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Deconstruction of the green alga *Ulva rigida* in ionic liquids: Closing the mass balance

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ABSTRACT

Algae are known to grow at high rates compared to terrestrial plants that contain comparable amounts of carbohydrates by weight. Therefore, this renders them attractive in terms of any biorefinery concept. In this work the green alga *Ulva rigida*, containing 40 wt.% of carbohydrates was pretreated with a switchable ionic liquid (SIL), distillable ionic liquid (DIL) and low-viscosity ionic liquid (LVIL). The SIL DBU–MEA–SO_2 was prepared from a mixture of mono-ethanolamine (MEA) and 1,8-diazabicyclo-[5,4,0]-undec-7-ene (DBU) that was coupled with sulfur dioxide (SO_2), whereas the DIL [$TMGH^+$][$EtCO_2^-$] (1,1,3,3-tetramethylguanidine propionate) was synthesized by a simple acid–base neutralization reaction. Consequently, the LVIL [$HDBU^+$][$5OF^-$] protonated 1,8-diazabicyclo-[5,4,0]-undec-7-ene- 2,2,3,3,4,4,5,5-octafluoro-1-pentoxide was used as received. The treatments were carried out in the temperature range of 100–160 °C for 6 h. The products obtained after the treatments were analyzed using different techniques like ICP, OES, SEM, TEM, TGA, FTIR and carbohydrate determination by GC. Upon treatment with DIL up to 67 wt.% of carbohydrates could be dissolved. For the first time, processing of *U. rigida* was carried out in ionic liquids so that the mass balance of the process was obtained. It can be concluded that 1,1,3,3-tetramethylguanidine propionate shows significant potential when aiming at releasing carbohydrates from algal biomass that, consequently, can be applied in the production of platform chemicals and/or biofuels such as bioethanol.

1. Introduction

The scientific community agrees that when the Earth was formed, its atmosphere did not yet have enough oxygen to support more complex forms of life. Nonetheless, there is evidence that as early as 3700 million years ago a particular type of prokaryotic microorganism containing chlorophylls, known as cyanobacteria, started to perform photosynthesis by taking up organic and inorganic carbon sources to produce energy and oxygen. Consequently, these microorganisms were most likely responsible for creating our oxygen-rich atmosphere. Biologists believe that algae evolved from these microorganisms, as they are also organisms containing chlorophyll capable of performing photosynthesis to produce energy and oxygen [1]. Algae species present in the marine world can be classified into two major groups: microalgae and macroalgae. Microalgae are mainly unicellular microorganisms, while macroalgae are multicellular organisms. Multiple species of both types of algae have a high content of carbohydrates, as well as protein and lipids, all of which could be used in the production of bioethanol, biogas and biodiesel. In fact, Algenol Biofuels, Inc. in the United States is a good example of utilization of genetically modified microalgae for bioethanol production. Algae are also well known to possess a higher growth rate compared to terrestrial biomass. Macroalgae are divided into three big groups: green algae, red algae and brown algae. All of them are characterized by being composed of different kinds of carbohydrate species, but most importantly they completely lack lignin and hemicelulloses, thus diminishing the need for any aggressive preprocessing techniques aiming at deconstruction of the carbohydrate matrix to release fermentable sugars [2–6].

Green algae are characterized as containing starch applicable as a food resource and polysaccharides containing sulfate esters, mainly composed of arabinose, galactose, rhamnose, xylose and glucuronic acid [7,8]. Red algae, in turn mainly contain cellulose, glucan and galactan sulfates as polysaccharide species [4,7,8]. Ultimately, brown algae mainly contain alginate as one of their main carbohydrate

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components, but they have fucoidan as a polysaccharide containing sulfated esters, as well as mannitol and laminarin [4,6,7,9,10].

Ulva rigida (C. Agardh 1823) is a type of seaweed belonging to the group of green algae tending to grow near submerged marine rocks in many seas and oceans across the world [11]. The main constituents of *U. rigida* are carbohydrates, protein, ash and lipids. Further, starch and cellulose are among the carbohydrate species contained in *U. rigida*. Zemke-White & Clements [12] reported 3.5 wt.% of starch in *U. rigida* and Roesijadi et al. [6] and Percival [8] have reported the content of cellulose to be very low.

On the other hand, ulvan is also found in the *U. rigida* species, a polysaccharide containing rhamnose, xylose, glucuronic acid and sulfate esters. Fig. 1 depictures the chemical structure of ulvan, which shows great complexity by repetition of a number of disaccharides within different species. The most common repeating units are ulvanobiouronic acid 3 sulfate type A (As3) and B (Bs3) shown in Fig. 1A and B, respectively [13]. As3 is composed by the dimer β-D-glucuronosyluronic acid-(1 → 4)-L-rhamnose-3-sulfate, whereas Bs3 is formed by the dimer α-L-iduronosyluronic acid-(1 → 4)-α-L-rhamnose-3-sulfate. In turn Fig. 1C depicts ulvanobiose 3-sulfate (Us3) dimer composed by a xylose unit replacing uronic acids, which can also contain a sulfate group in positions C-2 or C-3 as shown in Fig. 1D. Nevertheless, its presence is reported to be fewer compared to ulvanobiouronic acid 3 sulfates. Oligosaccharides containing residues of glucuronic acid in As3 dimers can also be found in ulvan (see Fig. 1E). Further the presence of galactan sulfates as minor components has also been reported in some *Ulva* species [13–16].

Ionic liquids (IL) are known to be able to dissolve polysaccharides and carbohydrates such as cellulose as well as to disrupt the complex linkages of pristine biomass [17,18]. In fact, dissolution of algae biomass in ionic liquids has been reported earlier for different types of algae. Species such as *Sargassum fulbellum*, *Laminaria japonica* and *Undaria pinnatifida* in the group of brown algae were processed in 1-n-butyl-3-methylimidazolium chloride ($[BMIM^+][Cl^-]$) containing different types of acids, whereupon yields approaching 99 wt.% of total reducing sugars were reported, at temperatures within the range of 100–150 °C and processing times up to 250 min. Further, it was claimed that, when treating algae at temperatures below 120 °C, the hydrolyzate purity was greater than 95 wt.%, albeit decreasing when exposure time increased. The authors claimed that carbohydrate hydrolysis in ionic liquid mediated systems rendered better results when compared to dilute sulfuric acid treatments in an autoclave [19]. *Saccharina*

