CHAPTER 12

Emerging seaweed extraction techniques using ionic liquids

Margarida Martins, Sónia P.M. Ventura
Department of Chemistry, CICECO—Aveiro Institute of Materials, University of Aveiro, Campus Universitário de Santiago, Aveiro, Portugal

1 Introduction

The use of seaweeds was spread worldwide and under different forms and applications. From a technological point of view, seaweeds have been used as sources of colorants and phycocolloids, as well as thickening and gelling agents applied in the food industries (Syad et al., 2013). More recently, their exploitation has been seen as a source of new chemicals, products, and drugs, potentially comprising terpenoids, proteins, carbohydrates, vitamins, sterols, and pigments, just to mention a few (Francavilla et al., 2013).

Besides the cultivation strategies that have been adopted to induce and maximize the production of primary and secondary metabolites of commercial relevance (Francavilla et al., 2013), the development of efficient methodologies to recover these compounds from seaweed biomass is essential to boost their industrial potential even more while maintaining the sustainability of the whole process. Comprising a sustainable exploitation of biomass, there are three main tasks in need of optimization under mild conditions. These are the aquaculture, harvesting, and downstream processes applied to separate each compound or class of compounds of interest. By each turn, the downstream processes normally combine two steps. A first step of extraction is where the main components of the biomass are released and, depending on the application or purity level required by the final end of the product, a second step of purification should be assessed; this includes fractionation of bioactive compounds present and concentrated in the extract. The most common schemes of cell disruption as well as extraction of intracellular biomolecules from biomass are based on mechanical or chemical treatments. These normally require long periods of time, specific equipment, and/or higher economic investment, making the scale-up process a much more difficult task. In addition, most of the conventional techniques are not specific for the target compound to extract, compromising the yield

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of extraction and the selectivity of the whole process (Passos et al., 2014; Simpson, 2000).

Despite the value of extracting some specific high-value compounds from seaweeds, one of the most promising ways to create a biomass industry is now based on the biorefinery concept. By applying this concept, the scientific community, supported mainly by emergent industries and sectors, intends to convert the biomass into fine chemicals and task-specific products with high commercial value and away from the nonrenewable and fossil-based economy path (Chew et al., 2017; Ruiz et al., 2013). The biorefinery strategy and Circular Economy philosophy, allied with the encouragement of international commissions (e.g., European Commission through Horizon 2020 and the recent Horizon Europe) to diversify and explore other raw materials and residues, have created an opportunity to develop a marine biorefinery platform. Indeed, following the concept of marine biorefinery, microalgae and seaweeds have been pointed out as some of the most promising matrices to be explored. Following the same approach of biorefinery, also in the exploration of seaweeds, the downstream processes comprise the same main steps, including (i) harvesting of (cultivated or native) biomass, (ii) cell disruption, (iii) recovery of the bioactive compounds, (iv) separation of the different molecules, depending on the criteria of the application, and, if needed, (v) isolation (also defined as polishing) of the target biomolecules from the main solvents applied (Fig. 1). Up to now, steps (iii), (iv), and (v) are the major bottlenecks to overcome. If some processes interfere negatively with the structure of biomolecules, others may require harmful solvents, complex steps, and poorly selective processes. The scientific community is searching for downstream schemes capable of maintaining both the chemical structure and the biological activity of the target biomolecules, while keeping high yields of extraction and low costs and environmental effects. It is this context that prompts some authors to look for implementation of new processes by applying alternative solvents in the different steps of the downstream processes (Markou and Nerantzis, 2013).

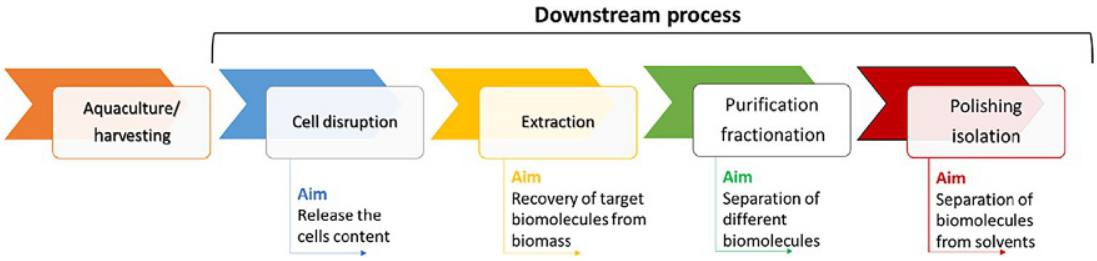

Fig. 1 Schematic representation of the main steps included in a downstream process.

2 Ionic liquids: Alternative solvents in extraction techniques

The application of ionic liquids (ILs) as alternative solvents to extract high value compounds from biomass is not new (Passos et al., 2014), as alkaloids (Cláudio et al., 2013), flavonoids (Chowdhury et al., 2010), terpenoids (Bica et al., 2011), proteins (Martins et al., 2016), and lipids (Kim et al., 2012) are some examples. In many cases, the use of ILs as solvents can greatly enhance the commercial potential of biomass by their application on the extraction and purification of different bioactive compounds with high industrial and commercial interest, under the biorefinery concept and as already done for microalgae (Orr and Rehmann, 2016; Ruiz et al., 2018).

ILs are no more than salts with low-charge density and low symmetry among their ions. These features decrease their melting points compared with common salts, as these are, in most of the cases, liquid at room temperature. Also ILs are typically composed of large organic cations and organic or inorganic anions (see Table 1), and due to the very high number of combinations of ions that can be formed, their properties can be tunable to a specific application, which is recurrently described by the designation of "designer solvents" (Kunz and Häckl, 2016).

ILs are also recognized by their unique properties, namely their negligible vapor pressure, low flammability, high thermal and chemical stabilities, broad liquid temperature range, high ionic conductivity, and high solvation ability for organic, inorganic, and organometallic compounds (Kunz and Häckl, 2016). Taking into account their unique characteristics, their role on the extraction processes is quite crucial because different interactions can occur among the solvent and the compound to extract (Passos et al., 2014) that may improve their selectivity to different molecules according to the IL chosen. Moreover, the use of aqueous solutions of ILs instead of pure ILs have proven that, for some biomolecules, higher yields of extraction are achieved due to the higher solubility of the compounds in the solvent (hydrotropic nature of ILs in water) (Cláudio et al., 2015; Sintra et al., 2018). Nevertheless, the use of ILs as extractive solvents can also lead to lysis of the cell wall, a phenomenon that also helps to improve the biomolecules extraction (Martins et al., 2018). It is noteworthy that some ILs are able to self-buffer the aqueous medium, meaning that they have a buffer-like behavior in the pH control (Taha et al., 2014, 2015). This special class of compounds is called Good's buffer ILs. These are usually synthesized with the combination of the conventional ILs cations and commercial Good's

Table 1 Name and respective acronym of the cations and anions of ILs addressed in this book section.

