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Research Article

Extraction and Fractionation of Pigments from *Saccharina latissima* (Linnaeus, 2006) Using an Ionic Liquid + Oil + Water System

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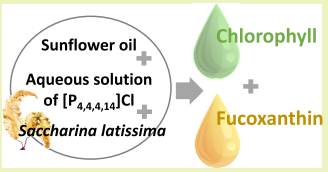
ABSTRACT: There is a strong industrial interest in the development of greener and more sustainable processes based on the use of renewable resources, and a biorefinery based on marine resources, such as macroalgae, stands as a major opportunity toward that end. In this work, *Saccharina latissima* (Linnaeus), a brown macroalga, was used as a source of pigments to develop an integrated platform that is able to promote the extraction and separation of chlorophyll and fucoxanthin in one single step. The process was studied, and its operational conditions were optimized with yields of extraction of chlorophyll and fucoxanthin of 4.93 ± 0.22 $mg_{chl}{\cdot}g_{dry\ biomass}^{-1}$ and 1956 ± 84 $\mu g_{fuco}{\cdot}g_{dry\ biomass}^{-1}$, respectively. These results were achieved with extraction systems composed of 84% of an aqueous solution of a tensioactive phosphonium-based ionic liquid (IL) at 350 mM + 16% of sunflower oil, during 40 min, using a solid−liquid ratio of 0.017 $g_{dry\ biomass}{\cdot}mL_{solvent}^{-1}$. After the separation of both aqueous IL-rich and oil-rich phases, the IL content in both phases was investigated, the oil phase being free of IL. Envisioning the industrial potential of the process developed in this work, the recovery of the IL from the aqueous IL-rich phase of the initial system was attempted by a back-extraction using organic solvents immiscible in water, being shown that 82% of the IL can be recovered and reused in new cycles of extraction. The environmental and economic impacts of the final process proposed for the extraction and fractionation of chlorophyll and fucoxanthin were evaluated. Different scenarios were considered, but summing up the main results, the solvents' recycling allowed better results, proving the economic and environmental viability of the overall process.



KEYWORDS: *Saccharina latissima (Linnaeus), pigments, one-pot, liquid−liquid extraction, ionic liquid, vegetable oil*

INTRODUCTION

The ever-stronger regulations on sustainability of manufactured goods are also creating opportunities for industries using natural raw materials and biomasses to manufacture consumer goods and are an excellent alternative for a more sustainable society. Algae are a good example of a natural, renewable resource on which a bioeconomy can be built on.[1] Besides their fast growth rates; lack of freshwater, fertilizer, and pesticide requirement; and possibility of cultivation even on non-arable areas, algae are a very interesting raw material not only from the point of view of composition but also considering the flexibility of applications of their bioactive compounds as a source of various food ingredients, e.g., colorants and phycocolloids with thickening and gelling properties. Moreover, they have been shown to be a rich source of different bioactive compounds with commercial interest in different commercial sectors.[2−4] Pigments are a very good example to highlight due to their large array of potential applications not only from food, as functional ingredients (as colorants and/or antioxidants),[5] but also in photodynamic therapy, imaging, solar energy conversion, and hydrogen production.[6,7] Pigments also stand out due to their biological activities. Chlorophyll and derivatives have been reported as having antimutagenic, chemo-preventive, antioxidant, anti-inflammatory, and gut microbiota regulator activities,[8] while carotenoids are known for their antioxidant function (preventing oxidative stress), immune response stimulation, and pro-vitamin A activity, allowing them to act in the prevention of tumors and other diseases.[9,10]

Despite the high diversity of macroalgae species, most have been poorly explored so far. The brown macroalga *Saccharina latissima* (Linnaeus), also known as sugar kelp or kombu royal, is one of the two algal species studied on the framework of the European project GENIALG (GENetic diversity exploitation for Innovative macro-ALGal biorefinery). The genus *Saccharina* is the most cultivated in the world with 17.5 M t fresh weight in China alone,[11] and more specifically, *S. latissima* is the most cultivated seaweed species in Europe, with Norway being at the forefront, hosting about 40 registered companies (17 of them are productive), 97 cultivation sites, and 178 t fresh weight harvested in 2018 (Jorunn Skjermo, SINTEF, pers. Comm.). It has a high biomass yield and, simultaneously, a high farming expansion potential, already validated in

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Europe.[12] Contrary to what was observed for *Ulva* species, which are abundant in chlorophylls,[13] this brown alga (besides chlorophyll *a* and chlorophyll *c*)[14] also produces significant amounts of carotenoids, namely, the xanthophylls and, particularly, fucoxanthin as the most abundant.[15,16] However, its recovery is still not straightforward or carried out in a large scale due to the lack of cost-efficient processes with low environmental footprint and good scalable potential.[17] Also, since carotenoids and chlorophylls have similar polarity and are usually present in the same cellular sites (chromoplasts, chloroplasts, leucoplasts, and fat globules),[18] their simultaneous extraction is very common,[19] impairing the selectivity of the extraction process and, consequently, compromising the purification of the compounds.