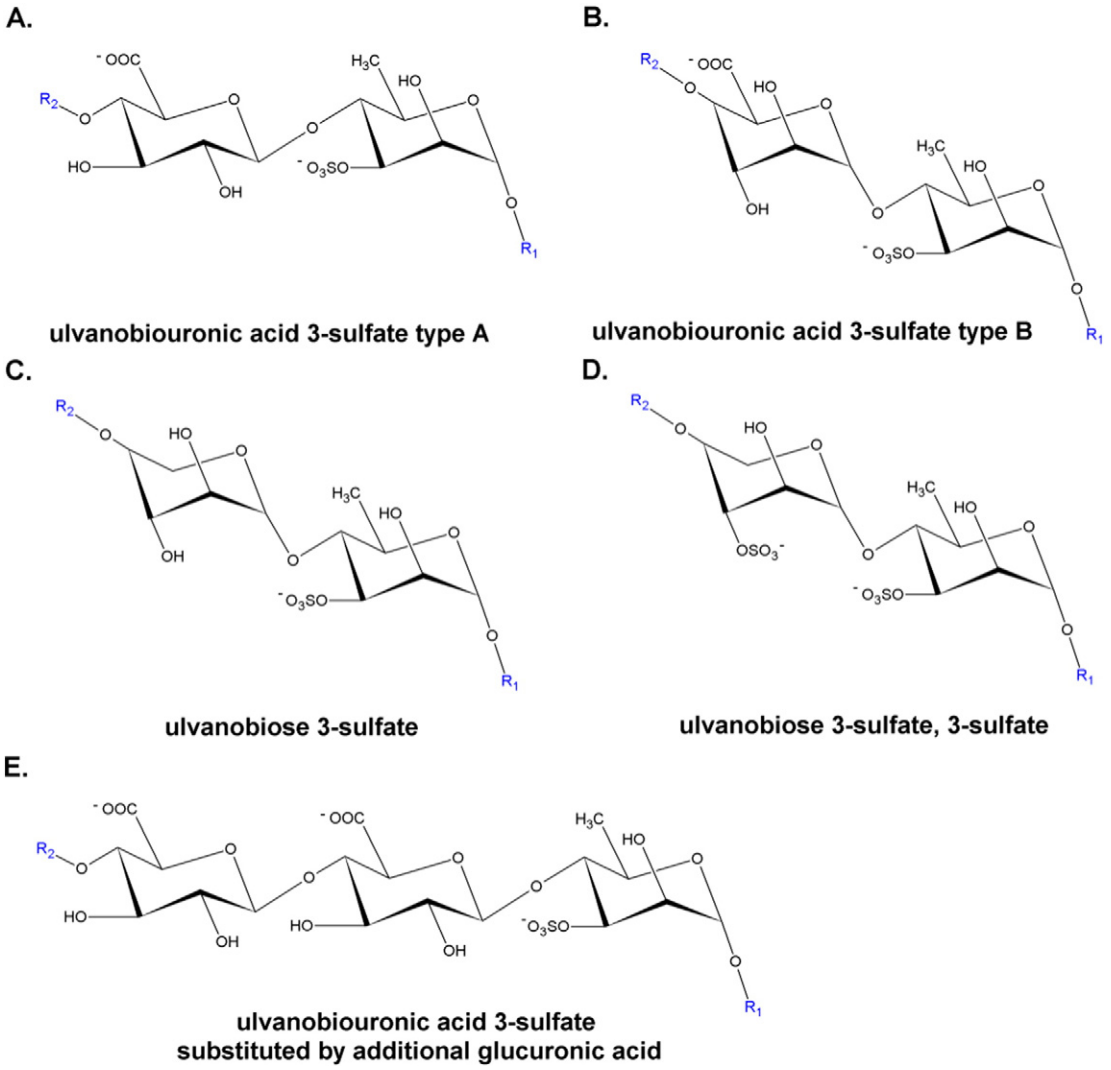

Fig. 1. Chemical structure of ulvan. * A. Ulvanobiuronic arid 3-sulfate type A, composed by [→4)-β-D-GlcpA-(1 → 4)-α-L-Rhap-3S-(1→]$_n$. B. Ulvanobiuronic acid 3-sulfate type B, composed by [→ 4)-α-D-IdopA-(1 → 4)-α-L-Rha-3S-(1→]$_n$. C. Ulvanobiose 3-sulfate, composed by [→ 4)-β-D-Xyl-(1 → 4)-α-L-Rha-3S-(1→]$_n$. D. Ulvanobiose 3,3-sulfate, composed by [→ 4)-β-D-Xyl-3S-(1 → 4)-α-L-Rha-3S-(1→]$_n$. E. Ulvanobiuronic acid 3-sulfate type A containing GlcA residue. Adapted from Jiao, Yu, Zhang, & Ewart [15] and Wang, Wang, Wu, & Liu [16].

japonica, a brown alga mainly cultivated in the coasts of China, South Korea and Japan, was also subject to processing with different kinds of ionic liquids such as 1-ethyl-3-methylimidazolium tetrafluoroborate ($[EMIM^+][BF_4^-]$), n-butyl-4-methylpyridinium tetrafluoroborate ($[BMPy^+][BF_4^-]$) and n-methylmorpholinium hydrogensulfate ($[NMM^+][HSO4^-]$) containing zinc chloride ($ZnCl_2$). As a result, the authors reported hydrolyzates with reducing sugars concentrations of 6.2, 6.4 and 6.0 g/L, respectively [20]. Besides the ionic liquid mediating process, *S. japonica* and *L. japonica* were exposed to low concentration, diluted sulfuric acid treatments, whereby subsequent simultaneous saccharification and fermentation (SSF; only for *S. japonica*) was performed in order to produce bioethanol. The acid concentration was varied within the range of 0.02–0.14 wt.%, at temperatures ranging from 150 to 180 °C and reaction times from 5 to 20 min. The enzymatic digestibility for *S. japonica* and *L. japonica* (enzymatic hydrolysis) was enhanced compared to untreated algae, whereby yields of 84.0 and 83.4 wt.% were reported, respectively [21,22].

Earlier reported ionic liquid preprocessing of green algae has mainly been focused on *Chlorella* biomass, a unicellular microorganism within the group of microalgae. *Chlorella* biomass is characterized as constituting high amounts of lipids, proteins and carbohydrates. Depending on the species, the typical values are about 20 wt.% fat, 45 wt.% protein and 20 wt.% carbohydrates [23]. *Chlorella* biomass treated with 1-ethyl-3-methylimidazolium chloride ($[EMIM^+][Cl^-]$) at 105 °C for 3 h, coupled to a subsequent treatment with concentrated 7 wt.% hydrochloric acid, relative to biomass content, at 105 °C for 3 h, resulted in about a 90 wt.% yield of sugars [24]. Choi et al. [25] treated *Chlorella* biomass with 1-ethyl-3-methylimidazolium acetate ($[EMIM^+][OAc^-]$) at 110 °C for 2 h, which resulted in a lipid extraction of 219 mg/g of cells. *Chlorella* biomass was also treated with 2 wt.% hydrochloric acid and 2.5 wt.% magnesium chloride ($MgCl_2$) at 180 °C for 10 min, resulting in about an 83 wt.% yield of sugars [26]. Interestingly, Lovejoy et al. reported a series of ionic liquids capable of extracting isopropenoids contained in the cellular wall of the green microalga *Botryococcus braunii*. The treatment reported minimal cellular death and allowed the recovery of the extracted isopropenoids via distillation [27]. Unlike the microalgae, the green macroalga *U. rigida* has only been characterized in terms of its protein, lipids and carbohydrate content so far, and practically no detailed analysis of its depolymerized and deconstructed products after processing was published to date. Nevertheless, there have been efforts for carbohydrate dissolution using aqueous systems that have accomplished ulvan extraction to a partial extent [28–30].

In this work, green alga *U. rigida* was processed in ionic liquids, whereupon the goal was to isolate carbohydrates that can be used in the production of platform chemicals or biofuels like ethanol. Consequently, the selection of the solvents was made on the basis of their carbohydrate dissolution potential. The first IL tested was DBU–MEA–SO_2 (1,8-diazabicyclo-[5.4.0]-undec-7-ene, monoethanolamine, sulfur dioxide) the so called switchable ionic liquid (SIL). The term switchable stands for the ability of this type of solvent to be back switched from ionic form to a mix of molecular liquids by bubbling an inert gas (such as nitrogen) to obtain the starting materials (in this case superbase and alkanol amine). This IL has been successfully utilized in e.g. solubilization of lignin and hemicelluloses [31]. The second IL used was 1,1,3,3-tetramethylguanidine (TMG) propionate ($[TMGH^+][CO_2Et^-]$), known as a distillable ionic liquid (DIL), capable of being distillated at temperatures around 130 °C under relatively high vacuum. This IL has been shown to successfully dissolve microcrystalline cellulose (MCC) with dissolution times as short as 10 min [32]. The third IL used was a protonated 1,8-diazabicyclo-[5.4.0]-undec-7-ene 2,2,3,3,4,4,5,5-octafluoro-1-pentoxide ($[HDBU^+][5OF^-]$). This IL was selected because of its very low viscosity (LVIL) which allows for better mixing and improved mass transfer compared to high viscosity ILs. Further, the IL pertains more hydrophobic properties, since its anion is a bulky fluorous, organic molecule which facilitates separation from water. Notwithstanding, this IL has not yet been reported in the literature as a biomass pretreatment or dissolution solvent. Fig. 2 depicts the chemical structure of the ionic liquids utilized in this work. Deconstruction of algae biomass in terms of carbohydrate, proteins and ash in order to determine the mass balance of the process was also another target of this work. No earlier reports on *U. rigida* processing in ionic liquids were found in open literature and the product mix obtained was comprehensively characterized.

2. Experimental

2.1. Materials

U. rigida (C. Aghard 1823 Chlorophyta, Ulvaceae) was kindly donated by Prof. Mario Edding from the Research and Technological Center in Applied Phycology – CIDTA – Northern Catholic University, Chile. The algae were collected in 2009 from submerged marine rocks located in La Herradura de Guayacán Bay, in the city of Coquimbo, Región de Coquimbo in Northern Chile. Algae were dispersed on plastic carpets and left to air dry for 48 h avoiding contact with sunlight. As the next step, algae were further dried in oven at 40 °C overnight and finally milled to 90%-# 30 mesh in a cross beater mill Retsch SK10. Once received, the algae samples were stored in a freezer and freeze dried prior to analyses.