Cations		Anions	
Name	**Acronym**	**Name**	**Acronym**
1-Butyl-1-methylpiperidinium	$[C_4C_1pip]^+$	Acetate	$[C_1CO_2]^-$
1-Butyl-1-methylpyrrolidinium	$[C_4C_1pyr]^+$	Bis(trifluoromethylsulfonyl)imide	$[NTf_2]^-$
1-Butyl-3-methylimidazolium	$[C_4C_1im]^+$	Bromide	Br^-
1-Butyl-3-methylpyridinium	$[C_4C_1py]^+$	Chloride	Cl^-
1-Decyl-3-methylimidazolium	$[C_{10}C_1im]^+$	Dibutyl phosphate	$[(C_4)_2PO_4]^-$
1-Dodecyl-3-methylimidazolium	$[C_{12}C_1im]^+$	Dicyanamide	$[N(CN)_2]^-$
1-Ethyl-1-methylpiperidinium	$[C_2C_1pip]^+$	Diethyl phosphate	$[(C_2)_2PO_4]^-$
1-Ethyl-3-methylimidazolium	$[C_2C_1im]^+$	Dimethyl phosphate	$[(C_1)_2PO_4]^-$
1-Ethyl-3-methylmorpholinium	$[C_2C_1mo]^+$	Hexafluoro phosphate	$[PF_6]^-$
1-Hexyl-3-methylimidazolium	$[C_6C_1im]^+$	Hydrogen sulfate	$[HSO_4]^-$
1-Methyl-3-octylimidazolium	$[C_8C_1im]^+$	L-(+)-lactate	$[L\text{-}L]^-$
Cholinium	$[N_{1,1,1,2OH}]^+$	Methanosulfonate	$[CH_3SO_3]^-$
Ethylpyridinium	$[C_2py]^+$	Methylsulfate	$[MSO_4]^-$
Hexadecylpyridinium	$[C_{16}py]^+$	Nitrate	$[NO_3]^-$
Hexyltrimethylammonium	$[N_{1,1,1,16}]^+$	Tetrafluoroborate	$[BF_4]^-$
Myristyltrimethylammonium	$[N_{1,1,1,14}]^+$	Thiocyanate	$[SCN]^-$
Tetrabutylammonium	$[N_{4,4,4,4}]^+$	Tosylate	$[TOS]^-$
Tributyl(methyl)phosphonium	$[P_{4,4,4,1}]^+$	Trifluoroacetate	$[CF_3CO_2]^-$
Trihexyltetradecylphosphonium	$[P_{4,4,4,4}]^+$	Trifluoromethanesulfonate	$[CF_3SO_3]^-$
Trihexyltetradecylphosphonium	$[P_{6,6,6,14}]^+$		

buffers (Gupta et al., 2015). They are especially useful to maintain the pH in the range of 6–11, which is an interesting property when the process of extraction focuses on the proteins (Gupta et al., 2015). Fig. 2 shows the summary of the main mechanisms acting in the extraction of biomolecules from fresh biomass when ILs are used as alternative solvents.

Although some ILs can be applied as pure compounds/solvents, their aqueous solutions seem to be a more reasonable and efficient choice, as they increase performance, simultaneously decreasing viscosity, and the environmental and economic effects of the integrated downstream processes (Passos et al., 2014). In spite of the advantages described, some ILs are still the focus of controversy mainly due to their cost and sustainability (Kunz and Häckl, 2016). There are some approaches to counter these disadvantages, namely the preference for aqueous solutions and more benign ILs, allied with the possible recovery of ILs for their reuse in new cycles of extraction. Aqueous solutions of ILs have water as their main solvent, the most biocompatible, greenest, and cheapest solvent. Then, more benign and cheaper ILs are being developed such as carboxylate-, amino-acid-, carbohydrate-, and cholinium-based ILs, which can be used as more sustainable alternatives (Passos et al., 2014). Lastly, although there is still a big lacuna, many efforts have been done to recover the target molecules from the IL, thus allowing its reuse in new cycles of extraction. Depending on the IL used or depending on the compound extracted, different techniques can be applied, for instance, hydrodistillation, back-extraction using organic solvents, precipitation with water, or using an anion-exchange resin, as reviewed by Passos et al. (2014).

In this section, three major steps will be addressed regarding the use of ILs in seaweed downstream processes: extraction, hydrolysis/biomass dissolution, and purification. In Fig. 3, details of the published works using ILs

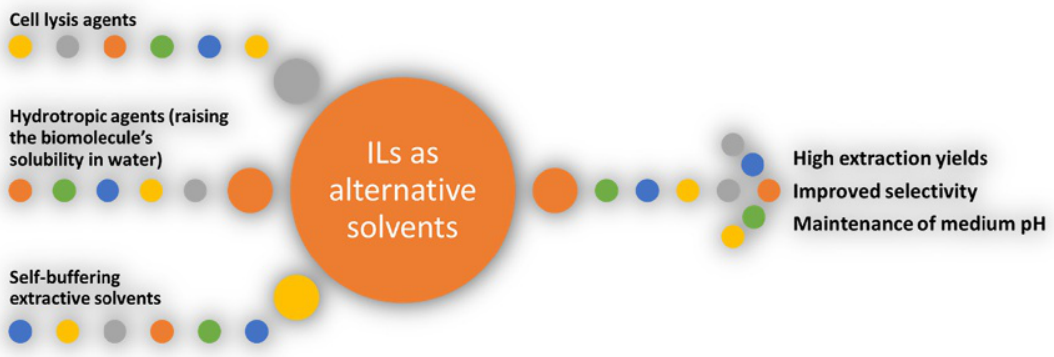

Fig. 2 Summary of the main mechanisms acting in the extraction of biomolecules from biomass by applying ILs.

in each process of downstream are presented, where the compounds targeted in each work are also highlighted.

Most published works regard the extraction and hydrolysis/biomass dissolution. Although different genera of red, brown, and green seaweeds were used, there seems to be a higher interest in the brown species. Regarding the target compounds, there is a large spectrum of different biomolecules recovered from seaweeds. However, there is a lack of studies reporting the extraction of lipids and fats, which seems to be a drawback to overcome in the coming years. Lastly, it is interesting to observe that, along with the use of ILs, different techniques were applied looking for synergistic effects. Details about each work will be further presented in next sections of this work.

