Ionic liquids (ILs) are salts with low melting points that can be used as solvents for mild extraction and selective fractionation of biomolecules (e.g., proteins, carbohydrates, lipids, and pigments), enabling the valorisation of microalgal biomass in a multiproduct biorefinery concept, while maintaining the biomolecules' structural integrity and activity. Aqueous biphasic systems and emulsions stabilised by core-shell particles have been used to fractionate disrupted microalgal biomass into hydrophobic (lipids and pigments) and hydrophilic (proteins and carbohydrates) components. From nondisrupted biomass, the hydrophobic components can be directly extracted using ILs from intact cells, while the most fragile hydrophilic components can be obtained upon further mechanical cell disruption. These multiproduct biorefinery concepts will be discussed in an outlook on future separations using IL-based systems.

Ionic Liquids as Solvents for the Separation of (Fragile) Biomolecules

Microalgae (see Glossary) are attractive organisms as an alternative feedstock for fossil fuel replacements; they have a high lipid productivity by using sunlight, CO₂, and sea or fresh water as growing conditions and do not compete for arable land [1,2], making them efficient organisms. However, it has recently become clear that the costs for biofuel production alone are economically unfeasible [2–4]; isolating other high-value components in microalgae (e.g., proteins, carbohydrates, and pigments) for the feed, chemical, cosmetic, and food industries [5–11] can create enough value to make the process economically feasible. The main challenge is to isolate these products from the lipids following sequential but sustainable strategies. Unfortunately, lipid extraction mostly occurs by solid–liquid or liquid–liquid extraction processes using volatile organic solvents [12–14], which is straightforward for a multitude of lipids but not always useful for other high-value products like proteins and carbohydrates. To obtain the remaining high-value compounds (proteins and carbohydrates) this strategy is not always feasible, due to the incompatibility between their chemical structures and properties and the conditions imposed by the use of organic solvents. Unlike hydrophobic pigments, the hydrophilic compounds will denature, precipitate, or degrade in some of the most used organic solvents under the harsh conditions they require, namely of temperature (e.g., > 40°C). Proteins are particularly fragile, having a compact 3D structure in which hydrogen bonding, ionic interactions combined with the secondary structure elements as β-sheets and α-helices define their native tertiary and/or quaternary structure in aqueous solutions. Organic solvents distort these protein structures irreversibly in a way that these high-value products can no longer be used as active ingredients [9,10]. In this context, more sustainable and mild (compatible with the target compounds in terms of media conditions) separation techniques are needed to recover the hydrophilic components (e.g., proteins and carbohydrates) before the hydrophobic components (e.g., lipids and pigments) out of the microalgal cells without compromising the chemical structure and main activities of the target compounds, thus allowing their application as refined products in different sectors of activity (e.g., cosmetics, nutraceuticals, and pharmaceuticals). A few possible

Highlights

Hydrophobic components can be extracted from intact microalgae.
Separation of hydrophobic and hydrophilic components is feasible with intact and disrupted microalgae.
Extraction can be performed without compromising the proteins structural integrity.
Multiproduct microalgae biorefining is a feasible approach.
combinations have been mentioned \([9,10]\), including cell disruption (e.g., bead milling and pulsed electric field), extraction [e.g., ionic liquids (ILs) and polymers] and fractionation (e.g., membrane filtration and chromatography) for a multiproduct biorefinery approach.

Following this rationale, attractive extraction techniques with ILs have gained a lot of interest in the past couple of years due to their high extraction efficiency and yields of extraction focusing the most interesting bioactive compounds obtained from biomass \([15,16]\). There is a clear need to develop greener, more sustainable, cost- and energy-effective, and environmentally safer processes by improving extraction and purification technologies to meet the requirements of a viable bioeconomy. With the plethora of available ILs (Box 1), it is possible to develop extraction methods tailored for specific microalgae products. In the past years, ILs have attracted considerable attention as medium for catalysis \([17–20]\) in extraction and separation processes \([21–24]\), and for the dissolution of biomaterials \([25]\).