Chemicals used for the synthesis of ionic liquids as well as for biomass analyses were used as received: 1,8-diazabicyclo-[5.4.0]-undec-7-ene (DBU, from Sigma Aldrich, 99.0% purity, PubChem CID: 81184), monoethanolamine (MEA, from Sigma Aldrich, 99.0% purity, PubChem CID: 700), sulfur dioxide (SO_2, from AGA, 99.998% purity, H_2O < 3 ppm, PubChem CID: 1119), 1,1,3,3-tetramethylguanidine (TMG, from Sigma Aldrich, 99.0% purity, PubChem CID: 66460), propionic acid (CO_2Et, from Acros Organics, 99.0% purity, PubChem CID: 1032), ethanol (from Altia Oyj, Aa quality, min. 99.5 wt.%, PubChem CID: 702), hexamethyldisilazane (HDMS, from Fluka, ≥99.0% purity, PubChem CID: 12838), chlorotrimethylsilane (TMCS, from Aldrich, ≥99.0% purity, PubChem CID: 6397), pyridine (from Aldrich, ≥99.0% purity, PubChem CID: 1049), N,N-dimethylformamide (DMF, from VWR, min 99.8% purity, CID: 6228).

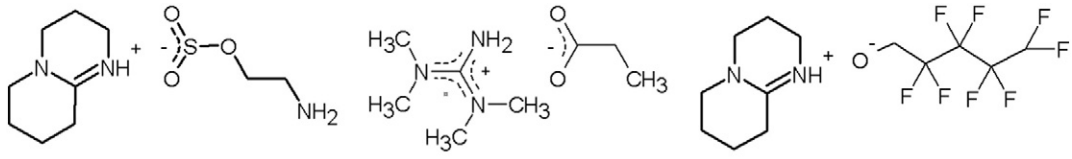

Fig. 2. Chemical structure of ionic liquids utilized within this work.

2.2. Ionic liquid processing

Three ionic liquids were used in alga processing. DBU–MEA–SO_2 (SIL) was prepared by mixing the superbase DBU and monoethanolamine followed by exothermic reaction upon bubbling of sulfur dioxide as described by Anugwom et al. [31]. The second IL used was TMG propionate ($[TMGH^+][EtCO_2^-]$), prepared by simple acid–base neutralization reaction as described by King et al. [32], whereas the third IL was protonated 1,8-diazabicyclo-[5.4.0]-undec-7-ene 2,2,3,3,4,4,5,5-octafluoro-1-pentoxide ($[HDBU^+][5OF^-]$) and kindly provided by the Institute of Heavy Organic Synthesis "Blachownia" (ICSO, used as received). The experimental procedure is illustrated by Fig. 3. After treatment with IL, a number of washing and precipitation steps for recovery of solubilized fractions were performed, as described in the following sections.

2.2.1. Switchable ionic liquid (SIL)

A total of 2.0 g of freeze dried algae was disposed in a 100 mL reactor where 18.0 g of the SIL DBU–MEA–SO_2 was added. The experiments were performed at 100 and 120 °C. The reactor was placed in an oil bath at the required temperature with continuous magnetic stirring for 6 h. All the experiments were carried out in duplicate unless otherwise stated.

2.2.2. Distillable ionic liquid (DIL)

A total of 11.0 g of TMG and 7.0 g of propionic acid were slowly added to a 100 mL reactor. The DIL formation took place upon mixing. Once mixed and cooled down, 2.0 g of freeze dried algae were disposed into the reactor. The experiments were performed at 100, 120, 140 and 160 °C, respectively. The reactor was placed in an oil bath at the required temperature with continuous magnetic stirring for 6 h. All the experiments were carried out in duplicate unless otherwise stated.

2.2.3. Low viscosity ionic liquid (LVIL)

A total of 100 mg of freeze dried algae was disposed in a 15 mL test tube where 900 mg of the LVIL was added. The test tube was placed in an oil bath at 120 °C with continuous magnetic stirring for 6 h. The experiments were carried out in duplicate.

2.2.4. Filtering and washing of fibers

Once the preset treatment time was reached, 50 mL of deionized water (DI, Millipore) was added to the reactor in order to facilitate the removal of ionic liquid. As the next step, the undissolved material was removed by using glass fiber filters and washed twice with 50 mL of deionized water. The samples treated with LVIL were filtered without the help of water due to their very low viscosity. Subsequently, a total of 20 mL of deionized water (DI, Millipore) was added for further IL removal. Due to its higher density compared to water, the LVIL decanted at the bottom of the funnel, allowing for a better IL removal from the undissolved material. The remaining solids were freeze dried at −50 °C and 0.1 mbar of vacuum overnight and kept in a freezer prior to FTIR analysis, acid methanolysis, acid hydrolysis or any other further analyses and treatments. The liquid filtrates (ionic liquid and washing water) were kept in the freezer until further analyses were performed.

2.2.5. Precipitation of dissolved algae biomass in ionic liquids

The water contained in the ionic liquid phase was evaporated using a Büchi R114 Rotavapor at 40 °C under vacuum. Once the viscosity of the samples notably increased because of water removal (especially for SIL and DIL) and the vacuum was below 0.1 mbar, the treatment was continued for an additional 20 min for maximum water removal. Subsequently, 40 mL of ethanol (Aa grade) was added and the mixture was gently stirred for 30 min (in the case of LVIL 5 mL of ethanol was used). Precipitated mass was separated by filtration using glass filters, and further washed with 20 mL ethanol for removal of trace ionic liquids (in the case of LVIL 5 mL of ethanol was used). Once filtered, the solid precipitate was dried in a furnace at 70 °C for 1 h and then left in freeze drying equipment at −50 °C and 0.1 mbar of vacuum overnight.

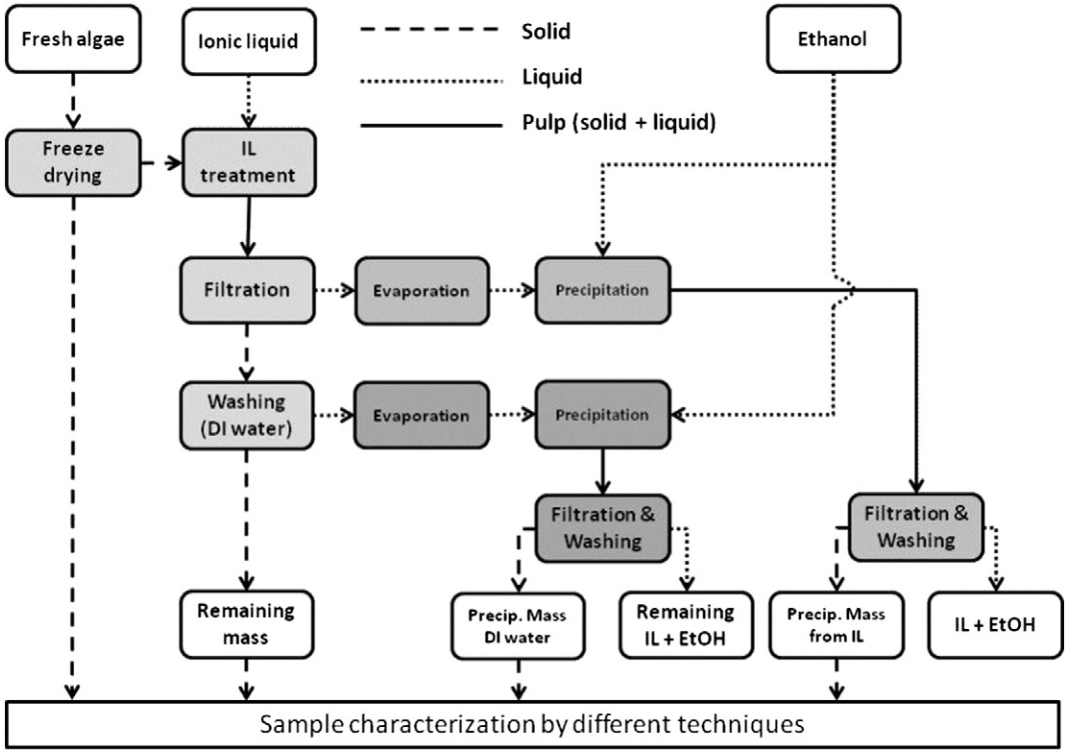

Fig. 3. Experimental procedure.

2.2.6. Precipitation of dissolved algae biomass in wash water

The water contained in the washing liquid was evaporated using a Büchi R114 Rotavapor at 40 °C under vacuum. Once a thin layer of solids was seen in the balloon and the vacuum was below 0.1 mbar, the samples were left for an additional 20 min for maximum water removal. Subsequently, 20 mL of ethanol (Aa grade) was added and the mixture was gently stirred for 30 min (in the case of LVIL 5 mL of ethanol was used). The precipitate was separated by filtration using glass filters, and further washed with 10 mL ethanol to ensure removal of any ionic liquid traces (in the case of LVIL 5 mL of ethanol was used). Once filtered, the solid precipitate was dried in a furnace, at 70 °C for 1 h, and then left in freeze drying equipment at −50 °C and 0.1 mbar of vacuum overnight.