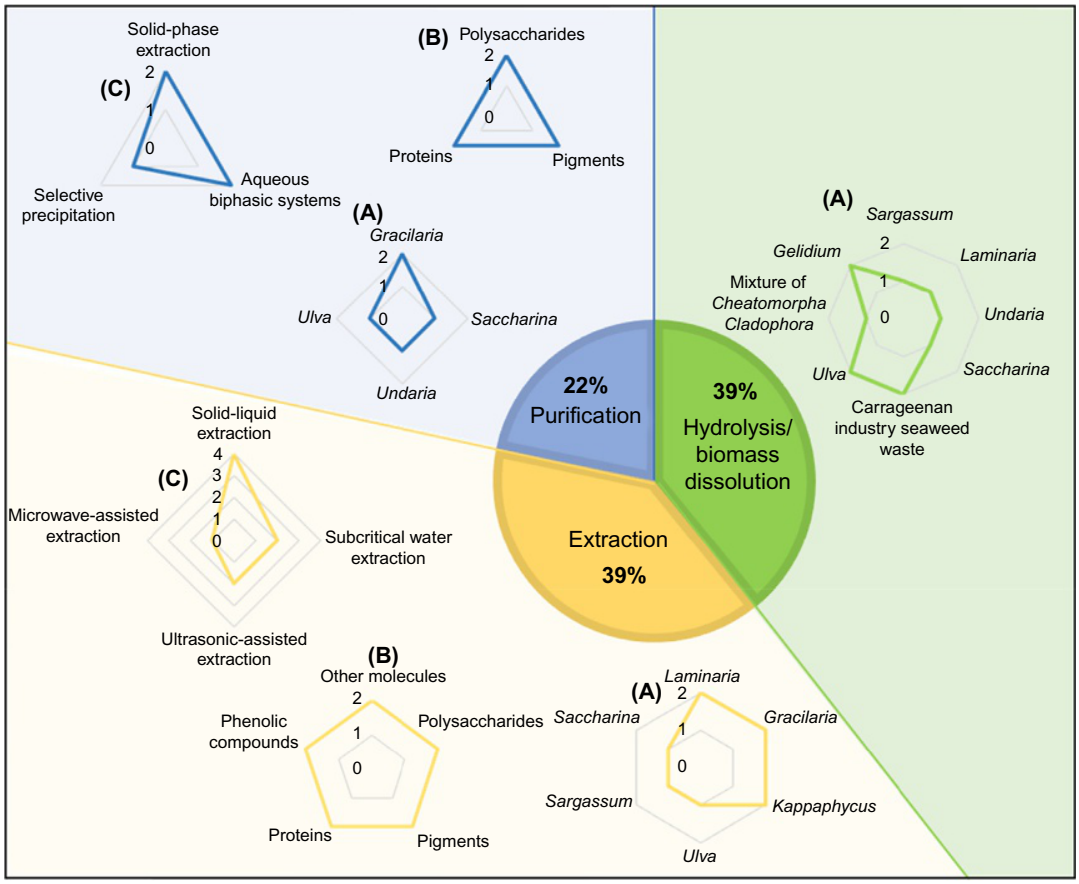

Fig. 3 Distribution of the published works dealing with ILs in downstream processes regarding seaweeds. The radial graphs display the number of publications for each downstream step, addressing the (A) genera of seaweed, (B) target compound, and (C) and technique applied. *ISI Web of Knowledge (Accessed June 2019).*

3 Extraction of biomolecules from seaweed

There is a large range of parameters that need to be considered during the extraction step. The pretreatment applied to the biomass and the use (or not) of mechanical approaches, such as microwave- and ultrasound-assisted extractions, are some examples. Other parameters regarding extraction itself cannot be neglected, namely the time of extraction, pH, temperature, solid-liquid ratio (SLR), agitation, and the solvent and its concentration (Esquivel-Hernández et al., 2017). When it comes to the use of ILs as solvents in extraction procedures, all the referred parameters are still valid. However, it is important to understand the IL role in the extraction, i.e., either in the cell lysis, in the target compound solubilization, or even as a self-buffering agent (Fig. 2). At this point, the combination of the IL cation, anion, and alkyl side chain length is of utmost importance, because it determines their interaction (or not) with the biomass and/or the target compound. Also, the use of the IL either pure or in aqueous or organic solutions (and its concentration) should also be considered (Passos et al., 2014). Up to now, several approaches were used to extract various biomolecules from different seaweed species using ILs, as detailed in Table 2. Despite the low number of works, the data from Table 2 suggests that the pigments (the most expensive compounds when in a pure state) (Martins et al., 2016; Vieira et al., 2017), carbohydrates (widely applied in food and feed) (Gereniu et al., 2018; Trivedi and Kumar, 2014), and phenolic compounds (Han et al., 2011; Vo Dinh et al., 2018) are the principal types of biomolecules studied up to now.

In 2011, for the first time, ILs were used as extracting solvents for biomolecules from seaweed. Han et al. (2011) performed the extraction of phenolic compounds, important antioxidant molecules, using an ultrasonic-assisted extraction based on aqueous solutions of imidazolium ILs from *Laminaria japonica* Aresch. The extraction was successfully performed using 1-butyl-3-methylimidazolium tetrafluoroborate ($[C_4C_1im][BF_4]$) with recovery values up to 88.3%, and these were reported as higher than what was obtained using water or methanol. The same IL was used some years later for the same propose but using a subcritical water extraction for *Saccharina japonica* (Vo Dinh et al., 2018). Comparing the effect of water as solvent in a conventional solid-liquid extraction or in a subcritical water extraction, the yield of extraction and antioxidant activity were enhanced when the IL-assisted subcritical water extraction was performed, perhaps due to a

Table 2 Summary of the literature works sorted by year of publication and dealing with the extraction of the different compounds from seaweeds based on ILs.

Seaweed specie (pretreatment)	Target compound(s)	Screening of ILs (IL concentration)	Extraction techniques/ parameters	Comparison with other solvents/ techniques	Best operational conditions	Best result/yield	Ref.
Laminaria japonica Aresch (dried and grinded)	Phenolic compounds	$[C_2C_1im][BF_4]$, $[C_4C_1im][BF_4]$, $[C_4C_1im]Cl$ (0.5 M in water)	Ultrasonic-assisted (50–200 W, 20 kHz), 15–120 min, SLR 1:15	Conventional: same procedure using water and methanol as solvents	Ultrasonic assisted with $[C_4C_1im][BF_4]$, 200 W, 60 min, pH 1.25	Recovery values up to 88.3%	Han et al. (2011)
Gracilaria dura (dried and grinded)	Agarose	$[C_2C_1im][C_1CO_2]$, $[N_{1,1,1,2OH}][C_1CO_2]$, $[C_2C_1im][(C_2)_2PO_4]$ (100%)	Microwave assisted for 2 min + agitation at 80–100°C for 2 h, SLR 1:20	Agitation at 80–100°C for 2 h	Microwave assisted with $[C_2C_1im][C_1CO_2]$; 80°C	39% of the total agarose content	Trivedi and Kumar (2014)
Kappaphycus alvarezii (fresh and grinded)	Plant growth regulators (trans-zeatin and indole-3-acetic acid)	$[C_4C_1im][PF_6]$, $[C_8C_1im][BF_4]$, $[C_4C_1im][NTf_2]$ (100%)	Agitation (450 rpm) at 25–50°C, 5–120 min, SLR 4:1	No data	IL-based extraction using $[C_4C_1im][PF_6]$; 50°C	65% of the total trans-zeatin and 18% of the total indole-3-acetic acid	Das and Prasad (2015)
Gracilaria sp. (fresh and grinded)	Phycobiliproteins	$[C_2C_1im]Cl$, $[C_4C_1im]Cl$, $[C_6C_1im]Cl$, $[C_{10}C_1im]Cl$, $[C_{12}C_1im]Cl$, $[C_2C_1im][C_1CO_2]$, $[C_4C_1im][N(CN)_2]$, $[C_4C_1im][CF_3SO_3]$, $[C_4C_1im][C_1CO_2]$, $[C_4C_1im][(C_1)_2PO_4]$ $[C_4C_1im][TOS]$, $[C_4C_1im][CH_3SO_3]$, $[C_4C_1im][SCN]$, $[C_4C_1im][CF_3CO_2]$, $[C_2C_1im][C_1CO_2]$,	Agitation (250 rpm) at room temperature, 5–120 min, SLR 0.7	Conventional: same procedure using sodium phosphate buffer (20 mM, pH 7) as solvent	IL-based extraction using $[N_{1,1,1,2OH}]Cl$ at 1 M in McIlvaine buffer (pH 5.9); 20 min	46.5% higher than conventional	Martins et al. (2016)