The use of aqueous solutions of ILs in the extraction of biomolecules from different biomass matrices is not new.[20,21] ILs have been recognized as powerful task-specific solvents for this purpose.[22−24] The mechanisms behind their success are normally assigned to an increased solubility of the target molecule in the IL media, their improved ability to disrupt the cell membranes, or the combination of both.[13,25] Following this rationale, and considering the properties of carotenoids and chlorophylls, aqueous solutions of tensioactive ILs have been successfully employed.[13,25−27] Nevertheless, pure extracts are difficult to obtain when molecules with similar structures and/or polarities are present in the same cellular site (e.g., chlorophylls and carotenoids),[18] and thus, additional steps of purification are required, involving higher costs, energy consumption, and specific equipment and/or materials.[28] The use of liquid−liquid extraction techniques is well known in purification processes. They have many advantages in comparison with other purification techniques, such as conventional chromatography and saponification,[29,30] due to their simplicity and familiarity, being easy to implement and to scale up. There are many variations, ranging from the use of volatile organic solvents, aqueous systems, and oily systems.[31−33] Vegetable oils are a food ingredient very well accepted in many industries[5] that can work not only in the extraction of compounds from biomass such as pigments, namely, chlorophylls and carotenoids, but also in the formation of liquid−liquid extraction systems.[5,34−36] In addition, edible oils are non-volatile, cost-effective, and excellent solvents to be applied in the food sector since they may be directly used in food formulations[5] without the need of performing the pigment−solvent separation.[17,35]

In this work, a new process able to extract and separate both chlorophylls and fucoxanthin from *S. latissima* (Linnaeus) by an integrated solid−liquid and liquid−liquid extraction process using aqueous solutions of a tensioactive IL and a common vegetable oil was designed as an alternative to the conventional extraction processes. After a first screening of different ILs as solvents to extract the pigments from the biomass, the best solvent was selected, and the principal operational conditions were optimized. Envisioning the industrial potential of the process developed, the IL recovery was tested, enabling thus the analysis of the environmental and economic impacts of the final process.

EXPERIMENTAL SECTION

Biomass. The biomass used in this work was kindly provided by one of the industrial partners of project GENIALG, Algaia SA (Saint-Lô, France) and the Station Biologique de Roscoff, CNRS. *S. latissima* (Linnaeus) C.E. Lane, C. Mayes, Druehl, and G.W. Saunders, 2006, was collected in Roscoff, France (48°43′54″N, 3°59′23″W). The fresh biomass was harvested in February 2019, washed, frozen with liquid nitrogen and ground in a coffee grinder, freeze dried, and sifted to achieve a particle size of <1 mm afterward. The biomass was kept at −20 °C until needed.

Chemicals. The series of 1-alkyl-3-methylimidazolium chloride-based ILs [$C_nC_1$im]Cl, as well as 1-hexyl-3-methylimidazolium chloride ([C_6C_1im]Cl, 98 wt %, CAS 171058-17-6), 1-dodecyl-3-methylimidazolium chloride ([$C_{12}C_1$im]Cl, >98 wt %, CAS 171058-18-7), 1-methyl-3-tetradecylimidazolium chloride ([$C_{14}C_1$im]Cl, 98 wt %, CAS 171058-21-2), were acquired from Iolitec (Heilbronn, Germany). The decyltrimethylammonium chloride ([$N_{1,1,1,10}$]Cl, 98 wt %, CAS 10108-87-9) and the decyltrimethylammonium bromide ([$N_{1,1,1,10}$]Br, 99 wt %, CAS 2082-84-0) were from Tokyo Chemical Industry (Fukaya, Japan). The dodecyltrimethylammonium bromide ([$N_{1,1,1,12}$]Br, 99 wt %, CAS 1119-94-4) and tetradecyltrimethylammonium bromide ([$N_{1,1,1,14}$]Br, 98 wt %, CAS 1119-97-7) were purchased from Alfa Aesar (Kandel, Germany). The tributyltetradecylphosphonium chloride ([$P_{4,4,4,14}$]Cl, 95 wt %, CAS 81741-28-8) was purchased from Iolitec (Heilbronn, Germany). All molecular structures of the ILs used in the screening of solvents are depicted in Figure S1 in the Supporting Information.

Refined sunflower oil (brand Auchan) purchased at an Auchan supermarket (Aveiro, Portugal) was used on the pigment extraction. Standard fucoxanthin (≥95%, CAS 3351-86-8) was acquired from Sigma-Aldrich (Darmstadt, Germany). The organic solvents used in the screening of solvents and back-extraction step, namely, ethanol (HPLC grade, CAS 64-17-5), toluene (HPLC grade, CAS 108-88-3), and ethyl acetate (HPLC grade, CAS 141-78-6), were purchased from Fisher Scientific (Porto Salvo, Portugal), while diethyl ether (99.8%, CAS 60−29-7) was acquired from Panreac (Barcelona, Spain).

Screening of Solvents. The extractions were performed at room temperature (20−25 °C) under a constant vertical rotation of 80 rpm in a shaker IKA TRAYSTER digital for 30 min. The ILs were screened at a 250 mM aqueous solution, the list of ILs screened and initial concentration being chosen according to previous works.[13,27,37] Water, sunflower oil, and ethanol were tested as control systems. A solid−liquid ratio (SLR) of 0.017 $g_{dry\ biomass}{\cdot}mL_{solvent}^{-1}$ was used, meaning 0.2 g of dry biomass and 12 mL of the respective solvent. All extractions were done in triplicate. In order to separate the cell debris from the supernatant, a centrifugation step was carried in a Thermo Scientific Heraeus Megafuge 16R centrifuge at 4700 *g* for 15 min at 10 °C, and the supernatant was recovered and analyzed.