An extensive review on the past, present, and future trends of IL-mediated extraction and separation of bioactive compounds shows the different possibilities compared with traditional solvents \([16]\). Future challenges should address process and product life cycle analysis, develop scalable and economical feasible separation processes, and decrease energy and solvent consumption \([16]\). The efficiency of IL-based systems in the extraction of fragile and complex biomolecules such as proteins, is mostly focused on model proteins (e.g., albumin, cytochrome c, haemoglobin, and lysozyme) \([8,16]\) or on isolating specific proteins from different biomass sources (e.g., algae, yeast, and bacteria) \([16]\). However, a multiproduct approach focusing on the biorefinery concept for the sequential isolation of several biomolecules with commercial interest (e.g., proteins, lipids, pigments, and carbohydrates) mediated by ILs under mild conditions is so far lacking.

In agriculture, a consistent amount of waste is generated that could account for at least 50% of the fresh harvested crops \([26]\). In this scenario, the circular economy and the bioeconomy intersect in their common aims to add value to biological wastes and residues through the smart management of agrowastes and their conversion via multipurpose biorefineries platforms into biobased products and biofuels, thus avoiding harmful consequences on soils, water, and air quality. For the agrowaste lignocellulosic biomasses, a multiproduct approach is already further in development not only in theory by assessing their economic evaluations \([27–32]\) but also in practice by developing processes for gasses, sugars, and fatty acids \([33,34]\). However, these biorefineries operate under harsh conditions, which are acceptable for the particular case of lignocellulosic feedstock-based biorefineries but not for microalgae biorefineries. Regarding microalgae processing, recent reviews have discussed microalgae biorefining by applying a variety of different downstream techniques \([7–9,11,35–38]\). However, microalgal multiproduct processes are still too expensive. For instance, typical downstream processing for industrial biotechnological bulk products accounts for 20–40% of the total production costs, while for the microalgae multiproduct scenario, these costs are substantially higher: between 50% and 60% of the overall cost. This discrepancy of results is attributed to the lack of cheap, selective, and mild technologies to access the complexity of different product fractions (proteins, carbohydrates, lipids, and pigments) in their native or at least, in their functional state. To reduce the costs, simplified and integrated processes \([11,35]\) under the strategy of a multiproduct scenario need to be developed.

Recently, (mild) multiproduct approaches mediated by ILs have been investigated (Table 1). The focus is still on separating different classes biomolecules (proteins, lipids, pigments, and carbohydrates) and not yet on specific compounds, except for the selective extraction of certain pigments (e.g., lutein and astaxanthin) as indicated in Table 1. Taking into account the state of the art, this
review intends to focus on novel IL-based systems for the extraction and separation of multiple biomolecules (proteins, pigments, lipids, and carbohydrates) from microalgae supporting the development of a mild multiproduct approach aligned with the demands of the biorefinery concept.

**Product Stability**

ILs have been used for different applications, such as crystallization, solubilisation, separation, extraction, enzymatic reactions, and stabilisation of proteins [19,39]. The stability of proteins from microalgae in IL-based systems is one of the primary requirements as their functionality depends forming a microgel-stabilised emulsion with a hydrophobic environment inside the nanoparticle and a hydrophilic environment outside the nanoparticle. The hydrophobic components (e.g., pigments) are transferred inside the nanoparticle whereas the hydrophilic components (e.g., proteins) remain outside the nanoparticle.

**Box 1. Ionic Liquids Have Separation Potential**

ILs are salts with low melting temperatures. They are composed, in general, of a large organic cation with dispersed charge, and a small organic or inorganic anion. The dispersed charge minimises the electrostatic interactions between the ions, and the large dimension of its ions make organisation into a crystalline structure difficult, so they are liquids at lower temperatures than conventional salts. The interest in ILs as separation media can be attributed to their interesting chemical and physical properties such as low vapour pressure, high thermal and chemical stability, nonflammability, and good solvation properties [15,51,90,91]. These are also referred to as designer solvents [92], as their properties such as polarity, viscosity, and hydrophobicity can be tuned by the adequate combination of cations and anions. These properties make them a desirable class of solvents for liquid-liquid extraction (Table I). The most commonly used anions and cations are depicted in Figure I, and are separated as toxic and harmless or practically harmless following the categorisation defined by GHS [74]. Recently, ILs have been used for extraction of value-added components from biomass [15,93], in synthesis [94], and in material chemistry as electrolytes for the electrochemical industry and liquid crystals. Moreover, ILs also demonstrate good performance in biocatalysis [18], while providing a nondenaturing environment for biomolecules and maintaining the protein structure and enzymatic activity [16].