2.3. Aqueous carbohydrate extraction

In addition to ionic liquid processing, experiments using aqueous solvents were performed to study carbohydrate dissolution yields. Two experiments in duplicate were carried out using DI Millipore water and dilute sulfuric acid 1.0 vol.% in an autoclave at 125 °C for 1 h. 200 mg of freeze-dried alga *U. rigida* was disposed in 15 mL test tubes at 10 wt.% mass loading. The test tubes were placed in the autoclave, and once the pretreatment temperature was achieved the reaction was continued for 60 min. Upon finishing, the autoclave was turned off and pressure was allowed to decrease to atmospheric condition. Subsequently, the test tubes were removed from the autoclave and allowed to cool down. The samples were filtered and the undissolved fraction and liquid phase were kept in the freezer before carbohydrate analyses were performed.

2.4. Proximate composition

Proximate analyses of *U. rigida* samples were carried out in order to determine the initial composition of the algae, in terms of protein, lipids, carbohydrates and ash content. Analyses were carried out by the Research and Technological Center in Applied Phycology – CIDTA – Northern Catholic University, Chile. The moisture content and protein, lipid, ash and insoluble fiber contents were quantified following the official methods of the Association of Official Analytical Chemistry (AOAC): 930.04, 978.04, 991.36, 930.05 and 962.09, respectively, [33]. The carbohydrate content was calculated by obtaining the difference between initial mass and the sum of values reported for proteins, ashes, lipids and fibers [34].

2.5. Chlorophyll analyses of fresh and processed algae

Chlorophyll content was analyzed in fresh and IL processed *U. rigida* samples. The experiments were carried out in duplicate using N,N-dimethylformamide (DMF) as the solvent. 120 mg of sample and 2.5 mL DMF were disposed in a flask and left in a refrigerator for 72 h as described by Schumann et al. [35]. The flasks were covered with aluminum foil in order to prevent chlorophyll decomposition by UV. Samples were homogenized in vortex every 24 h. Once extraction time was completed, the solution was filtered and analyzed in a spectrophotometer at 646.8, 663.8 and 750.0 nm for calculation of chlorophyll concentration using the equation proposed by Porra et al. [36].

2.6. Thermogravimetric analysis of fresh and processed algae

Thermogravimetric analyses of fresh and processed *U. rigida* biomass was performed under synthetic air with the SDT Q600 (V20.9 Build 20) instrument. About 6.00–8.00 mg of sample was weighed to carry out the analyses. The sample was inserted in a platinum pan and heated up from room temperature to 625 °C with a 10 °C/min ramp. The purge gas feed rate into the system was 100 ml/min.

2.7. Organic elemental analysis

Organic elemental analyses were performed on fresh and treated *U. rigida* biomass in order to quantify the content of nitrogen, carbon, hydrogen, sulfur and oxygen, respectively, using a Flash 2000 Thermo Scientific equipment. About 2.00 mg of standards and samples was weighed into tin cups. Helium (He) and oxygen (O_2) gases were used in the experiment. The instrument was calibrated using a commercial cystine standard. In addition, the commercial standard 2,5-(Bis(5-tert-butyl-2-benzo-oxazol-2-yl) thiophene (BBOT) was used as the reference sample.

2.8. Fourier transform infrared spectroscopy – FTIR – analysis of fresh and processed algae

The IR spectrum of fresh and pre-processed algae samples was recorded on an ATI Mattson Infinity Series IR spectrometer at room temperature. Samples were freeze-dried prior to FTIR analyses. The samples were blended with potassium bromide (KBr) powder (3.0 wt.%), and pressed with the force of 10 tons for 1 min into tablets before measurement. A region of 4000–400 cm^{-1} was used for scanning.

2.9. Carbohydrate analysis

2.9.1. Acid methanolysis method

The concentrations of hemicelluloses and pectins were determined by acid methanolysis method. 2 mL of methanolysis reagent containing 2 M of hydrochloric acid (HCl) in methanol was added to 10 mg of freeze dried algae samples and a calibration solution containing known carbohydrates. As the next step, the tubes were inserted into an oven operating at 100 °C for 3 h. Once the reaction was completed, 200 μL of pyridine was added to neutralize any excess of HCl, and 1 mL of each internal standard solution containing 0.1 mg/mL of sorbitol and resorcinol in methanol, respectively, was added to each sample. After mixing, methanol was evaporated at 50 °C under nitrogen stream and the sample was further dried under a vacuum (Heraeus VTR 5022) at 42 °C below 50 mbar for 20 min prior to the derivatization of the samples [37].

2.9.2. Acid hydrolysis method

Cellulose content was determined by acid hydrolysis method. 200 μL of 72 vol.% sulfuric acid was added to each 10 mg algae sample. Also, 10 mg cellulose powder was used as the standard. The standard and the algae samples were placed in a vacuum oven and degassed until below 50 mbar. This step was repeated three times. As the next step, the samples were stored under a fume hood for 2 h, whereupon 0.5 mL of distilled water was added to each sample. Again, 4 h later 6 mL of distilled water was added to each sample and the samples were stored under the fume hood overnight, at room temperature. Next day samples were placed in an autoclave at 125 °C for 90 min and then cooled down to room temperature. Once finished, two droplets of bromo-cresol green were added to each sample as an indicator and barium carbonate was added to neutralize the samples until the liquid phase turned blue. 1 mL of internal standard containing 5 mg/mL of sorbitol in distilled water was added to each sample, and then centrifuged at 1200 U/min for 10 min. 1 mL of liquid phase was taken from each sample and transferred to another test tube, where 1 mL of acetone was added. Finally, the samples were evaporated under nitrogen gas stream at 60 °C and further dried under vacuum (Heraeus VTR 5022) at 42 °C below 50 mbar for 15 min prior to the derivatization of the samples [38].

2.9.3. Derivatization of samples

Once the samples were completely dry, silylation was commenced by adding 150 μL of pyridine, 150 μL of hexamethyldisilazane (HMDS) and 70 μL of chlorotrimethylsilane (TMCS), followed by a thorough

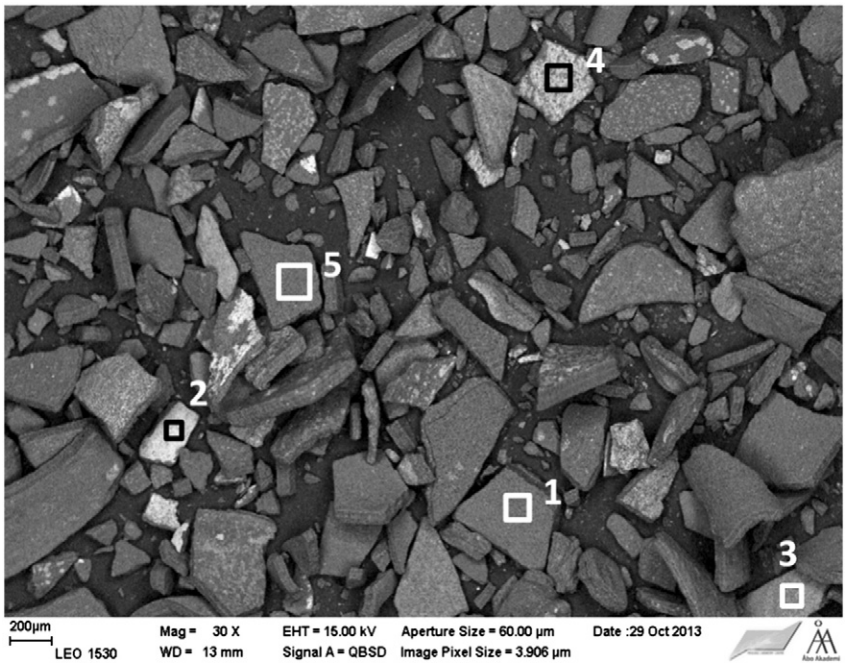

Fig. 4. SEM picture of fresh *Ulva rigida* biomass.

mix using a high-sheer vortex mixer. Further, the samples were left in a fume hood overnight and the clear liquid phase was analyzed in order to determine the sugar content of the samples by gas chromatography [37].

2.9.4. Gas chromatography

About 1 µL of a silylated sample was injected via a split injector (260 °C, split ratio 1:15) into a 30 m/0.32 mm i.d. column coated with dimethyl polysiloxane (HP-1, Hewlett Packard), the film thickness being 0.17 pm. The column temperature program was as follows: a temperature ramp from 100 to 175 °C (4 °C/min), followed by a ramp of 175 to 290 °C (12 °C/min). The detector (FID) temperature was 290 °C. Hydrogen was used as the carrier gas.