Pigment Fractionation: From a Two-Step to a Single-Step Approach. After the first step of selecting the best solvent to extract the pigments, a second step followed, which consisted of a liquid−liquid extraction system obtained by adding and mixing sunflower oil to the pigment-based IL aqueous extract in a proportion (in volume) of 60% of the aqueous solution of IL ($\%_{IL}$) to 40% of sunflower oil. The two phases were formed in a Thermo Scientific Heraeus Megafuge 16R centrifuge at 4700 *g* for 15 min at 10 °C, and both phases were analyzed.

In the single-step approach, the dry biomass (0.2 g) was mixed with a fresh aqueous solution of IL (instead of the crude extract) and with oil in a system using the same volume ratio than before (60% IL aqueous solution + 40% sunflower oil). The temperature, agitation, time of extraction, and concentration of the IL in the aqueous solution were kept as described for the screening of solvents. In order to separate the various phases, a centrifugation was done in a Thermo Scientific Heraeus Megafuge 16R centrifuge at 4700 *g* for 15 min at 10 °C, and both phases were analyzed.

Optimization of the Process Conditions by a Response Surface Methodology. Each system was composed of the biomass, an aqueous solution of the best ILs screened, and sunflower oil. The optimization of the process was done by applying a central composite rotatable design (CCRD, 2^4 plus axial), totalizing 28 extractions with four replicates at the central point. The independent variables optimized were the time of extraction (*t* in min), the concentration of IL in water (C_{IL} in mM), the volume of aqueous solution of IL toward the oil volume ($\%_{IL}$ in %), and the solid−liquid ratio (SLR in

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$g_{dry\ biomass}{\cdot}mL_{solvent}^{-1}$), considering both aqueous and organic fractions as solvents. The temperature and agitation were kept constant as described for the screening of solvents, i.e., room temperature (20−25 °C) and 80 rpm, respectively. The conditions are presented in Tables S1 and S2 in the Supporting Information. All the experimental planning analysis was performed following the theory exposed by Dean et al. and Rodrigues and Lemma.[38,39] The obtained results were analyzed using the Statista 12.0 and statistically verified for a confidence level of 95%.

Pigment Quantification. The absorption spectra of the aqueous phases were measured between 300 and 700 nm using a UV−vis microplate reader (Synergy HT microplate reader − BioTek). The chlorophyll and fucoxanthin contents were evaluated according to calibration curves previously determined and depicted in Figure S2 in the Supporting Information [R^2 = 0.9389, R^2 = 0.9805, and R^2 = 0.9986, for chlorophyll in aqueous solutions (at 667 nm), chlorophyll in ethanol (at 667 nm), and fucoxanthin in aqueous solution (at 457 nm), respectively]. The absorption spectra of the oil phases were analyzed between 350 and 750 nm using a UV−vis spectrophotometer (SHIMADZU UV-1700 PharmaSpec spectrometer). In this case, the chlorophyll content was quantified following an equation that allows the determination of chlorophyll in vegetable oils adopted from Pokorný et al.[40] The results are expressed in terms of yield of extraction ($mg_{chl}{\cdot}g_{dry\ biomass}^{-1}$ and $\mu g_{fuco}{\cdot}g_{dry\ biomass}^{-1}$).

Statistical Analysis. The analysis of variance (ANOVA) followed by Bonferroni post hoc was performed using the BIOESTAT 5.3 to compare the significance of the obtained extraction yields of fucoxanthin and chlorophyll using a degree of significance of 95% ($p < 0.05$, $n = 3$).

IL Recovery and Quantification. The pigments were separated from the IL using back-extraction with organic solvents with low miscibility in water. Systems composed of an aqueous extract of IL (rich in fucoxanthin) and different organic solvents (toluene, ethyl acetate, and diethyl ether) were tested in a ratio of 3:2 (v/v). The mixtures were centrifuged in a Thermo Scientific Heraeus Megafuge 16R centrifuge at 3300 *g* for 30 min at 25 °C, and a two-phase system was obtained. In order to quantify the recovery of the IL in the extracts, an ion-selective electrode (Metrohm) able to detect the chloride anion (electrode reference: 6.0502.120) was used after preparing the respective calibration curve (R^2 = 0.9999). The chloride content in the oil phase was calculated by the difference between the chloride content in the initial aqueous solution of IL and the aqueous phase (bottom phase) in the system with oil.

Environmental Analysis. Life cycle assessment was applied following the ISO 14040 standard[41] to determine the environmental impacts of the $[P_{4,4,4,14}]$Cl-based process proposed for the extraction and fractionation of chlorophyll and fucoxanthin from *S. latissima* (Linnaeus). Two scenarios were analyzed, one where $[P_{4,4,4,14}]$Cl is not reused and the other where the $[P_{4,4,4,14}]$Cl remaining in the aqueous phase (82% of the initial amount) is reused. The impacts derived from the production of electricity, $[P_{4,4,4,14}]$Cl, toluene, sunflower oil, and distilled water were calculated based on the amounts consumed during the experimental procedure (Table S3 in the Supporting Information) multiplied by the respective impact factors (e.g., mass of greenhouse gas emissions expressed as carbon dioxide equivalent (CO_2 equiv) per mass of toluene). These impact factors were taken from the World Food LCA Database 3.5[42] for sunflower oil and Ecoinvent 3.5 database[43] for the other inputs.