![Figure I. Illustration of the Toxic and Nontoxic Nature of Different ILs Following the Classification Defined by GHS [75]. Various cations and anions were selected and the respective example of each family of IL is chemically represented as well. The results depicted represent the (logarithmic function) EC50 data obtained by Microtox (toxicity against Allivibrio fischeri) at 15 min of exposure. Data were collected from literature for imidazolium [95], pyridinium, pyrrolidinium, and piperidinium [95], phosphonium [96], morpholinium [97], guanidinium [96], cholinium, [98], and betainium [99]. Abbreviations: GHS, Globally Harmonized System of Classification and Labeling Chemicals; IL, ionic liquid.](image-url)
on noncovalently stabilised 3D structures that can be disrupted by elevated temperatures and various solvents. In the past decade, the stability and activity of many proteins were tested in ILs [19,39,40]. The potential of some ILs to stabilise proteins by maintaining their 3D structure because of the complex interactions they establish (e.g., electrostatic interaction, H-bonds, and dispersion forces) was demonstrated [19,40]. Improved stability (longer storage at room temperature in the presence of ILs) when compared with the same biomolecules in an aqueous buffer was shown for proteins such as lysozyme, bovine serum albumin (BSA), cytochrome c,

| Table I. IL-Mediated (Mild) Extraction of Microalgal Components |
|---------------------------------------------------------------|
| **IL** | **Organism** | **Extraction conditions** | **Valorisation** | **Yield** (~%) | **Refs** |
|-----------------|--------------|-------------------------|-----------------|---------------|-----|
| Trihexyl(tetradecyl)phosphonium bis (trifluoromethanesulfonyl)amide | Haematococcus pluvialis | Disrupted cells RT ionic liquids | Proteins Pigments (astaxanthin) | 68/82 | [57] |
| 1-methyl-3-octylimidazolium chloride | Isochrysis galbana | Disrupted cells organic solvents 70°C | Proteins, carbohydrates (arabinose/glucose-rich polysaccharides) | 100/71 | [24] |
| 1-Butyl-3-methylimidazolium dibutylphosphate | Neochloris oleoabundans | Intact and disrupted cells RT ILs | Lipids Proteins Carbohydrates | 44/77/49 | [64] |
| Tributylmethylphosphonium methyl sulfate | N. oleoabundans | Intact and disrupted cells RT ILs | Lipids Proteins Carbohydrates | 68/80/77 | [64] |
| Iolityle 221PG | N. oleoabundans/Tetraselmis sueca | Disrupted cells RT ILs | Proteins Carbohydrates | 43–48/22–37 | [47] |
| Choline dihydrogenphosphate | N. oleoabundans | Disrupted cells RT ILs | Proteins Pigments (lutein) | 92/97 | [55] |
| Tributyl-1-tetradecylphosphonium | N. oleoabundans | Intact cells RT ILs | Proteins Carbohydrates Pigments (lutein) | 100/80/98 | [65] |
| Choline dihydrogenphosphate | N. oleoabundans | Disrupted cells RT ILs | Lipids Proteins Pigments (lutein, chlorophyll) Carbohydrates | 71/82/98/93 | [56] |

*Recovery mainly based on biomolecules (proteins and carbohydrates) available in cytoplasm or biomolecules (pigments and lipids) available in cytoplasm/cell wall.

*bAbbreviation: RT, room temperature (15–25°C).
ribonuclease A, and enzymes such as lipases, alcohol dehydrogenase, and various proteases in IL structures [8,19,39–41]. Due to the diverse nature of proteins and ILs, not all proteins are stable in ILs [8,19,39,40,42]. Depending on the IL and its concentration, the structural integrity of proteins may be affected with regard to unfolding or protein aggregation [43–46]. Some ILs consisting of large organic cations and organic or inorganic anions (Box 1) are able to interact strongly with the amino acids that comprise proteins, consequently affecting the integrity of the proteins. Protein/IL interactions depend on the size and complexity of these macromolecules. BSA, for example, is a compact tertiary globular folded protein with a molecular weight of ~66 kDa consisting of one subunit and is stable in an IL solution of up to 50% lollyte 221PG (w/w) in water. At higher concentrations of lollyte 221PG, BSA starts to aggregate [46], due to the interaction of the IL with the surface amino acids, resulting in BSA unfolding and, consequently, interactions inducing aggregation. Ribulose-1,5-biphosphate carboxylase/oxygenase (RuBisCo) present in microalgae is a large ternary multimeric protein with a molecular weight of ~540 kDa consisting of eight large and eight smaller subunits noncovalently interacting with each other. This protein, in contrast to BSA, forms aggregates at lower concentrations (up to 25%) of lollyte 221PG (w/w) in water. Moreover, the enzymatic activity of RuBisCo drastically diminishes due to the enzyme inactivation promoted by the IL [46,47] due the competition of the IL with the noncovalently interacting subunits (e.g., surface exposed amino acids) of RuBisCo, in addition to the subunit dissociation and/or aggregation that may occur. Furthermore, it is concluded that protein destabilization may occur at different IL concentrations depending on the size, structure, hydrophobicity, and surface charges of the protein.