3. Results and discussion

3.1. Fresh algae characterization

3.1.1. Proximate composition of the fresh alga

According to proximate analysis, *Ulva rigida* contains high amounts of carbohydrates, i.e. 50.1 wt.%. This value is in accordance with earlier reported values [39]. In terms of its potential nutritional value, special attention should be paid to the high protein content of 20.6 wt.%. Consequently, *Ulva rigida* species are used as a source of food in some regions across Asia because of its high carbohydrate and protein content [40,41]. This substantial amount of carbohydrates also motivates its industrial potential, since it is a feasible feed stock for biofuel production (e.g. ethanol), as well as for the production of platform chemicals such as sugar alcohols. Finally, the ash content in *U. rigida* was measured to 19.5 wt.%, whereas its lipid content only amounted to 4.1 wt.%.

3.1.2. Scanning electron microscopy (SEM) analysis

Scanning electron microscopy analyses revealed disposition of small particles of *U. rigida* biomass. In terms of its elemental composition, average elemental composition (carbon excluded) for *U. rigida* biomass presented in Fig. 4 unravels a high relative amount of oxygen present, certainly due to a high content of carbohydrates also suggested by proximate analysis. Not unsurprisingly *U. rigida* also contains high amounts of sodium and chloride (harvested from sea water) as well as sulfur. Particularly the white spots depicted in Fig. 4 (marks 2 and 4) are high in sodium and chloride, presumably because of the presence of salts due to the marine nature of the sample.

3.1.3. Organic elemental analysis OEA

Table 1 presents the results of OEA for pristine *U. rigida* samples. The values confirm the high content of carbon (C) and oxygen (O) (more

Table 1
Results of OEA and ICP analysis showing elemental composition of *Ulva rigida*.

Organic elemental analysis					
N [wt.%] 3.5 ± <0.1	C [wt.%] 37.0 ± <0.1	H [wt.%] 5.7 ± <0.1	S [wt.%] 3.6 ± 0.2	O [wt.%] 34.9 ± 0.1	Others [wt.%] 15.3 ± 0.3
ICP-OES					
Al [mg/kg] 120 ± n.d	As [mg/kg] ND.	B [mg/kg] 70 ± n.d	Fe [mg/kg] 390 ± n.d	Si [mg/kg] 500 ± n.d	Sr [mg/kg] 50 ± n.d
Ca [wt.%] 0.4 ± n.d	K [wt.%] 1.6 ± n.d	Mg [wt.%] 2.0 ± n.d	Na [wt.%] 2.6 ± n.d	P [wt.%] 0.2 ± n.d	S [wt.%] 3.4 ± n.d

* ICP-OES values represent average ± standard deviation of triplicates. Others have been calculated as 100 − N(content) − C(content) − H(content) − S(content) − O(content).
** ICP-OES analyses were carried out doing only one run. Aluminum (Al) content might be overestimated because of the preparation of sample was performed using an Al_2O_3 mortar. n.d.: not determined.
*** ND.: not detected

Table 2
Monosaccharide content in *Ulva rigida*.

Monosaccharide	Value mg/g of dry alga
Arabinose	0.7 ± <0.1
Fucose	0.1 ± <0.1
Galactose	11.7 ± 0.1
Galacturonic acid	1.4 ± 1.5
Glucose	183 ± 7.8
From acid hydrolysis*	183 ± 7.8
From acid methanolysis**	184 ± 1.1
Glucuronic acid	62.3 ± 0.9
Fructose	1.4 ± 0.1
Mannose	ND ± ND
Mannitol	0.9 ± 0.8
Mannuronic and guluronic acids	18.0 ± 6.1
Rhamnose	81.2 ± 2.5
Xylose	38.5 ± 1.1

Values represent average ± standard deviation of duplicates. Arabinose, fucose, galactose, galacturonic acid, glucuronic acid, fructose, mannose, mannitol, mannuronic and guluronic acids (constituents of alginate), rhamnose and xylose were calculated by acid methanolysis method (see Section 2.9.1). Glucose was analyzed following two methods: * acid hydrolysis to analyze cellulose content, and ** acid methanolysis to analyze other hemicelluloses and pectins. ND: not detected.

than 30 wt.%) which is characteristic for materials with abundant carbohydrate content. The relative amount of nitrogen (N) was equal to 3.6 wt.%, corresponding to the protein content of 182 mg/g for dry algae, calculated using the conversion value 5.12 for *U. rigida* reported by Shuuluka et al. [42]. This value is in agreement to the one obtained when calculating protein content on the basis of proximate analyses (206 mg/g for dry algae).

3.1.4. ICP-OES analysis

Table 1 reports the results of elemental composition for pristine *U. rigida*. A high concentration of sodium (Na) present 2.6 wt.%, was again confirmed. Further, a significant iron (Fe) concentration of 390 mg/kg for dry algae was indicated. In fact, these results are in accordance with the analyses performed by Frikha, et al. [43], who reported a Na content of 0.43 wt.% for *U. rigida* and Fe content of 520 mg/kg for dry algae. Interestingly, magnesium (Mg) content reported by them (8.6 wt.%) by far exceeded that indicated by our analysis (2.0 wt.%). However, the species were not exactly the same algae and the salts concentrations can change a lot in marine environments.

3.1.5. Thermo gravimetric analysis (TGA)

As a result of TGA, an inorganic ash content amounting to 16.0 wt.% for fresh *U. rigida* biomass was found. This result is within the same magnitude of order obtained from the proximate analysis (18 wt.% of ash in fresh *U. rigida*) and is in accordance with the values reported in literature. Frikha et al. [43] characterized *U. rigida* samples harvested in Tunisia (an ash content of 25 wt.% by proximate analysis). On the contrary, Negreanu-Pîrjol et al. [39] reported an ash content of 18 wt.% for *U. rigida* collected at the Romanian Black Sea coast after calcination at 550 °C for 12 h.

3.1.6. Monosaccharide content in algae

Table 2 enlists the monosaccharide content for *U. rigida*, determined by GC after derivatization via acid methanolysis and/or acid hydrolysis. *U. rigida* contains a very small amount of mannuronic and guluronic acid, 18.0 mg/g for dry algae. On the contrary, glucose content in *U. rigida* is high, accounting for 183 mg/g for dry algae. Both acid hydrolysis and acid methanolysis methods gave similar results, suggesting that the alga contains almost no cellulose. This conclusion is also supported by earlier studies [6,8]. Consequently, the glucose released probably comes from starch, a polysaccharide reported to be present in green algae by some authors [7,8]. Rhamnose is the second most abundant monosaccharide present in *U. rigida* according to this study, accounting for 81.2 mg/g for dry alga. Also, relatively high concentrations of glucuronic acid and xylose were found, accounting for 62.3 and 38.5 mg/g, respectively, for dry alga. The presence of these three monomers suggests that they originate from ulvan, a polysaccharide containing sulfate esters, as it has been reported before [7,8,14,44]. Molar ratio of uronic acids (UruAc), rhamnose (Rha) and xylose (Xyl) was equal to 1.64:1.93:1.00. Ray & Lahaye, [28] reported the UruAc:Rha:Xyl ratio in *U. rigida* as equal to 2.28:2.13:1.00 and 1.96:2.35:1.00, suggesting that our algae samples are enriched in xylose. Therefore, as glucose is the main sugar present in this matrix, this alga represents a suitable feedstock when aiming production of chemicals and/or bioethanol. High content of other sugars, even rare ones, also increases the potential to synthesize other valuable chemicals such as sugar alcohols which are premium platform chemicals for production of various valuable products [45–48].

3.2. Pre-processing results

3.2.1. Algae IL processing

The mass balance for algae processing with ionic liquids is tabulated in Table 3. When the alga was treated with the SIL complete mass recovery was achieved. Regardless of the treatment temperature (100–120 °C), the undissolved fraction constituted about 76.1–78.5 wt.% of the original mass, whereas the precipitated biomass recovered from the ionic liquid and wash water amounted to 25.6–25.8 wt.%. On the contrary, when the alga was treated with DIL, the residual undissolved biomass left amounted to 66.0 wt.% after treatment at 100 °C, while the fraction recovered via precipitation from the DIL and washing water fractions amounted to 22.8 wt.%. Further, when the processing temperature was increased to 120 °C for DIL processing, only 38.0 wt.% of the original mass remained undissolved and 46.9 wt.% of the original mass could be recovered from IL and the washing water via precipitation. Even higher temperature treatments revealed more severe alga decomposition, as 16.7 wt.% at 140 °C and 14.6 wt.% at 160 °C remained undissolved after treatment with DIL, and the masses recovered after precipitation from IL were 71.9 and 74.4 wt.%, respectively. Still, when the alga was treated with the LVIL at 120 °C, 66.9 wt.% of the alga remained undissolved, whereas 28.8 wt.% was recovered via precipitation from the IL. Obviously, the nature of the solvent was important and DIL was clearly superior in dissolving biomass of this type. The influence of temperature was clear as well. 42 wt.%

Table 3
Mass balance of dissolution experiments with ionic liquids.