		$[C_4C_1pip]Cl$, $[C_4C_1pyr][C_1CO_2]$, $[C_4C_1pyr]Cl$, $[C_4C_1py]Cl$, $[N_{1,1,1,2OH}][C_1CO_2]$, $[N_{4,4,4,4}]Cl$, $[N_{1,1,1,2OH}]Cl$, $[P_{4,4,4,4}]Cl$ (1 M in buffer)					
Ulva lactuca (dried and grinded)	Proteins	$[N_{1,1,1,2OH}][C_1CO_2]$, $[C_4C_1im][C_1CO_2]$, $[C_2C_1im][(C_4)_2PO_4]$, $[C_4C_1im][(C_4)_2PO_4]$, $[C_4C_1im]Cl$, $[N_{1,1,1,2OH}]Cl$, $[P_{6,6,6,14}]Cl$, $[P_{4,4,4,1}][MSO_4]$, $[C_4C_1im][N(CN)_2]$, $[P_{6,6,6,14}][N(CN)_2]$ (40% (w/w))	Tissue homogenizer and bead beaten for 3 cycles of 60 s at 6500 rpm, with 120 s break between cycles	Sequential aqueous and alkaline extractions; mechanical grinding under alkaline conditions; aqueous biphasic system w/ polyethylene glycol 1000/ Na_2CO_3	IL-based extraction using $[C_2C_1im][(C_4)_2PO_4]$	Recovery of 80.62% of the total protein	Miranda et al. (2017)
Sargassum muticum (fresh and dried, both grinded)	Carotenoids	$[N_{1,1,1,14}]Br$, $[N_{1,1,1,16}]Br$, $[C_{16}py]Cl$, $[C_{16}py]Br$, AOT[a], SDBS[a], SDS[a] (1–30 × CMC in water)	Agitation (250 rpm) at room temperature, 73–107 min, SLR 0.04	Conventional: successive extractions with ethanol-based solutions	Fresh algae; SDS[a] at 15 × CMC; 90 min	37.4% higher than conventional	Vieira et al. (2018a)
Kappaphycus alvarezii (dried and grinded)	κ-Carrageenan	$[C_4C_1im][C_1CO_2]$, $[C_4C_1im][(C_4)_2PO_4]$, $[C_4C_1im][BF_4]$, $[C_2C_1im]Br$, $[C_2C_1im][BF_4]$, $[C_4C_1im]Cl$, $[N_{1,1,1,2OH}]Cl$ (0.1%–2% IL in water)	Subcritical water extraction (5 MPa) at 60–180°C, 5 min, SLR 1:80	Conventional: soaked w/calcium hydroxide solution (0.25%) + 1.5 h at 107°C	Subcritical water extraction with $[C_4C_1im][C_1CO_2]$, at 1%; 150°C	1.42-fold higher than the conventional	Gereniu et al. (2018)
				Subcritical water extraction with water; 150°C		1.11-fold higher than subcritical water extraction with water	

Continued

Table 2 Summary of the literature works sorted by year of publication and dealing with the extraction of the different compounds from seaweeds based on ILs—cont'd

Seaweed specie (pretreatment)	Target compound(s)	Screening of ILs (IL concentration)	Extraction techniques/ parameters	Comparison with other solvents/ techniques	Best operational conditions	Best result/yield	Ref.
Saccharina japonica (dried and grinded)	Phenolics compounds (gallic acid, chlorogenic acid, gentisic acid, protocatechuic acid, *p*-hydroxybenzoic acid, vanillic acid, caffeic acid, and syringic acid)	$[C_4C_1im][BF_4]$ (0.25–1.00 M in water)	Subcritical water extraction (50 bar) at 100–250°C, SLR 1:32	Conventional: agitation (500 rpm) using organic solvents and water; 24 h; room temperature Subcritical water extraction (50 bar) using water at 100–250°C	Subcritical water extraction with $[C_4C_1im][BF_4]$ at 0.5 M; 175°C	Gallic, chlorogenic, protocatechuic, *p*-hydroxybenzoic, and caffeic acids were 7.33-, 154.9-, 572.8-, 54.8-, and 91.8-fold higher than conventional (water) Gallic, chlorogenic, gentisic, protocatechuic, caffeic, and syringic acids were 1.18-, 4.68-, 4.66-, 7.67-, 5.12-, and 5.08-fold higher than subcritical extraction with water	Vo Dinh et al. (2018)

Laminaria sp. (dried and grinded)	Inorganic and organic iodine compounds (I^-, monoiodo-tyrosine and diiodo-tyrosine)	$[C_2C_1im][L\text{-}L]$, $[C_2C_1im]Br$, $[C_4C_1im][CH_3SO_3]$, $[C_4C_1im][BF_4]$, $[C_6C_1im]Br$, $[C_2C_1mo]Br$, $[C_2py]Br$, $[C_2C_1pip]Br$, $[C_4C_1pyr]Br$, $[C_{12}C_1im][NO_3]$, $[C_{12}C_1im][HSO_4]$, $[C_{12}C_1im]Br$, $[C_{12}C_1im]Cl$ (100–300 mM in water)	Ultrasonic assisted (100 W, 40 kHz) at 40°C, 15–75 min, SLR 1:10	No data	Ultrasonic assisted with $[C_2Py]Br$ at 200 mM (pH 6.5); 30 min	Recovery values of I^-, monoiodo-tyrosine and diiodo-tyrosine among 88% and 94%	Peng et al. (2018)

[a]Anionic surfactants.