In conclusion, IL protein interactions are crucial for the maintenance of protein functionality during extraction of high value biomolecules (proteins, carbohydrates, lipids, and pigments) in a microalgae multiproduct biorefinery scenario.

The next section discusses IL-based systems for the (back) extraction of microalgae components within the biorefinery concept, and the fractionation of the different components by manipulating the affinity between target products and the different phases investigated. The recovery of biobased compounds dissolved in ILs is an important aspect that demands more research.

**IL-Based Systems**

This section discusses two innovative IL-based systems for the separation of (fragile) biomolecules from microalgae: IL-based biorefineries of disrupted microalgae biomass (Figure 1) and nondisrupted microalgae biomass (Figure 2).

**IL-Based Biorefinery of Disrupted Microalgal Biomass**

IL-based **aqueous biphasic systems (ABSs)** have an advantage over traditional ABSs in terms of the process conditions (e.g., lower viscosity and fewer diffusional and mass and heat transfer problems) and process outputs [16,48]. The range of variability on the hydrophobicity and polarities differences between the phases in traditional ABSs is limited whereas with IL-based ABSs this range can be extended [16,48,49]. Using IL-based ABSs, specific interactions can be introduced in the system allowing for a much better extraction performance (in terms of extraction efficiency, recovery, and/or purity) or instead to design systems with tuneable selectivity for the different biomolecules of interest, thus improving the process performance. For example, the extraction of RuBisCO using IL-based ABSs shows a higher partition coefficient for RuBisCO, a major microalgal protein, towards the IL-rich phase in comparison to the conventional polyethylene glycol-based ABS [8,46,50,51]. While IL-based ABSs are widely studied for extraction of biomolecules such as lipids, amino acids, pigments, proteins, nucleic acids, etc. [16] using model systems, their potential for extracting biomolecules like proteins from raw materials has only been described.
in a few cases [40,52–54]. This ability of IL-based ABSs holds a huge prospect in extracting fragile biomolecules from raw materials such as microalgae, which contain molecules of different polarity and solubility (proteins, pigments, lipids, and carbohydrates). Beyond one study [24] that used harsh extraction conditions, only a few studies reported the separation of multiple (fragile) microalgae biomolecules using IL-based ABSs under mild conditions.
Although these studies showed promising results, additional work is required before this approach renders suitable strategies for a microalgal multiproduct biorefinery. More extensive studies using different ILs and the proper, careful design of IL-based ABSs should be performed, in which the role of IL recycling to reduce costs and environmental impact needs to be scrutinised.

Another strategy recently proposed in this field was the use of Cyphos 109-based systems in water-based emulsion stabilised by microgel particles to simultaneously separate fragile biomolecules of different polarities from microalgae [57]. Earlier studies were analytically focused [58–60]. More recently, preparative applications have shown promising results. When mixing with microalgae biomass (disrupted cells), the proteins remain in the aqueous phase outside the microgel particles whereas astaxanthin (the most abundant pigment present in the microalgae cells) partitions to the most hydrophobic medium in the microgel particles. The most interesting aspect of these systems is that they allow the proteins to retain their structural integrity. The microgel particles used to stabilise the emulsions are porous, allowing the transfer of small molecules (e.g., astaxanthin) and prevent direct contact between proteins and the IL, thus assisting with the maintenance of the structural integrity of the proteins. A proof of concept for novel continuous separation was recently reported [57]. The microgel particles were thermoresponsive, a property which was unexplored in this work. However, when the temperature was raised above 32°C, the particles collapsed and the emulsion broke [61]. This property of the emulsion could be explored in the separation process in the future for the development of formulations to be applied in other fields of application, namely in cosmetics, food ingredients, or pharmaceuticals [62], given the innovative profile of this approach towards the microalgae field. Although promising, this technique is still in its infancy and should be tested with different ILs and smaller IL droplets to improve mass transfer phenomena. Additionally, although the emulsion facilitates the simultaneous separation of different components from the biomass, the process still needs to be optimised in terms of efficiency and reusability of the IL and microgel particles. Future research should focus not only on the optimization of the process parameters (e.g., selection of the IL, ratio of IL to microgel particles, and ratio of IL to water content) and scalability, but also on finding newer applications for this emulsion, which provides a complete toolbox for the creation of high performant downstream processes.