Economic Analysis. The economic analysis done in this work is based on two equations published before.[13,44] Briefly, eq 1 calculates the production cost per unit of mass of the product obtained.[13] Eq 2 was used to calculate the potential return given that the products generated in this process can be sold for a profit.[21]

$$\frac{\text{production cost of materials}}{\text{unit mass of pigment}} = \frac{\sum_{i=1}^{n} \frac{\text{use of material}_i}{\text{batch}} \times \frac{\text{price of material}_i}{\text{unit of material}_i}}{\frac{\text{amount of pigment}}{\text{unit of dry biomass}}} \times \text{mass of dry biomass used} \quad (1)$$

$$\text{return}(EU{\cdot}g_{dry\ biomass}^{-1}) = [C_{prod} \times \$_{prod}] - \$_{biom} - [\alpha \times \text{production cost per g of biomass}] \quad (2)$$

Using eq 1, it is possible to obtain the production cost per mass of any product (i.e., chlorophyll and fucoxanthin) but considering only the materials employed for its production. Data generated in this work relates only to a laboratory-scale development, so there is no real information on how a potential scaled-up version of the process will behave, and thus, in this work, it was only possible to make projections. Through these projections, the overall cost considered the capital contribution, materials/consumables, labor, and others (waste disposal, insurance, and utilities). In order to calculate the theoretical total production cost, and as there is no data for this process, the capital contribution was decided to be fixed at 50% of the total cost. From the literature, labor was established at 15% and others (waste disposal, insurance, and utilities) at 4%.[45] This allows for materials/consumables to take up the remaining 31% of the total projected cost. Using these proportions, it was possible to have the total production cost per mg of the pigment (or cost of goods per mg of pigment, $CoG{\cdot}mg^{-1}$). Other aspects, such as those required for final product polishing (freeze drying, transportation, packaging, etc.), were not considered in this work as the aim is to determine the areas of opportunity for the liquid−liquid extraction systems developed here.

Eq 2 requires the input of five variables, which can vary to enhance the potential of the analysis, thus containing a comprehensive collection of possibilities. C_{prod} considers the concentration of the product per unit of mass of dry biomass processed (yield of extraction for each pigment in the best operational conditions), and $\$_{prod}$ is the market price of each product (pigment) based on the suppliers. It is important to note that commercial prices might be higher than the possible actual selling price of the product developed here, and for this reason, this analysis considered prices 10- and 100-fold lower as well. $\$_{biom}$ is the variable to capture the cost of obtaining the biomass; in this study, this cost was fixed at 0 $EU{\cdot}g_{dry\ biomass}^{-1}$. The production cost per dry biomass is obtained by multiplying the complete production cost per mg of the pigment ($CoG{\cdot}mg^{-1}$) and the yield of extraction ($mg_{pigment}{\cdot}g_{dry\ biomass}^{-1}$). The term α is used to express a multiplier of the production costs. This term allowed to analyze the impact of having a higher or lower production cost in practice. This study analyzed an α of 0.1, 1, and 10, representing an increase and decrease by 10-fold, besides the base scenario.

The calculation of the values for the production costs using eq 1 and the subsequent total costs was based on the materials used, their respective costs being included in the Supporting Information (Table S4). Additionally, one of the aims of the work is to provide an insight on the economic aspects of including the IL's recycling on the step of extraction and the toluene recycling on the final polishing (and back-extraction) step. To capture this, several scenarios were included after the base calculation was completed. These scenarios include (i) no recycling (benchmark), (ii) recycling of only IL, (iii) recycling of IL and the water where it is contained, (iv) recycling only toluene, and (v) recycling everything (IL, water, and toluene). Moreover, each of these scenarios (except for when no recycling is included) was evaluated for different recycling scenarios, namely, 20, 40, 60, 80, and 100%. By doing all these combinations, it is possible to have a comprehensive collection of data to include potential real-life scenarios and set up a benchmark for future developments.

Through eq 2, the potential profit possible to be achieved from these two products (chlorophyll and fucoxanthin) is determined. First, the return of each scenario was analyzed individually as a benchmark to determine the potential areas of improvement. Then, a combined return (total return) was calculated through eq 3, which is an updated version of eq 2. This can reflect the more realistic scenario as both products are generated in the same process and with an overall production cost.

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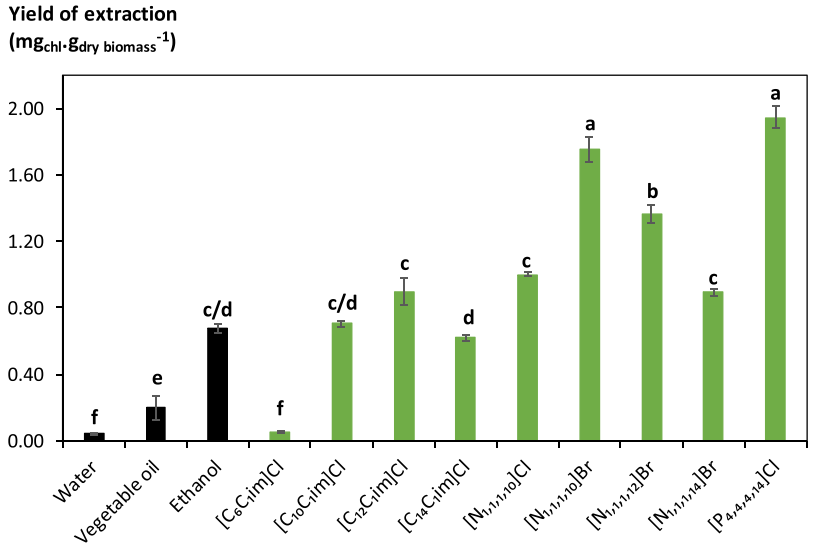

Figure 1. Screening of different solvents in the extraction of pigments from *S. latissima* (Linnaeus) in terms of yield of extraction of chlorophyll (mg$_{chl}$·g$_{dry\ biomass}^{-1}$). Black bars are solvents tested as controls. Different letters represent statistically different values ($p < 0.05$).