AOT, dioctyl sulfosuccinate sodium salt; *CMC*, critical micelle concentration; *SDBS*, sodium dodecyl-benzenesulfonate; *SDS*, sodium dodecylsulfate.

synergistic effect between the process conditions of temperature, pressure, and the solvent itself (Vo Dinh et al., 2018).

Specific polysaccharides were also targeted as interesting biomolecules to extract, mainly from red seaweed, recognized as a rich source of polysaccharides. Agarose was first extracted by dissolution of the biomass with 1-ethyl-3-methylimidazolium acetate ($[C_2C_1im][C_1CO_2]$) in a microwave-assisted extraction, followed by precipitation of the highly pure agarose using methanol (Trivedi and Kumar, 2014). A similar IL, 1-butyl-3-methylimidazolium acetate ($[C_4C_1im][C_1CO_2]$), was also found among others as the most efficient in the extraction of κ-carrageenan using a subcritical water extraction after the biomass dissolution. Once again, the extraction yields were enhanced when compared with conventional techniques and solvents, with remarkably high purity of the polysaccharide (Gereniu et al., 2018).

The application of ILs in the extraction of biomolecules from seaweeds was also extended to the extraction of plant growth regulators (trans-zeatin and indole-3-acetic acid) in 2015 (Das and Prasad, 2015). Among the three imidazolium-based ILs studied, mostly hydrophobic, the importance of both the cation and anion selected in the extraction was clear. Actually, and despite the inefficient extractive behavior of 1-butyl-3-methylimidazolium bis(trifluoromethylsulfonyl)imide ($[C_4C_1im][NTf_2]$), when this was replaced by 1-butyl-3-methylimidazolium hexafluorophosphate ($[C_4C_1im][PF_6]$), higher yields were achieved with 65% of the total trans-zeatin and 18% of the total indole-3-acetic acid being recovered. In this specific work, as well reported and previously discussed for the agarose extraction (Trivedi and Kumar, 2014), pure ILs were used, which in some cases seems to be a limiting factor for the processes performance. Indeed, the range of ILs available to be applied in a pure state is limited, as some are solid or present high viscosities, which is an obstacle to the mass transfer, increasing the costs and environmental effects.

Our group is being very active on the use of ILs, but mainly IL-based aqueous solutions as tunable solvents. Due to all the expertise created in the last 15 years on applying ILs as alternative ecosolvents, we have developed a deep interest for the development of appropriate and sustainable technologies to contribute toward a more efficient marine biorefinery. In 2016, Martins et al. (2016) reported on the selectivity of aqueous solutions of ILs and proved their ability to extract different pigments, namely phycobiliproteins and chlorophylls, from the same biomass, which was performed just by tuning the IL chemical structure regarding its alky side chain length (Martins et al., 2016). Phycobiliproteins are fluorescent proteins produced

by red seaweeds and some cyanobacteria and microalgae to facilitate the capture of light used for photosynthesis. In this work (Martins et al., 2016), the authors proved the potential of manipulating the aqueous solutions properties to simultaneously extract hydrophobic and hydrophilic biomolecules by simply tuning the ILs properties. This was the first insight on the design of a water-based process to selectively extract hydrophilic and hydrophobic biomolecules from seaweeds without compromising their structural integrity, under the scope of a multiproduct scenario (Martins et al., 2016). Also, the use of more biocompatible (cholinium-based) families of ILs as solvents to extract phycobiliproteins from the red seaweed *Gracilaria* sp. was tested. It enhanced the extraction of phycobiliproteins by more than 46.5%, in comparison with the conventional methodology tested.

More recently, the recovery of carotenoids and particularly fucoxanthin was evaluated by applying aqueous solutions of ILs. Fucoxanthin is a hydrophobic pigment, which is present in high quantities in brown macroalgae (Vieira et al., 2018a) and mainly in *Sargassum* species. In this work, aqueous solutions of common surfactants, ammonium and pyridinium based-tensioactive ILs were tested and compared with ethanol as the conventional solvent. Although four ILs were tested, the aqueous solutions of sodium dodecylsulfate (SDS), a common surfactant, achieved the best results, not only when compared with the ILs but also with ethanol. This problem can probably be overcome by the choice of other ILs or by the addition of a mechanical force that helps the solvent reach those molecules in the cells.

In the search for new sources of protein, Miranda et al. (2017) developed a strategy to recover the total proteinic content of green seaweed *Ulva lactuca* found to be at circa of 18% (w/w) in a dry basis. A set of imidazolium-, phosphonium-, and ammonium-based ILs were used at 40% (w/w) in a solid-liquid extraction. Moreover, sequential aqueous and alkaline extractions, mechanical grinding under alkaline conditions, and aqueous biphasic systems were also performed to extract the total protein content from this seaweed. In the end, the extraction yields obtained by means of sequential aqueous and alkaline solutions, mechanical grinding under alkaline conditions, and aqueous biphasic system (polyethylene glycol 1000 and sodium carbonate; PEG1000/Na_2CO_3) led to extraction efficiencies of 49.1%, 6.68%, and 10.5%, respectively, whereas for the 1-ethyl-3-methylimidazolium dibutyl phosphate ([C_2C_1im][$(C_4)_2PO_4$]), a recovery of 80.6% of the total proteins was assessed (Miranda et al., 2017).

Lastly, the extraction of inorganic and organic iodine compounds (I-, monoiodo-tyrosine and diiodo-tyrosine) from the genus *Laminaria* was reported. These types of molecules play an essential role as a micronutrient and are crucial in the healthy development of animals and humans. Up to now, iodine compounds were commonly extracted using strong alkaline and toxic solvents for long times of extraction. During the search for alternative processes and solvents, a wide number of ILs were screened as well as ultrasonic-assisted extraction. As reported, the ethylpyridinium bromide ($[C_2Py]Br$) at 200 mM (pH 6.5) achieved recovery values around 88% and 94% in only 30 min, results that proved to be reproducible when applied in different commercial samples of *Laminaria* sp. (Peng et al., 2018).