IL-Based Biorefinery of Nondisrupted Microalgal Biomass

The performance of IL solutions on the extraction of biological compounds from nondisrupted microalgal cells has also been investigated. Microalgae have a rigid cell wall that requires mechanical disruption to release the intracellular components. However, a more recent study [63] showed that with 40% of an aqueous solution of 1-ethyl-3-methylimidazolium dibutylphosphate it was possible to permeabilise vegetative cells of *Haematococcus pluvialis* under mild conditions, allowing the release of astaxanthin that could subsequently be extracted with ethyl acetate. Microscopic studies confirmed that the cells were not ruptured. Based on these observations, a possible mechanism was postulated wherein the permeabilisation of the microalgal cells occurs by the action of the ILs. However, it remains unclear how this effect takes place and what specific action of the IL makes the cell wall porous. A similar observation was also made when *Neochloris oleoabundans* cells were treated with imidazolium-based ILs [64,65] and a phosphonium-based IL, Cyphos 108. Despite the good solubility achieved with Cyphos 108 towards the *N. oleoabundans* cells, the same effect was not observed in the *H. pluvialis* cell wall, which indicates the relevant role played by the cell wall composition. Of course, while the results seem to be promising, in-depth knowledge and understanding of the effect of ILs on cell walls, which appears to be microalgal-specific, is required. When the more hydrophobic components (lipids and pigments) are extracted out of intact cells by cell wall permeabilisation with ILs and mediated
by organic solvents (e.g., ethylacetate), the more hydrophilic components (proteins and carbohydrates) remain in the cells and can be extracted by mechanical disruption to recover these components in suitable aqueous buffer solutions maintaining the structural integrity of the components (i.e., keeping their native form). These results show the potential of ILs to fractionate and separate the microalgal biomass components in a multiproduct biorefinery approach.

The most logical approach would be to start by studying the cell wall composition and then to use this characterisation to select the most appropriate solvent and technique to proceed. No less relevant is the need for advanced analytical methods to properly identify and quantify the cell components that can cope with IL solutions. This is not straightforward since often the ILs cause massive interference with the methods of quantification used. These studies would help in selecting and designing ILs that are better suited to the cells’ morphology and biochemistry and the target compounds’ dissolution.

Overall, extraction of proteins with ILs shows good recovery [16,46,55,64]. However, future work should focus on the back-extraction of proteins from ILs and investigate the protein–IL interactions to understand the solvation of proteins in IL solutions.

Concluding Remarks and Future Perspectives
ILs have become popular as remarkable solvents in various fields of application, such as biocatalysts for bioactive compounds and for separation processes. Different combinations of ILs can be prepared (Box 1) and used in separation techniques as mobile or stationary phase modifiers in HPLC, as running buffer additives in capillary electrophoresis, as crystallising agent in protein crystallisation, protein solubilisation in formulation studies, or as new extraction solvents [39,66]. Most of the IL-based separation techniques reported focus on analytical separation and sample preparation [66], whereas the extraction and separation of bioactive compounds from complex matrices, such as biomass, at preparative scale is step by step and focuses on specific components [16]. Recently, more multiproduct biorefinery approaches are coming up as well (Table 1), but will take more time to implement as their complexity increases. The expectation is that this will further improve if multiple products can be recovered with simple IL-mediated extraction methods. However, more research and development is needed to achieve large scale production processes (see Outstanding Questions), not only for microalgae but also for other organisms (e.g., yeast, macroalgae, bacteria, and fungi).