Ionic liquid	Temperature [°C]	Remaining mass [mg]	Precipitated mass from IL [mg]	Precipitated mass from DI water [mg]	Total recovered mass [mg]
SIL	100	785 ± 27.7	239 ± 44.1	18.2 ± 17.7	1043 ± 1.2
DIL	100	660 ± 8.3	140 ± 16.4	88.3 ± 47.9	888 ± 56.0
SIL	120	761 ± 1.2	208 ± 15.4	48.4 ± 35.9	1018 ± 19.4
LVIL	120	669 ± 15.8	288 ± 17.8	n.d ± n.d	957 ± 2.0
DIL	120	380 ± 0.4	450 ± 41.9	18.5 ± 1.7	848 ± 39.8
DIL	140	167 ± n.d	716 ± n.d	4.0 ± n.d	887 ± n.d
DIL	160	146 ± n.d	739 ± n.d	5.4 ± n.d	890 ± n.d

* Values represent average ± standard deviation of duplicates. Results are normalized to 1000 mg initial biomass. n.d.: not determined (only one experiment performed).

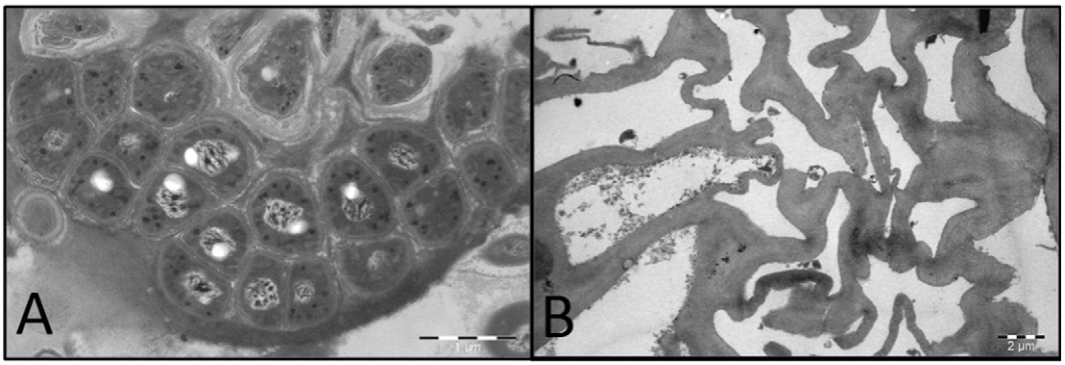

Fig. 5. Transmission electron microscopy image of *U. rigida* samples. A: Fresh *U. rigida*. B: Treated *U. rigida* with TMG propionate at 120 °C for 6 h.

more biomass was dissolved using DIL when the processing temperature was increased from 100 °C to 120 °C. When the temperature was increased up to 160 °C 61.5 wt.% more biomass was dissolved, however, carbohydrate decomposition was observed as will be discuss later. When observing the transmission electron microscopy (TEM) images, intact cell walls and intracellular organs could be seen for fresh *U. rigida* (see Fig. 5A). However, after the processing steps only the cell wall was retained confirming internal disruption (see Fig. 5B).

3.2.2. Carbohydrate dissolution

The carbohydrate composition of undissolved and precipitated fractions after ionic liquid processing is summarized in Table 4. In terms of monosaccharide content, one can see that treatments at 100 °C were not able to dissolve much of the carbohydrates, neither in the case of SIL nor DIL ionic liquids. Nevertheless, 27.4 wt.% of the monosaccharides present in fresh alga was extracted using DIL at 100 °C and 19.0 wt.% of the carbohydrates was precipitated (27.4 mg/g dry fresh alga). On the contrary, 21.1 wt.% of the carbohydrates was extracted in SIL and 1.4 wt.% of the carbohydrates was precipitated (5.7 mg/g dry fresh alga). At 120 °C, 25.9 wt.% of the carbohydrates present in fresh alga was extracted using SIL and 2.6 wt.% of the carbohydrates was precipitated (10.4 mg/g dry fresh alga). Further, when using LVIL 36.6 wt.% of the carbohydrates was extracted and 34.1 wt.% was precipitated, whereas the DIL was able to extract 67.2 wt.% of carbohydrates and 61.8 wt.% of carbohydrates was precipitated (248 mg/g dry fresh alga), representing 92.2 wt.% recovery yield. We performed a statistical

Table 4
Carbohydrate composition of undissolved and precipitated fractions recovered after ionic liquid treatment of green alga *U. rigida*.

Fractions	Fresh alga	Water	Dilute sulfuric acid	SIL DBU–MEA–SO_2	
		125 °C	125 °C	100 °C	120 °C
Undissolved					
TOC	399 ± 20.9	190 ± 25.8	21.2 ± 1.4	316 ± 29.5	297 ± 4.5
Glu	183 ± 7.8	135 ± 21.9	8.4 ± 0.8	147 ± 14.4	164 ± 13.1
Rha	81.2 ± 0.5	10.3 ± 0.6	0.1 ± <0.1	61.3 ± 3.4	45.3 ± 10.3
GluA	62.3 ± 0.9	7.6 ± 0.9	0.8 ± 0.1	48.8 ± 6.6	31.6 ± 8.1
Xyl	38.5 ± 1.1	22.0 ± 0.7	4.3 ± 0.2	32.8 ± 2.7	32.7 ± 1.2
Precipitated/dissolved					
TOC	–	197 ± n.d	374 ± 6.3	5.7 ± 0.7	10.4 ± 0.2
Glu	–	30.1 ± n.d	178 ± 4.4	2.1 ± 0.4	5.0 ± 1.5
Rha	–	77.5 ± n.d	80.8 ± 0.5	0.7 ± <0.1	1.9 ± 0.9
GluA	–	60.4 ± n.d	62.0 ± 0.1	0.8 ± 0.2	1.1 ± 0.3
Xyl	–	11.3 ± n.d	25.5 ± 0.8	0.6 ± 0.1	1.0 ± 0.2
	LVIL [$HBDU^+$][$5OF^-$]	DIL [$TMGH^+$][$EtCO_2^-$]			
	100 °C	100 °C	120 °C	140 °C	160 °C
Undissolved					
TOC	254 ± 37.8	291 ± 2.2	131 ± 19.7	44.8 ± n.d	36.0 ± n.d
Glu	132 ± 3.4	148 ± 0.1	57.6 ± 13.3	27.8 ± n.d	28.0 ± n.d
Rha	44.4 ± 16.6	48.9 ± 1.7	22.0 ± 0.4	1.6 ± n.d	0.3 ± n.d
GluA	28.7 ± 8.5	39.9 ± 0.1	16.7 ± 1.9	1.4 ± n.d	0.5 ± n.d
Xyl	25.8 ± 5.6	31.8 ± 0.4	26.3 ± 3.9	11.2 ± n.d	3.9 ± n.d
Precipitated/dissolved					
TOC	137 ± n.d	76.2 ± 24.0	248 ± 3.9	229 ± n.d	103 ± n.d
Glu	34.5 ± n.d	24.1 ± 5.6	128 ± 7.2	107 ± n.d	56.4 ± n.d
Rha	40.2 ± n.d	24.2 ± 11.3	47.1 ± 4.3	47.4 ± n.d	15.3 ± n.d
GluA	28.3 ± n.d	14.9 ± 7.4	38.5 ± 3.9	33.6 ± n.d	7.7 ± n.d
Xyl	14.8 ± n.d	6.7 ± 2.8	17.5 ± 0.4	19.4 ± n.d	13.4 ± n.d

* Values represent average ± standard deviation of duplicates. Results of composition of products obtained after treatment. Precipitated stands for the sum of the precipitated mass from ionic liquid and washing water in the case of ionic liquid processing, whereas for the aqueous treatment direct analyses of the liquid phase was performed prior to neutralization and filtering of the samples. TOC: total carbohydrate content, Glu: glucose, Rha: rhamnose, GluA: glucuronic acid, Xyl: xylose. Results were analyzed by acid methanolysis method. Acid hydrolysis method was not performed to analyze these samples, as fresh algae showed no difference in glucose content when analyzed by acid hydrolysis and acid methanolysis (see Section 3.1.6). Results are normalized to 1.0 g of initial dry biomass. n.d.: not determined.

Table 5
Benchmarking of carbohydrate dissolution from Ulva rigida using different solvents.