Although there are plenty of different families of ILs identified in the diverse studies analyzed here, almost all extraction studies focused on imidazolium-based systems combined with Cl^-, Br^-, and $C_1CO_2^-$ anions (Fig. 4) that, for sure, are not representative of the full potential of ILs, although very interesting results were shown regarding the yields of

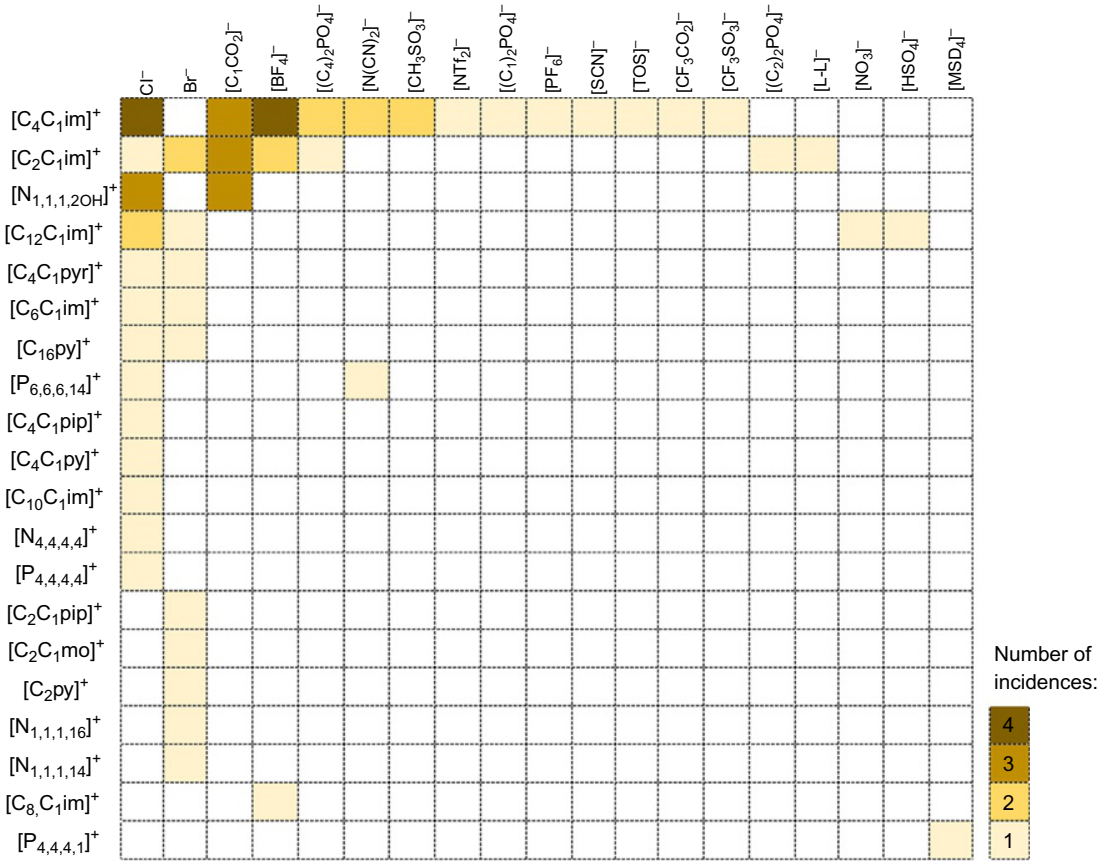

Fig. 4 Incidence of combinations of anions-cations used in the extraction of biomolecules from seaweeds.

extraction using ILs in comparison with the conventional methodologies displayed (Gereniu et al., 2018; Martins et al., 2016; Vo Dinh et al., 2018).

Some of the reviewed studies also report the use of ILs combined with mechanical extraction techniques such as microwaves (Trivedi and Kumar, 2014), ultrasounds (Peng et al., 2018), and subcritical water extraction (Gereniu et al., 2018; Vo Dinh et al., 2018). There seems to be a synergistic effect, achieved when the mechanical techniques and ILs as solvents are combined. Some of the IL-based extraction schemes reported lead to better results of extraction, also enhancing the antioxidant activity of phenolic compounds (Vo Dinh et al., 2018) than either conventional solid-liquid extraction using conventional solvents or ILs or the respective techniques using water as a solvent can achieve (Gereniu et al., 2018; Vo Dinh et al., 2018).

With the higher demand for more sustainable processes, the use of ILs as solvents was not an exception. From the last publications analyzed, a huge decrease of the amount of IL used is clear; the pure IL is replaced by their respective aqueous solutions (Gereniu et al., 2018; Martins et al., 2016; Peng et al., 2018; Vieira et al., 2018a; Vo Dinh et al., 2018). In this case, and in close agreement with the literature, the use of aqueous solutions of ILs (Passos et al., 2014) had higher yields of extraction reported, but also systems with lower viscosity, economical, and environmental effects, were developed (Cláudio et al., 2015). Notwithstanding the concern to reduce the amounts of IL in the extractions, there is still a big gap regarding the recycling of IL and its reuse in subsequent cycles of extraction. From the publications presented in Table 2, there is a single study reporting the recycle of IL in which four cycles of IL reuse were successfully developed without compromising the yield of extraction of agarose, the targeted compound (Trivedi and Kumar, 2014).

Besides the use of ILs in their pure state or in aqueous solution, ILs and IL-derivatives can be utilized as part of eutectic mixtures. These mixtures have emerged as another class of alternative solvents in the extraction of biomolecules. These are binary or ternary mixtures that usually result from the formation of strong hydrogen bonds between two (or more) solid materials (an acceptor and a donor) from renewable resources (Abbott et al., 2004). They are characterized by having melting points lower than those of the starting compounds, often becoming liquid at conditions close to room temperature (Vieira et al., 2018b). Like ILs, these mixtures are recognized by their high solvation ability for a wide variety of compounds (Passos et al., 2016), because these can be structurally manipulated to acquire the properties of both starting materials (Abbott et al., 2003). Up to now, many

compounds were already successfully extracted from different biomass matrices, namely lignocellulosic-based compounds, phenolics, and proteins, among others (de Faria et al., 2017; Morais et al., 2018; Rommi et al., 2017; Soares et al., 2017; Vieira et al., 2018b; Yoo et al., 2018). However, the number of works dealing with seaweeds and eutectic mixtures as solvents is much scarcer. In 2014, eutectic mixtures of cholinium chloride-oxalic acid (1:2) were successfully used to extract metals (Ghanemi et al., 2014) from the macroalgae *Enteromorpha intestinalis*. In this work, a microwave-assisted digestion in eutectic mixtures was performed and compared with conventional acid digestion. The results obtained for the studied metals/analytes are extremely similar for both alternative and conventional digestions. However, the extraction time was, at least, 100 times lower when compared to that consumed by the conventional methods, thereby, leading to a reduction in energy consumption. The same eutectic solvent was also reported for biomass dissolution to extract polycyclic aromatic hydrocarbons also from *E. intestinalis* (Helalat-Nezhad et al., 2015). The individual recovery of polycyclic aromatic hydrocarbons rounded 100% using lower temperatures and simpler steps in comparison with conventional methodologies (Helalat-Nezhad et al., 2015).

4 Hydrolyses of carbohydrates and biomass dissolution using ILs

Algae are known to grow at high rates compared to terrestrial plants that contain comparable amounts of carbohydrates by weight (Pezoa-Conte et al., 2015). Besides many other applications, sugars and other seaweed components can be converted into chemicals and biofuels that can be very helpful for supplying energy, increasing the product's value and reducing pollution risks under a biorefinery concept (Uju et al., 2015). Conventionally, this type of process of biomass dissolution or carbohydrate hydrolysis is usually promoted by very strong and harmful acids, which mandatorily require qualified human resources and much care when applied (Malihan et al., 2012; Park and Jeong, 2013).