Novel IL-based systems for the extraction of fragile molecules from complex matrices are herein discussed, showing new directions towards multiproduct biorefinery approaches of microalgae [56,64]. Figures 1 and 2 summarise the possibilities to selectively extract hydrophobic components (lipids and pigments) and hydrophilic components (proteins and carbohydrates) from (non-)disrupted microalgae (R. Desai, PhD thesis, Wageningen University, 2016). Unfortunately, as previously mentioned, the exact mechanism of extracting the hydrophobic biomolecules (lipids and pigments) from intact cells, and which interactions play major roles to explain the results obtained when using ILs and/or organic solvents, remain unknown. The same holds true when disintegrating microalgal cells (e.g., bead milling) and recovering the biomolecules with IL-based extractions. However, despite the potential of ILs in the pretreatment and separation of biomolecules from microalgal cells, some bottlenecks need to be overcome before their successful use at larger scale. For instance, the cost and environmental impact (e.g., biodegradability and toxicity) of using ILs as extractants or solvents (for biomass pretreatment) needs to be considered. Despite the overgeneralised idea that ILs are toxic [67–73], this is not necessarily correct. The toxicity of ILs mainly depends on the length of their alkyl chains, and generalisations that all ILs are toxic are neither straightforward nor supported by recent literature data.

Outstanding Questions
Recycling of ILs is challenging. How can we develop efficient recycling technologies for ILs in microalgal biorefineries?

The multiproduct biorefinery is important to develop cost-effective processes for microalgae, but how can we scale-up the reported bench scale processes?

Will it be possible to improve thermoresponsive solvents, or microgel particles, for a multiproduct biorefinery approach?

Will the development of cost-effective, biobased, biodegradable, and non-toxic ILs be feasible?

What is the mechanism of interaction, and what interactions play a role, between ILs and microalgal cell wall components that allow the extraction of intercellular biomolecules?
legislation from Registration, Evaluation, Authorization, and Restriction of Chemicals (REACH) [74] requires ecotoxicological data for all chemicals produced or imported into the EU above 1 ton/year. In accordance with the United Nations Globally Harmonized System of Classification and Labelling Chemicals (GHS) [75], these data should be classified in categories [76], for which compounds with median effective concentration (EC50) values above 100 mg/l should be considered as practically harmless, and compounds with EC50 values below this value should be considered hazardous. Imidazolium ILs are not toxic per se (the same is true of ILs with some other head groups), with some already having been registered (e.g., 1-ethyl-3-methylimidazolium acetate) by REACH and industrially applied (e.g., by 525 Solutions). However, simultaneously considering biocompatibility, low toxicity, price, and availability, some structures that can be considered as more advantageous, are those based on cholinium [47,56,73–80] and betainium cations [81]. In this context, Box 1 depicts several IL chemical structures and demonstrates their toxic or nontoxic nature with respect to the GHS categorisation, allowing researchers to select the most appropriate solvents focusing on the target products and their final applications (Table 1).

Beyond the appropriate selection of both the process conditions and IL chemical structure, the environmental impact of ILs could also be lowered by their efficient recycling and reuse in the process so that the recovered biomolecules contain no, or only traces of, ILs, allowing the potential application of the final products as ingredients in cosmetic, nutraceutical, or pharmaceutical formulations. Therefore, the final preparation step should be a formulation in which the different biomolecules remain stable for specific periods of time, at room temperature (15–25°C) or, if necessary, refrigerated (2–8°C).

The most common strategies to recycle ILs used as solvents were recently reviewed [82]. These include ultrafiltration/diafiltration (mainly applied to isolate proteins and carbohydrates), back-extraction with sustainable organic solvents followed by the evaporation of the organic solvents if needed (a protocol normally applied to lipids and hydrophobic pigments), and precipitation of the compounds extracted followed by the precipitate redissolution in the appropriate media for further application, a strategy commonly applied to proteins but also small compounds.

In addition to these standard techniques, recycling and reuse of ILs may be defined considering the particularities of the optimised downstream process. In the case of processes using IL-based emulsions, from the approaches reported, the thermoresponsive properties of the IL emulsions can easily break the emulsions to recycle ILs [57]. However, and as previously discussed, this technology needs further improvement, such as the use of more biocompatible (less toxic and more biodegradable) ILs, which is the case for cholinium- [47,56,79,80] or betainium [81]-based structures, mainly considering their good scalable potential observed from millilitre to litre scales.

