whereas glucuronic acid occurs in the ulvan structure in the dimer ulvanobiuronic 3-sulfate type A [8]. These two dimers have different configuration in the ulvan backbone [33], which might influence the conformation of ulvan fractions with different sizes, resulting in potential differences in the activation energy values.

Unfortunately, there are no publications in the open literature reporting the parameter values for the ulvan extraction kinetics from green algae to which these data can be compared to or even for other types of sulfated polysaccharides from different seaweed. Vauchel and co-workers studied the extraction of alginate from *Lamarina digitata* using alkaline extraction, but the kinetic approach considered mainly the influence of stirring [48]. Recently, Leyton and co-workers studied the extraction of phlorotannins from *Macrocystis pyrifera* where the presence of fucose-containing carbohydrates (brown algal sulfated polysaccharide) was reported [51]. In turn, Yanomoto made a physicochemical study of sulfated polysaccharides extracted from green algae, where intrinsic viscosity and diffusion coefficients were reported [52]. Consequently, this data can only be compared to what has been reported for hemicellulose from terrestrial plants. Grénman and co-workers reported activation energy values for the extraction of hemicellulose from spruce to be around 120 kJ mol^{-1} [30], and around 82–251 kJ mol^{-1} for the extraction of hemicelluloses from different terrestrial plants [49, 50, 53, 54]. The activation energy values reported here for ulvan are between 34.3 and 78.5%

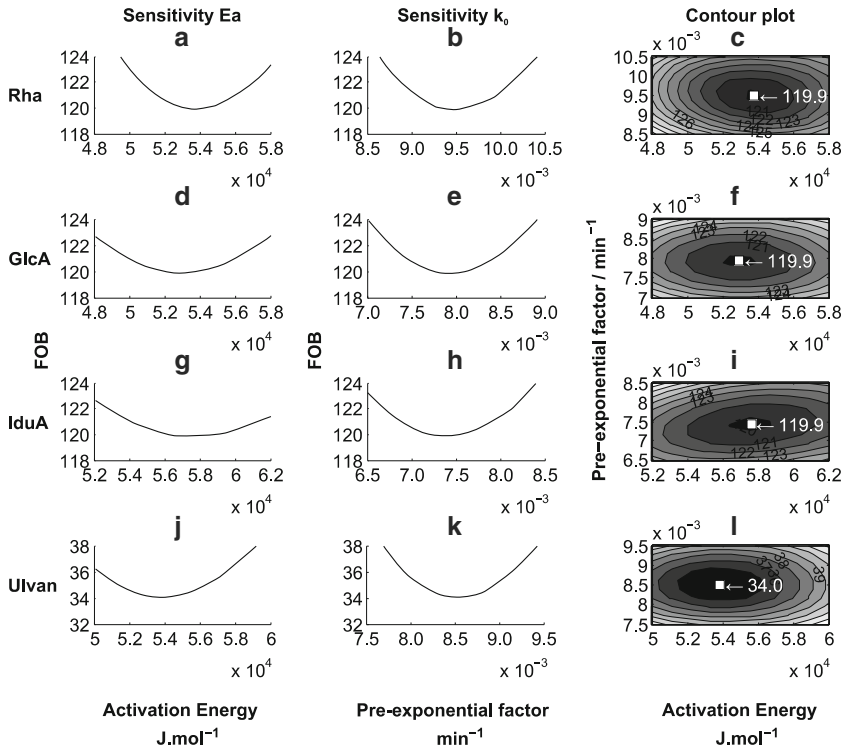

Fig. 6 Sensitivity analysis of the FOB function to the kinetic parameters for the extraction of rhamnose, glucuronic, and iduronic acids, as well as ulvan. Monomers were studied together in the same FOB function, while ulvan was studied separately. **a**, **d**, **g**, **j** The sensitivity of the FOB function to the E_a parameter for each constituent. **b**, **e**, **h**, **k** The sensibility of the FOB function to the k_0 parameter of each component. **c**, **f**, **i**, **l** The contour plots of the FOB function for the activation energy and the pre-exponential factor

lower than those reported for hemicelluloses from terrestrial pants. However, this seems rather logical as the hemicellulose in the cell wall of terrestrial plants is heavily linked to lignin, which hinders the extraction, and consequently, harsher conditions are required in terms of temperature in order to obtain acceptable extraction yields. In contrast, algal cell walls do not contain lignin; therefore, the conditions required to obtain higher extraction yields are milder than those for terrestrial plants.

Sensitivity Analysis of the Parameters

Figure 6 displays the results of the sensitivity analysis of the parameters estimated by the kinetic model for each of the main constituents of ulvan. The analysis was performed so that the value of one parameter was varied; the values of the rest of the parameters were fixed to the optimized value estimated by the model. Accordingly, the sensitivity of the objective function FOB to the activation energy displayed a clear minimum for all the components evaluated, as shown in Fig. 6a, d, g, j. Similarly, a global minimum was found for the pre-exponential factor values as shown in Fig. 6b, e, h, k. Additionally, contour plots were made for each component to evaluate if the pre-exponential factor and the activation energy correlate, and the results are shown in Fig. 6c, f, i, l. The sensitivity analysis demonstrated that the parameters are well defined under the experimental conditions evaluated in this work and no severe correlations exist.

Conclusions

The quantitative kinetics of the selective aqueous extraction of ulvan and its constituents rhamnose, glucuronic acid, and iduronic acid was studied in the temperature range of 60–130 °C. Yields up to 98 wt.% were obtained within 2 h of extraction and the extraction rate was observed to be heavily temperature dependent. A mathematical model was developed for the extraction kinetics, based on the experimental observations. An activation energy of 54 kJ mol^{-1} was attained for ulvan, which is considerably lower than the values obtained for terrestrial plants. Alternatively, the algal residue was fermented and a yield of 0.48 g ethanol g^{-1} sugars was attained. The results demonstrate that selective extraction of ulvan can be performed in high yields, at relatively mild conditions.

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