The use of ILs as biomass dissolution agents was already proven to be efficient. In 2015, Pezoa-Conte and collaborators used ILs to dissolve polysaccharides and carbohydrates, such as cellulose, and simultaneously disrupt the complex linkages of pristine biomass (Pezoa-Conte et al., 2015). As previously recognized, ILs can play different roles in the field. These can act as effective solvents for carbohydrates by dissolving them in IL media or by

promoting acid-catalyzed hydrolysis of a biomass, or a combination of both strategies. Indeed, this last approach was exploited for several reactions where traditional mineral acids like sulfuric and hydrochloric acids (Malihan et al., 2017) were replaced by acidic ILs. Again, the imidazolium-based ILs were the most studied for hydrolysis and seaweed dissolution procedures, an approach started in 2012 (Al-Zuhair et al., 2015; Jmel et al., 2018; Malihan et al., 2012, 2014, 2017; Park and Jeong, 2013; Uju et al., 2015, 2018). Later, pyridinium-based ILs were tested (Park and Jeong, 2013; Uju et al., 2015, 2018) and successfully found as an advantageous alternative to the imidazolium family with higher conversion rates in all reported studies. In this specific alternative procedure of hydrolysis and dissolution applying acidic ILs (Malihan et al., 2014, 2017; Park and Jeong, 2013), different conditions were tested, not only regarding changes on the anion but, for example, by adding small amounts of specific enzymes (Al-Zuhair et al., 2015) or acids to reduce the operational time and enhance the conversion yield (Malihan et al., 2012; Uju et al., 2015, 2018). Malihan et al. (2014) found that, although 1-*n*-butyl-3-methylimidazolium hydrogen sulfate ($[C_4C_1im][HSO_4]$) was found to be the most appropriate to provide the highest sugar yields, a system composed of two different ILs (by the addition of 1-*n*-butyl-3-methylimidazolium chloride ($[C_4C_1im]Cl$)) was more efficient in seaweed dissolution than the most efficient pure IL. Actually, many approaches were already described, and when comparisons with conventional acidic approaches were shown, ILs proved to be a most performant and profitable alternative (Malihan et al., 2012).

It is important to note that, in some studies, the biomass used was a mixture of different seaweed genera (Al-Zuhair et al., 2015), or even more interesting, seaweed-waste biomass from the carrageenan industry (Uju et al., 2015, 2018), proving the viability of the biorefinery concept. Taking into account the use of ILs in procedures of hydrolysis and dissolution, only one work has demonstrated the IL recovery through its precipitation, achieving a maximum of 92 wt% (Pezoa-Conte et al., 2015).

5 Purification approaches

Apart from the use of ILs as solvents from the solid matrix, these can also be used in the development or for improvement of established methods of purification from the raw extracts obtained after the first step of extraction. Once again, the interactions among the ILs and compounds to purify are crucial for the success of the purification process. The large amount of ILs available

allows us to find the perfect interaction with a wide variety of molecules. Indeed, high quality compounds were obtained using these alternative and more efficient methods compared with the conventional ones (Sharma et al., 2015). Sharma et al. (2015) reported agarose isolation from *Gracilaria dura* by precipitation under ambient conditions using cholinium laurate (Fig. 5A). The authors have proposed interactions among the IL anions and moieties of Na^+ and K^+ from extract impurities as the main driven force, justifying the results of purification. At the same time, agarose attached to the cholinium cations, and this bulky structure is precipitated. In the end, a washing step allowed the recovery of pure agarose, as the IL is recycled and reused without compromising the quality and yield of agarose.

Back in 2011, a chromatographic strategy using imidazolium-based modified silica for solid-phase extraction of astaxanthin from *S. japonica* extracts was reported (Fig. 5B) (Zhou et al., 2011). A complex of 1-ethyl-3-methylimidazolium ($[C_2C_1im]$)-silica was found to be an efficient stationary phase for the separation of astaxanthin from the extract solution leading to a purer fraction of astaxanthin according to the high performance liquid chromatography (HPLC) chromatogram (purity values are not available) (Zhou et al., 2011). A similar strategy was used some years later in which an IL-modified silica was used as a stationary phase to separate polysaccharides, namely fucoidan, alginic acid, and laminarin extracted from *Undaria pinnatifida* (Lee et al., 2018). The IL-modified silica exhibited very good size exclusion properties, especially in laminarin separation when the 1-butyl-3-methyl-imidazolium bis-(trifluoromethylsulfonyl)-amino silica column was used (Lee et al., 2018).

Lastly, two more approaches were developed using ILs in purification methods (Fig. 5C). An integrated process of extraction and purification was proposed using the IL-based extract rich in the bioactive compounds in an aqueous three-phase partitioning system (ATPPS) formed by $[C_2C_1im][(C_4)_2PO_4] + K_2HPO_3$. ATPPS is a specific type of aqueous biphasic system (ABS). ABS forms two water-phases in equilibrium due to the combination and dissolution of pairs of solutes in an aqueous environment when at the appropriate concentrations (Freire et al., 2012). In general, these systems are applied in the purification of compounds when more biocompatible separation processes are demanded. ABS may be composed of polymers (e.g., polymer-polymer or polymer-salt), recognized for their low interfacial tension, good biocompatibility, fast and high phase separation rates, and low cost (Freire et al., 2012; Ventura et al., 2017). However, their performance may be significantly influenced by the range of polarities of the