Table 2 gives an overview of the advantages and disadvantages of IL-based multiproduct approaches under the scope of the biorefinery concept. The main benefits include the capacity to recover multiple biomolecules (proteins, carbohydrates, pigments, and lipids), implying a high economic return, more products for more applications and less waste (more products recovered means less biomass residues wasted). By contrast, the process complexity increases to improve the separation of the different biomolecules.

The sustainable nature of the industry is a crucial concern. There is no commonly agreed upon definition of sustainability, but we believe that it should be the direct result of the balance between the process performance, environmental, and economic impacts. Process performance and the
environmental impact are already under discussion, but the economic impact analysis is still in its infancy. In the past year, a study focusing on analysing different industrial scenarios for multiproduct versus single-product approaches, considering the biomolecule selection, yields and economic performance, and considering the biological characteristics of algae species [4] was published. In this work, the authors showed the most promising economic gains of working under the multiproduct biorefinery approach [4].

Despite the increased enthusiasm for using ILs as solvents, some others, such as deep eutectic solvents (DESs) have emerged as alternative solvents for the extraction of biomolecules. DESs have been explored as potential alternatives to ILs [73,82–84]. Some components like choline can form deep eutectic solvents [73,85]. These mixtures may possess similar characteristics to ILs (e.g., low melting temperatures, low volatility, nonflammability, tunability, high solubility, and chemical and thermal stability), while benefitting from the availability and low cost of the starting materials. Moreover, they are easy to store and prepare, which consequently allows them to have lower costs. DESs result from the combination of a hydrogen bond acceptor (HBA) and a hydrogen bond donor (HBD) whereby the interactions between HBA and HBD mostly involve hydrogen bonding and, to a lesser extent, electrostatic forces and van der Waals interactions [86]. An interesting approach was reported [85] using a switchable-hydrophilicity solvent system consisting of a fatty-acid-based DES, and complemented by a biorenewable dilute amine solution. This study demonstrated that a DES can be made to reversibly switch from a hydrophobic phase to a hydrophilic phase, thereby being able to extract various biological fractions (proteins, carbohydrates, and lipids) making up the biomass [85,87]. This work was carried out according to a circular extraction paradigm [88] in two subsequent steps and in both the forward and backward mode. A synthetic matrix representing the microalgal biomass, made of selected proteins, lipids, carbohydrates, and water, was extracted by DES and the extraction streams were characterised. Despite their sustainable character, new developments in DES are required to demonstrate if they could become attractive alternatives for the recovery of multibiomolecules including proteins. Moreover, and considering the monopoly of chromatographic techniques to

| Table 2. Advantages and Disadvantages of a Future Microalgal Biorefinery with ILs |
|---------------------------------|---------------------------------|-----------------|
| Future microalgal biorefinery   | Advantages                      | Disadvantages   |
| Product functionality           | Functional multiple biomolecules | Each biomolecule needs specific conditions |
| Complexity                      | Development of new biorefinery concepts | Complex processing |
| High value products              | Recovery of different biomolecules (proteins, lipids, carbohydrates, and pigments) | — |
| Temperature                     | Room temperature              | — |
| ILs                             | Recycling                      | Toxicity        |
| Yield                           | Medium-high yield, extraction of biomolecules from complete cells | — |
| Selectivity                     | Specific for different biomolecules | Complex processing |
| Stability/storage               | Room/refrigerator temperature  | Storage at -20°C is required for less-stable compounds |
| Energy costs                    | Low temperature               | Multiple steps  |
| Targeted application            | Cosmetics, nutraceuticals, pharmaceuticals | — |
| Costs                           | High value economically competitive | — |
purify proteins, the use of IL-based ABSs should be focused on compounds for which the use of chromatography is not an option. ABSs, as separation techniques, have several advantages over chromatography when implemented properly [10,35]. They allow the development of multiproduct downstream processes, can be easily integrated with other techniques, and have high potential to be conjugated with miniaturised systems whenever needed [89]. Taking this into account, they have good potential for (micro)algal biorefinery, wherein there is a need to develop efficient processes to ensure the complete fractionation of the algal biomass, allowing the creation of more sustainable processes, which will be needed as the world moves towards a biobased economy.

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Declaration of Interests
No interests are declared by the authors.

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