Extraction solvent	Aim	Temperature	Pressure	Time	Precipitated carbohydrate [mg/g dry alga]	Reference
DIL—[$TMGH^+$][$EtCO_2^-$]	Carbohydrate extraction[(a)]	160 °C	Atmospheric	360 min	229	This work
DIL—[$TMGH^+$][$EtCO_2^-$]	Carbohydrate extraction[(a)]	140 °C	Atmospheric	360 min	103	This work
Water	Nanofiber production[(a)]	130 °C	Autoclaved, 2 atm	30 min	96.7	[30]
Water	Carbohydrate extraction[(b)]	125 °C	Autoclaved, 2 atm	60 min	197	This work
1.0 vol.% H_2SO_4	Carbohydrate extraction[(b)]	125 °C	Autoclaved, 2 atm	60 min	374.	This work
DIL—[$TMGH^+$][$EtCO_2^-$]	Carbohydrate extraction[(a)]	120 °C	Atmospheric	360 min	248	This work
SIL—DBU–MEA–SO_2	Carbohydrate extraction[(a)]	120 °C	Atmospheric	360 min	10.4	This work
LVIL—[$HDBU^+$][$5OF^-$]	Carbohydrate extraction[(a)]	120 °C	Atmospheric	360 min	75.6	This work
Three subsequent extractions	Carbohydrate extraction[(a)]	100 °C	Atmospheric		102	[28]
1. 50 mM sodium oxalate				90 min		
2. 50 mM sodium oxalate				60 min		
3. Water				120 min		
DIL—[$TMGH^+$][$EtCO_2^-$]	Carbohydrate extraction[(a)]	100 °C	Atmospheric	360 min	76.1	This work
SIL—DBU–MEA–SO_2	Carbohydrate extraction[(a)]	100 °C	Atmospheric	360 min	5.7	This work
Water	Carbohydrate extraction[(a)]	100 °C	Atmospheric	360 min	101	[29]
1. Cold water						
2. Hot water						
Water	Bioethanol production[(c)]	37 °C	Atmospheric	180 min	196	[49]
Enzymes under sonication						

(a) : Precipitation carried out with ethanol.
(b) : Carbohydrates analyzed in-situ through acid methanolysis method.
(c) : Glucose content analyzed by colorimetric enzymatic method [49].

study of the IL extraction results using the Welch test, with which significant differences were obtained among the ILs used at 120 °C. Thus, the DIL is clearly superior in its ability to dissolve carbohydrates, as expected. Still, when temperature was increased to 140 °C, the DIL was able to extract 88.8 wt.% and 57.2 wt.% of carbohydrates was precipitated (228 mg/g dry fresh alga). When temperature was increased to 160 °C, a slightly higher extraction yield was achieved resulting in 91.0 wt.% of carbohydrate dissolution, but only 25.7 wt.% of carbohydrates could be precipitated (103 mg/g dry fresh alga). These last two treatments clearly indicate further degradation of the extracted carbohydrates. When analyzing the composition of the pristine alga to the processed material obtained after DIL treatment at 100 °C, one can observe that rhamnose and glucuronic acid are more easily extracted from the alga compared to glucose and xylose. It is clear that higher temperatures are required to extract glucan, and in the case of ulvan, carbohydrates containing ulvanobiuronic residues are more easily extracted. At 140 °C and 160 °C, glucan and ulvan were substantially removed, but the amount of carbohydrates precipitated from the IL showed no significant difference between processing temperatures 120 °C (247 mg carbohydrates/g dry alga) and 140 °C (229 mg carbohydrates/g dry alga). However, the undissolved fraction obtained after the IL processing at 140 °C contained much less carbohydrates (44.8 mg carbohydrates/g dry alga) than the one obtained after the IL processing at 120 °C (131 mg carbohydrates/g dry alga), presumably because of further decomposition of the carbohydrates at higher temperatures. In the case of the IL processing at 160 °C much less carbohydrates were precipitated from the IL (103 mg carbohydrates/g dry alga), and the undissolved fraction obtained after the IL processing at this temperature contained less carbohydrates (36.0 mg carbohydrates/g dry alga) than the undissolved fractions obtained after the IL treatments at 120 °C and 140 °C, indicating that carbohydrate degradation occurred.

Anugwom et al. [31] used SIL to treat Nordic woody biomass in a 1:3 ratio with water at 160 °C and ~6.1 bar, achieving 90 wt.% of lignin removal in 2 h. Thus, cellulose and hemicelluloses mainly remained in the biomass matrix, as well as it was observed for the green alga *U. rigida* in this work. Comparing the results obtained with the ionic liquid processing to the ones involving aqueous systems, it can be observed that the water treatment at 125 °C for 60 min released 49 wt.% of carbohydrates. In this process ulvan was principally released rendering an 82.0 wt.% extraction yield, whereas the glucose yield was only 16.1 wt.%. Consequently, the water treatment shows high selectivity towards ulvan dissolution compared to glucan. On the other hand, the DIL processing rendered 69.2 wt.% glucose yield, being 4.2 fold higher than those obtained with water although a shorter treatment time was used. Toskas et al. [30] performed a treatment for *U. rigida* using water at 130 °C for 30 min in an autoclave, achieving a 24.3 wt.% carbohydrate extraction yield as shown in Table 5. Siddhanta et al. [29] reported treatment of *U. rigida* using cold water extraction at 4–5 °C followed by hot water extraction at 80 °C for 3 h. With this approach, the carbohydrate extraction yield reported was 101 mg/g dry alga, where mainly rhamnose and uronic acids were extracted, but very low amounts of glucose were obtained. Ray & Lahaye [28] made a subsequent extraction using sodium oxalate and water, achieving 102 mg/g dry alga carbohydrate yield, with mainly rhamnose and uronic acids. When the dilute sulfuric acid treatment at 125 °C for 60 min was performed to *U. rigida*, the carbohydrate extraction yield increased to 93.5 wt.%, with an almost complete digestion of the alga. This treatment was clearly the best in terms of carbohydrate extraction, as significant differences with all the treatments presented in this work were attained when the statistical Welch test was evaluated. However the recovery of the mineral acid containing solvent is an issue when aiming industrial applications.

Table 6
Mass balance for *Ulva rigida* dissolution at 120 °C.

Mass balance		Initial mass [mg]	Remaining mass [mg]	Precipitated mass [mg]
SIL	Mass	1000 ± ---	761 ± 1.2	257 ± 20.5
	Protein	183 ± 4.5	173 ± 8.3	9.3 ± 8.3
	Ash	163 ± n/d	28.3 ± 7.0	132 ± 7.0
	Carbohydrates	399 ± 20.9	297 ± 4.5	10.4 ± 3.4
DIL	Mass	1000 ± ---	380 ± 0.4	469 ± 40.2
	Protein	183 ± 4.5	106 ± 2.5	65.1 ± 5.1
	Ash	160 ± n/d	26.7 ± 1.4	113 ± 4.1
	Carbohydrates	399 ± 20.9	131 ± 19.7	248 ± 17.1

* Values represent average ± standard deviation of duplicates. Protein content was analyzed via nitrogen content reported in OES analysis for fresh algae (triplicate) and remaining mass after treatment (duplicate), using a conversion factor equal to 5.12 used by Shuuluka et al. [42]. Ash content was analyzed via TGA analysis for fresh algae (no replicates) and remaining mass after treatment (duplicate). Protein and ash contents for precipitated mass were calculated by difference. Precipitated mass includes both from ionic liquid and wash water. Carbohydrates content was analyzed via acid methanolysis method for all samples obtained. n.d: not determined.