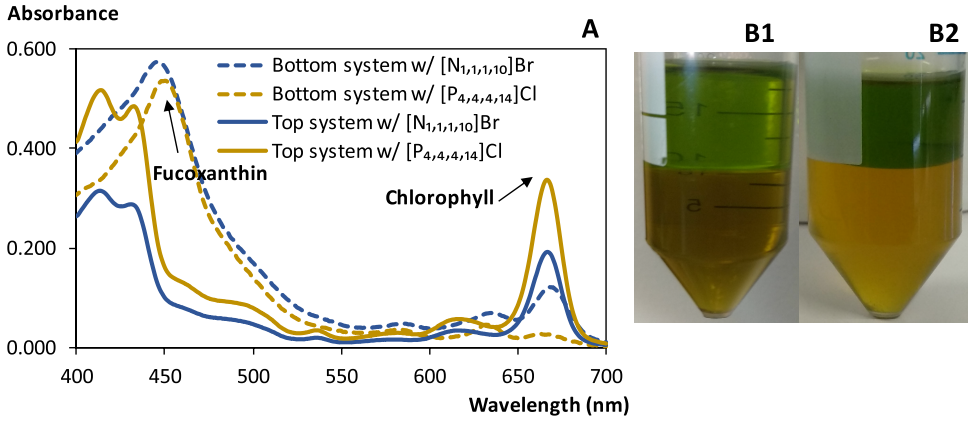

Figure 2. Liquid−liquid extraction systems composed of the extract obtained using the aqueous solutions of [N$_{1,1,1,10}$]Br and [P$_{4,4,4,14}$]Cl and the vegetable oil: (A) UV−vis spectra of top and bottom phases of both systems and photographs of the system with (B1) [N$_{1,1,1,10}$]Br and (B2) [P$_{4,4,4,14}$]Cl. Blue lines represent systems with [N$_{1,1,1,10}$]Br, while dashed lines represent the systems' bottom phases. Note that the top and bottom phases were analyzed in different spectrophotometers as described in the Experimental Section and by applying different dilutions.

$$\text{total return}(\text{EU}\cdot g_{\text{dry biomass}}^{-1}) = [C_{\text{prod chl}} \times \$_{\text{prod}} + C_{\text{prod fuco}} \times \$_{\text{prod}}] - \$_{\text{biom}} - [\alpha \times \text{production cost per g of biomass}] \quad (3)$$

RESULTS AND DISCUSSION

Screening of ILs as Extraction Solvents. The results of a screening of aqueous solutions of different ILs (at 250 mM), with the extracts quantified in terms of yield of extraction of chlorophylls (mg$_{chl}$·g$_{dry\ biomass}^{-1}$), are presented in Figure 1. Water, ethanol, and a vegetable oil were used at the same conditions of extraction as the control solvents. Photographs and the UV−vis spectra of the extracts are depicted in Figure S3 in the Supporting Information.

As it can be seen in Figure 1, and as expected due to the pigment hydrophobicity, water and aqueous solutions of [C$_6$C$_1$im]Cl are not efficiently extracting the pigment, which may be explained by the low capacity of these hydrophilic solvents to interact with the membrane phospholipids and, consequently, to disrupt the cells. A similar behavior was obtained with the use of vegetable oil, also showing a low performance in the extraction. This could be justified by its high viscosity that hinders the mass transfer of pigments from the biomass to the oil and by the low capacity of the oil components to interact with the cell structure, without any mechanical help, as reported elsewhere.[35]

On the other hand, ethanol, a well-known solvent in pigment extraction and membrane solubilization, and aqueous solutions of (cationic) tensioactive compounds are able to extract chlorophylls (and carotenoids) as already shown in previous works.[13,26] As previously discussed in the literature, the tensioactive ILs are able to form micelles in aqueous solutions above certain concentrations, named as the critical micelle concentration (CMC), as presented in Table S5 from the Supporting Information. The ability of these ILs to extract these hydrophobic pigments can be explained by two different phenomena: (i) the creation of a perfect environment for the solubilization of the pigments within the micelles since all ILs were tested at concentrations above their CMC and (ii) the ability of these cationic ILs to disrupt the phospholipid cell and thylakoid membranes protecting the pigments involved in the

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photosynthesis. This disruption happens due to the formation of electrostatic interactions between the cationic ILs with the negatively charged head of the phospholipids. Moreover, hydrophobic interactions have also an important role on this process as supported by the similarity in length of the IL alkyl chain and the lipidic part of the phospholipids that can lead to cell disruption and release of intracellular materials by mechanisms of expansion and permeabilization.[46,47] Among the ILs screened, aqueous solutions of $[N_{1,1,1,10}]Br$ and $[P_{4,4,4,14}]Cl$ stand out as the most efficient in the extraction of pigments, in accordance with previous works.[13,27,48]

Pigment Fractionation. Given the good performance of aqueous solutions of $[P_{4,4,4,14}]Cl$ and $[N_{1,1,1,10}]Br$, both were considered in further experiments. The extracts obtained using the aqueous solutions of the two ILs and a low-cost vegetable oil (sunflower oil) were combined to form a liquid−liquid extraction system (resulting from the immiscibility between oil and water). The two-phase system formed is composed of a top phase rich in oil, presenting a green color (rich in chlorophylls), and a bottom phase rich in the IL aqueous phase, with a yellow color (rich in fucoxanthin), as shown in Figure 2.