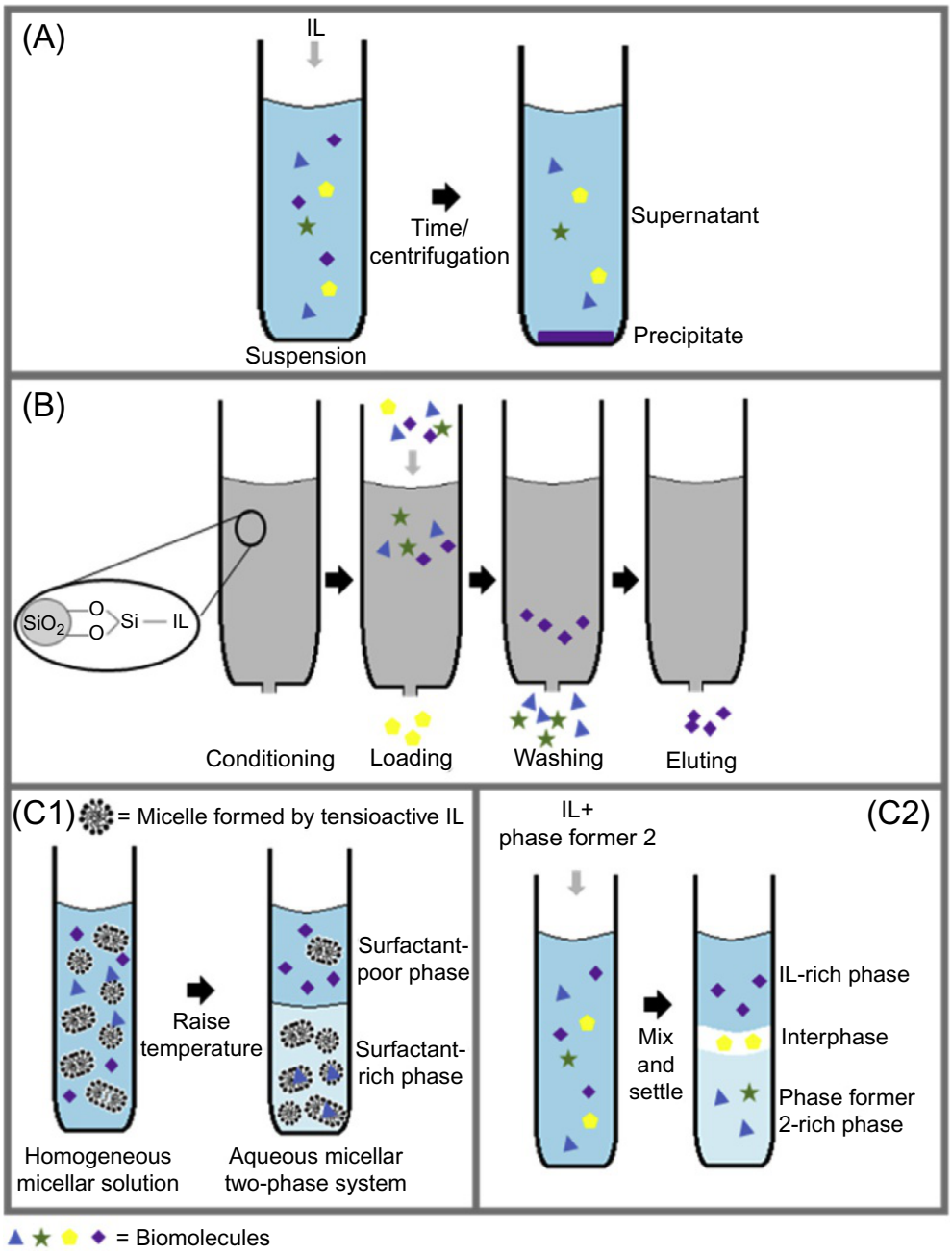

Fig. 5 Scheme of possible purification procedures of biomolecules extracted from seaweeds using ILs, namely by (A) selective precipitation, (B) solid-phase extraction using modified silica, and (C) aqueous biphasic systems; (C1) represents the aqueous micellar two-phase systems and (C2) the aqueous three-phase partitioning systems.

coexisting aqueous phases, thus imposing the appropriate optimization of the process conditions. ATPPS maintains the ABS advantages, resulting from the formation of a third phase (normally as a middle phase) where the compounds with low solubility are accumulated, normally in a purer state (Alvarez-Guerra and Irabien, 2014). This process allowed the protein-carbohydrate fractionation and concentration mainly in the top

(IL-rich phase) and interphase, respectively (Miranda et al., 2017). More recently, Vicente et al. (2019) proposed the use of aqueous micellar two-phase systems (AMTPS) using ILs as cosurfactants on the separation of phycobiliproteins. AMTPS are thermoresponsive systems in which molecules in water media can be fractionated by the formation of two macroscopic liquid phases above a certain surfactant concentration and temperature. ILs, and specifically, surface-active ILs, can be used as cosurfactants to enhance the extraction and selectivity, and also by reducing the use of other solvents (Vicente et al., 2017). In this particular work, the phycobiliproteins were extracted and purified from the red seaweed *Gracilaria* sp. (Vicente et al., 2019). In the first step of purification, it was proven the separation of phycobiliproteins from the extract impurities. Additionally, R-phycoerythrin, the most abundant phycobiliprotein in this red seaweed, was recovered in a second step of purification without compromising its structural integrity by applying ILs as cosurfactants. The proposed process also included the recycle and reuse of the surfactant through an ultrafiltration process, enhancing its profitability and decreasing its carbon footprint, as evaluated in the publication (Vicente et al., 2019).

6 Conclusions and critical analysis

In this work, it was shown how ILs can be used as alternative tools in the extraction and fractionation of compounds from red, brown, and green seaweeds. Several biomolecules, ranging from polysaccharides and phenolic compounds to proteins and pigments (among others), were proven to be successfully recovered. Moreover, although with scarcer results, ILs have also been proven to be a promising tool to develop new and efficient processes of hydrolysis of carbohydrates and seaweed dissolution. Besides the generally higher yields of extraction obtained in comparison with conventional solvents and common techniques, improved selectivity and biological activities were shown for the alternative methods based in ILs. In some particular cases, even more interesting results were obtained when ILs were conjugated with extractions assisted by ultrasounds, microwaves, and subcritical water. Some purification approaches have been developed as well, considering the need for purer extracts and products. Up to now, this approach is not significantly developed. Included in these approaches are some chromatographic and selective precipitation strategies, and more recently, the use of aqueous micellar two-phase systems and three-phase partitioning systems. Despite the high potential reported by the different

Fig. 6 SWOT analysis of seaweeds processing using ILs.

research groups on the use of ILs as solvents in downstream processes to recover high added-value compounds from seaweeds, the development of new studies is crucial under marine biorefinery purposes, as detailed in the SWOT analysis presented in Fig. 6.

7 Future perspectives

Although there is a large number of known ILs, the majority of the presented studies have mainly considered imidazolium-based ILs, which can be a barrier to their full potential. In this sense, more benign and cheaper ILs should be studied as more sustainable alternatives, such as carboxylate-, amino-acid-, carbohydrate-, and cholinium-based ILs, as briefly mentioned in one or two of the publications reviewed here. In future works, tests on the recycling of the IL should also be a matter of interest to increase the viability of the processes, not only by the optimization of the proposed methodologies already proposed (Passos et al., 2014) but also by the development of new experimental and processing tools. In the same context of process optimization, the IL concentration should also be considered as an important condition to test, to reduce costs, viscosities, and the environmental effects, while keeping the same extraction and/or purification performance.

Despite all the efforts, there is still a big lacuna on the complete fractionation of algae components to take maximum advantage of the seaweed

biomass, from the high value compounds to the complete biomass utilization, under the marine biorefinery approach. Up to now, there is no single study comprising a complete cascade of processes in which either the entire cost of production or the so-called life cycle assessment were analyzed to prove their viability (or not) to inspire others on the development of new products and industries.

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