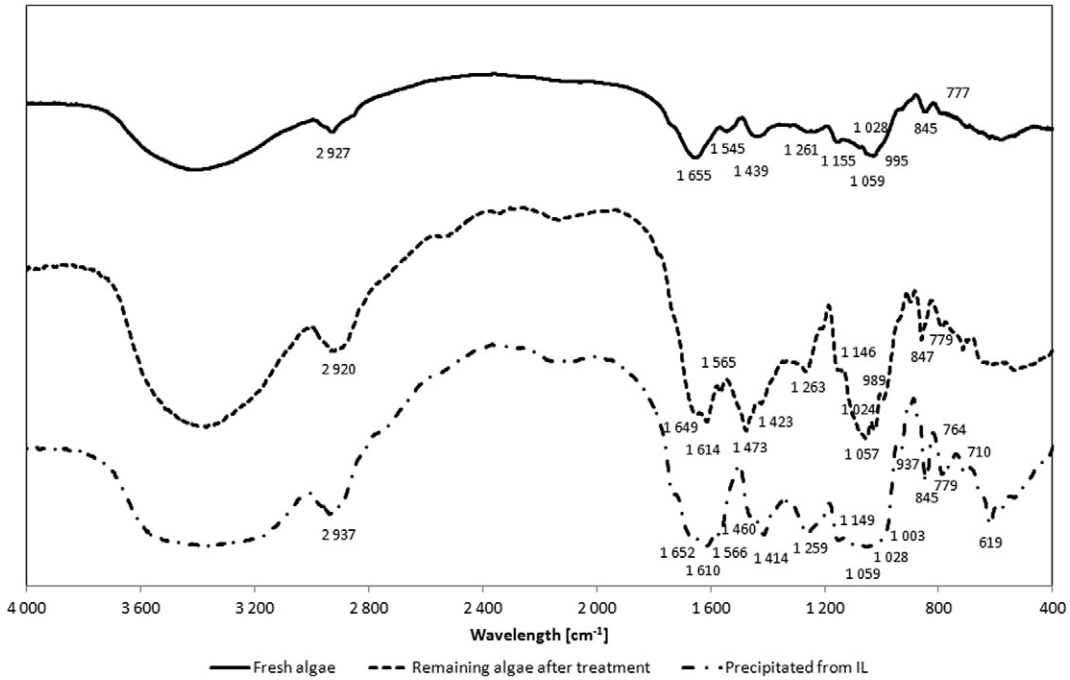

Fig. 6. FTIR spectra for fresh algae and products obtained after ionic liquid treatment. Treatment performed with TMG propionate at 120 °C for 6 h. ————: fresh algae,- - - - -: remaining algae after treatment, -·-·-·-: precipitated mass from ionic liquid phase.

3.2.3. Mass balance by component

Table 6 unravels the mass balance obtained for *U. rigida* processed at 120 °C. It can be seen that proteins mainly remain in the residual algae recovered after treatment. After processing of algae in SIL, the protein content in the residual algae was increased to 22.7 wt.%, whereas in the case of DIL the protein content in the residual algae was increased to 27.9 wt.%. In other words, 94.9 wt.% of the protein present in the pristine algae remained in the residual algae after processing with SIL, whereas in the case of DIL 58.0 wt.% of the total protein remained.

In terms of ash elements, it could be seen that the most part was dissolved and the minor part remained in the dissolved fraction; it was evident that the SIL was more prone to solubilize the ash elements since the residual biomass after SIL treatment only contained 3.7 wt.% whereas the sample treated with DIL still contained 7.0 wt.% of ash.

When accounting for protein, ash and carbohydrates in non-dissolved and solubilized fractions, some of the original mass is still unaccounted for, most probably insoluble fibers and lipids. Still, the carbohydrate content on the basis of the acid methanolysis method results in lower amounts than on the basis of proximate analyses, probably because of the presence of polysaccharides not fully accounted for upon acid methanolysis. Also, some carbohydrate loss likely occurred during ethanol washings (see Table 3). Analysis of monosaccharides is compromised if any ionic liquids are present, since chromatographic

Table 7
FTIR frequency assignments for green alga *Ulva rigida*.

Entry	Wavenumber [cm^{-1}]	Interpretation	Component	Reference
1	3400–3300	OH stretching	Carbohydrates, proteins, lipids (sterols fatty acids), nucleic acids, chlorophyll	[53–55]
2	2930–2915	CH_2 stretching	Lipids	[53–57]
		C–H stretching	Chlorophyll a & b (phytol)	
3	2720–2710	C–H stretching	Chlorophyll b (aldehydes)	[55]
4	1740–1720	C = O stretching	Chlorophyll a & b (ester)	[55,57,58]
5	1701–1610	C = O stretching	Proteins (amide I band)	[53–55,57,58]
		C = C stretching	Chlorophyll a & b (ketone, aldehyde, chelate)	
6	1565–1536	N–H bending	Proteins (amide II band)	[53,54,57]
		C–N stretching		
7	1485–1425	CH_2 stretching	Proteins	[53,54,57]
		CH_3 stretching	Methyl-lipid	
8	1440–1380	CH bending	Aliphatic groups	[53]
9	1356–1191	P = O stretching	Nucleic acids, other compounds containing phosphates and stretching of phosphodiesters	[53,54,57]
10	1160–980	C–O–C stretching	Carbohydrates of polysaccharides	[53,54,56,57]
11	933–928	–	3,6-Anhydro-D-galactose	[52]
12	850–840	$–SO_4$-binding	D-Galactose-4-sulfate	[52]
13	820–810	$–SO_4$-binding	D-Galactose-6-sulfate	[52]
14	630–620	CH_2 stretching	Unsaturated aliphatic chains (lipids or phenolic groups)	[53]
		C–H stretching		
15	470–450	C–H stretching	Aromatic chains	[54]

columns (e.g. HPLC and GC columns) don't tolerate high concentration of salts, and a common limit is 50 ppm only [50].

3.2.4. Chlorophyll analyses

Chlorophyll is an important pigment found in plants. The total content of chlorophyll a and b, was determined by extraction with DMF and subsequently analyzed by UV absorbance. Total chlorophyll content in fresh *U. rigida* accounted for 867 ± 72.9 μg/g dry alga, in accordance with the value reported by Yildiz et al. [51], who obtained 945 ± 450 μg of chlorophyll/g dry algae for fresh *U. rigida* using 90 vol.% acetone for extraction. Both SIL and DIL treatment at 120 °C demonstrated to be very prone for chlorophyll removal from pristine alga, since only 7.6 ± 0.2 μg/g dry alga of chlorophyll a and b remained in the processed alga with SIL, representing 99.1 wt.% chlorophyll removal. In turn, when the alga was processed with DIL at 120 °C, 21.4 ± 15.6 μg/g dry alga of chlorophyll a and b remained in the undissolved fraction, thus representing 97.6 wt.% chlorophyll removal.

3.2.5. Fourier transform infrared spectroscopy – FTIR – analysis

The results of FTIR analysis for the pristine alga, non-dissolved alga and recovered alga after precipitation from DIL (TMG propionate at 120 °C in 6 h) are shown in Fig. 6. Several peaks match to earlier reported data and can be assigned as proteins, lipids and carbohydrates (Table 7). Pristine *U. rigida* gives rise to less prominent peaks than the processed samples, particularly for the peaks corresponding to carbohydrates (1160–980 cm^{-1}) and sulfated carbohydrates (950–800 cm^{-1}) (see entries 10–13, Table 7). The last band is rather prominent in the precipitate obtained from IL, and likely corresponds to rhamnose, glucuronic acid, xylose and galactose interconnected by sulfate bonds. Such observations are common for sulfated carbohydrates such as agar found in red and brown algae species [52]. We should also keep in mind that 60 wt.% of carbohydrate was found in the precipitate fraction (see DIL 120 °C, precipitated/dissolved fraction, Table 4). On the other hand, it seems that the proteins (1485–1425 cm^{-1}, entry 8), the second abundant fraction in *U. rigida* according to proximate analysis, stayed in the non-dissolved fraction as also suggested by the OEA analysis. Several of these peaks overlap with those corresponding to chlorophyll as it is seen in Table 7, although chlorophyll is not an important constituent of the biomass matrix in *U. rigida* as our analyses demonstrated.

4. Conclusions

Hereby we report a mild ionic liquid mediated processing to facilitate *Ulva rigida* biomass deconstruction with extensive characterization of the phenomena occurring. It was demonstrated that TMG propionate ionic liquid is a potent candidate for removal of carbohydrate from the biomass matrix since 67 wt.% of the total carbohydrates was dissolved. The use of DBU–MEA–SO_2 SIL and [$DBUH^+$][$5OF^-$] didn't give as good results as the use of [$TMGH^+$][$EtCO_2$] and, only approximately 25 wt.% of the carbohydrates was solvated. It was also demonstrated that the process mediated in ionic liquids at 120 °C resulted in a 3 fold increase in the yield of carbohydrates dissolved compared to 100 °C. Further, 92 wt.% of solubilized carbohydrates were recovered by precipitation from IL.

Regarding the other components found in the *Ulva rigida* biomass, the results suggest that the proteins didn't readily dissolve since 58 wt.% remained in the non-dissolved fraction. On the other hand, up to 83 wt.% of ash was removed from the algae upon processing. Consequently, the mass balance of the process was obtained, and comprehensive characterization of the products obtained by ICP, OES, SEM, TEM, TGA, and FTIR and carbohydrate determination by GC was performed. However, more work remains in order to optimize the procedure to yield the maximum amount of carbohydrates. Further analyses of the ionic liquid phase need to be performed in order to study the reusability of the solvent. Notwithstanding, high water solubility of many ionic liquids complicates the design of a viable industrial process.

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