From the results depicted in Figure 2, it is possible to conclude that, besides the chlorophyll, a significant amount of fucoxanthin was also extracted from the biomass by using the ILs' aqueous solutions. Chlorophylls present a higher hydrophobicity than xanthophylls (miLogP around 9.8, whereas for fucoxanthin, it is around 8.5),[19] which explains the partition of the chlorophyll to the oil phase, highly hydrophobic, while fucoxanthin remains in the aqueous phase. Although the separation of phases starts to occur just a few minutes after homogenization, the centrifugation step allowed a faster and complete phase separation and consequent fractionation of pigments. Yields of extraction of fucoxanthin of 1397 ± 3 and 1376 ± 79 $\mu g_{fuco} \cdot g_{dry\ biomass}^{-1}$ and chlorophyll of 1.65 ± 0.02 and 2.9 ± 0.1 $mg_{chl} \cdot g_{dry\ biomass}^{-1}$ were obtained for $[N_{1,1,1,10}]Br$ and $[P_{4,4,4,14}]Cl$, respectively. Interestingly, the system with $[P_{4,4,4,14}]Cl$ is not only providing higher yields of extraction of chlorophyll but it is also contributing for a higher selectivity when compared to the system based on $[N_{1,1,1,10}]Br$. As can be seen in Figure 2, by the UV−vis absorption spectra and the photographs, there is a higher contamination of chlorophyll in the fucoxanthin-rich phase in the systems with $[N_{1,1,1,10}]Br$, showing the less efficient separation of the pigments. On the other hand, the bottom phase of the system with $[P_{4,4,4,14}]Cl$ (fucoxanthin-rich phase) is almost free of chlorophyll (only 3.9 ± 0.2 $mg.L^{-1}$, which corresponds to 2.11% of the initial amount of chlorophyll).

Aiming at simplifying the methodology, the previous assays were replicated but replacing the two-step procedure by a combined approach of extraction and purification in a single step. Briefly, fresh (i) aqueous solutions of $[N_{1,1,1,10}]Br$ or $[P_{4,4,4,14}]Cl$, (ii) vegetable oil, and (iii) biomass were mixed together under the same conditions of the agitation and IL/oil ratio previously used in the two-step approach. After the extraction, a centrifugation step was carried out, and as the previous, a liquid−liquid extraction system was obtained but with the biomass deposited as a solid pellet in the bottom of the vial. Subsequently, the quantification of chlorophylls and fucoxanthin released from the biomass and partitioned between the phases was carried out with the results presented in Table 1.

Table 1. Comparison between the Single-Step and the Two-Step Approaches in Terms of Yield of Extraction of Fucoxanthin and Chlorophyll for the Systems Based on $[N_{1,1,1,10}]Br$ and $[P_{4,4,4,14}]Cl$[a]

approach	yield of extraction of fucoxanthin ($\mu g_{fuco} \cdot g_{dry\ biomass}^{-1}$)	yield of extraction of chlorophyll ($mg_{chl} \cdot g_{dry\ biomass}^{-1}$)
$[N_{1,1,1,10}]Br$		
two-step	1397.4 ± 3.2[a]	1.649 ± 0.023[b]
single-step	1289 ± 18[a]	4.70 ± 0.23[a]
$[P_{4,4,4,14}]Cl$		
two-step	1376 ± 79[a]	2.88 ± 0.11[b]
single-step	1226 ± 91[b]	4.04 ± 0.54[a]

[a]Different letters represent statistically different values ($p < 0.05$). The analysis was performed considering a comparison of significance in the yield of extraction of each pigment, in separate, using the two different procedures proposed for the same IL.

Table 1 shows that the yield of extraction of fucoxanthin using the $[P_{4,4,4,14}]Cl$-based system decreased when the single-step approach was applied, while no significant changes were observed using the $[N_{1,1,1,10}]Br$-based system. In the other hand, the yield of extraction of chlorophyll was enhanced for the $[N_{1,1,1,10}]Br$- and $[P_{4,4,4,14}]Cl$-based systems using the single-step approach. Even though the two-step procedure showed the best results in extracting fucoxanthin when systems with $[P_{4,4,4,14}]Cl$ were used, considering the simplicity of the process and the lower energy spent, the single-step approach turned out to be the most promising.

The aqueous solution of the IL has allowed to decrease the viscosity of the system (in comparison with oil alone), allowing to demonstrate the advantages of combining solvents to enhance the yield of extraction of chlorophyll (in comparison with IL aqueous solution alone) and allowing the simultaneous separation of two different classes of pigments, which have no precedent in the scientific literature.

Optimization of the Process Operational Conditions. The optimization of the process conditions was performed considering (X_1) the volume fraction of the aqueous solution of IL in the system ($\%_{IL}$ in %), (X_2) the concentration of IL in water (C_{IL} in mM), (X_3) the solid−liquid ratio (SLR in $g_{dry\ biomass} \cdot mL_{solvent}^{-1}$), and ($X_4$) the time of extraction (t in min) based on a CCDR (2^4). Liquid−liquid extraction systems were obtained for all tested conditions for both the $[N_{1,1,1,10}]Br$ (Table S1 in the Supporting Information) and $[P_{4,4,4,14}]Cl$ (Table S2 in the Supporting Information). The same behavior was again observed, a yellowish bottom phase (IL-rich phase) and a greenish top phase (oil-rich phase), and the biomass was recovered as a solid pellet at the bottom of the vial.

The optimization was planned considering the single-step approach, the yield of extraction of fucoxanthin and chlorophylls (expressed in $\mu g_{fuco} \cdot g_{dry\ biomass}^{-1}$ and $mg_{chl} \cdot g_{dry\ biomass}^{-1}$, respectively) being the dependent responses used on the predictive model. The yields of extraction of the pigments experimentally that are determined are shown in Tables S1 and S2 in the Supporting Information along with the respective conditions of extraction. The model was fitted using pure error with the confidence level fixed at 95% in order to guarantee its high predictability. The parameters that are not statistically significant were incorporated into the lack of fit for calculation of the R^2 and F ratio. In each assay, the optimum conditions were chosen by the interpretation of the respective response surfaces.

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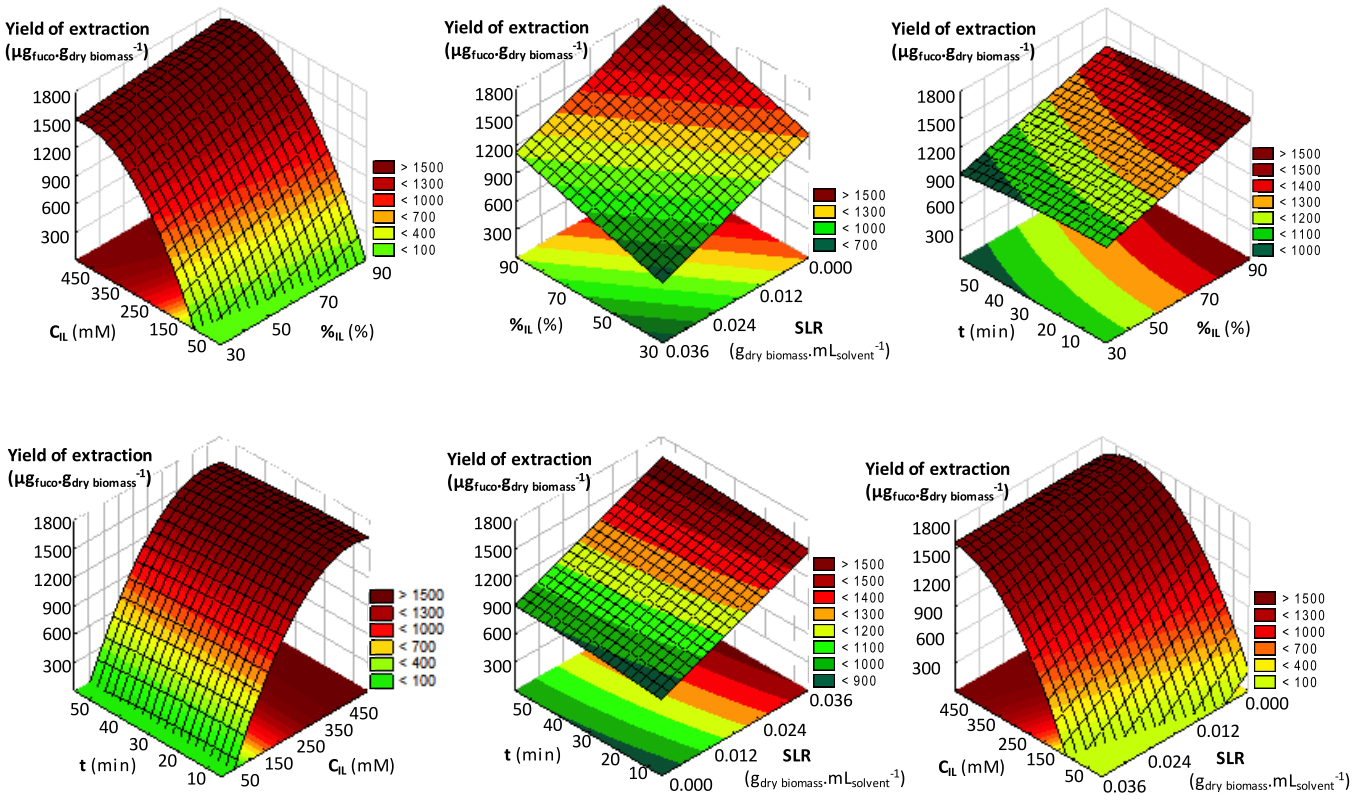

Figure 3. Response surface plots obtained for the CCRD (2^4) using a system with $[N_{1,1,1,10}]Br$ regarding the content of aqueous solution of IL ($\%_{IL}$ in %), time (t in min), IL concentration (C_{IL} in mM), and solid−liquid ratio (SLR in $g_{dry\ biomass}.mL_{solvent}^{-1}$) in terms of yield of extraction of fucoxanthin ($\mu g_{fuco}.g_{dry\ biomass}^{-1}$).

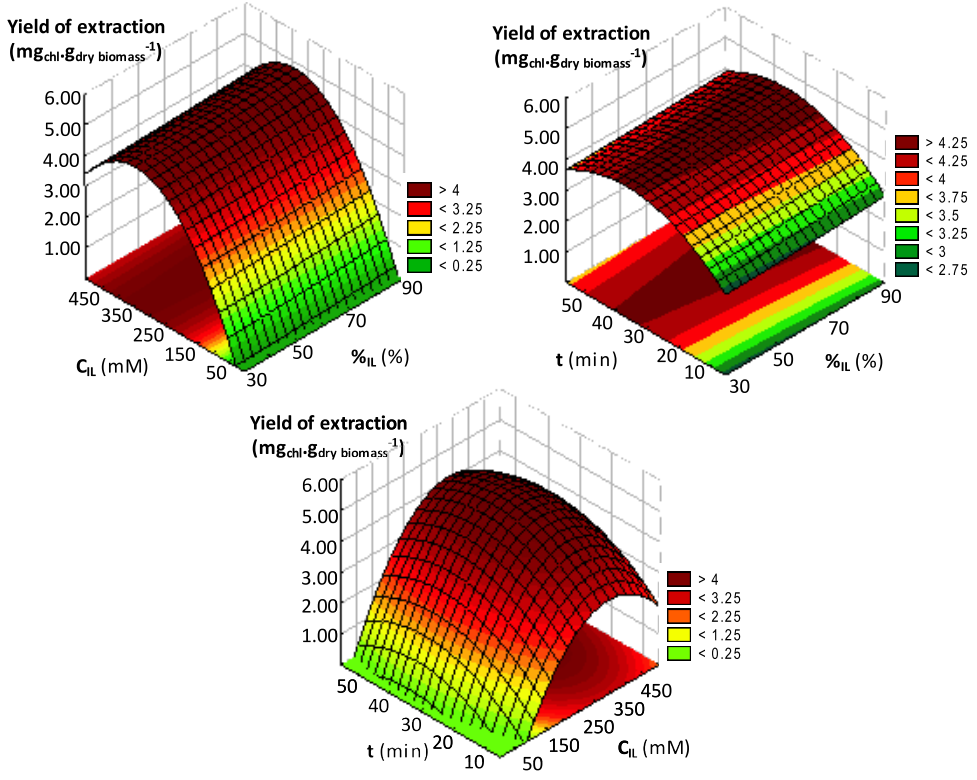

Figure 4. Response surface plots obtained for the CCRD (2^4) using the system based on an aqueous solution of $[N_{1,1,1,10}]Br$ regarding the content of aqueous solution of IL ($\%_{IL}$ in %), time (t in min), and IL concentration (C_{IL} in mM) in terms of yield of extraction of chlorophyll ($mg_{chl}.g_{dry\ biomass}^{-1}$). Graphs regarding the SLR are not depicted since this condition is not significant in this context.

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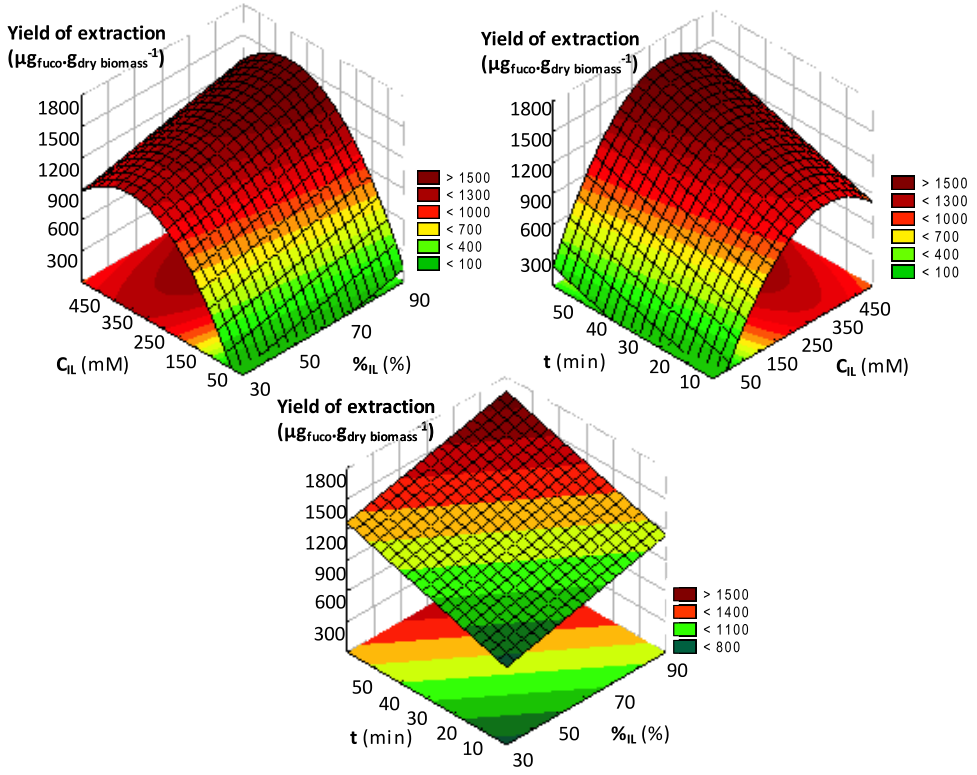

Figure 5. Response surface plots obtained for the CCRD (2^4) using a system with $[P_{4,4,4,14}]Cl$ regarding the content of aqueous solution of IL ($\%_{IL}$ in %), time (t in min), and IL concentration (C_{IL} in mM) in terms of yield of extraction of fucoxanthin ($\mu g_{fuco} \cdot g_{dry\ biomass}^{-1}$). Graphs regarding the solid−liquid ratio are not depicted since the condition is not significant in this context.

Regarding the $[N_{1,1,1,10}]Br$-based systems, the yield of extraction of fucoxanthin ranged between 133.4 and 1687.4 $\mu g_{fuco} \cdot g_{dry\ biomass}^{-1}$. As depicted in eq 4 and demonstrated in the predicted vs observed graph and Pareto chart (Figures S4 and S5 in the Supporting Information), three variables were significant in considering the extraction efficiency of carotenoids, namely, $\%_{IL}$ (X_1), C_{IL} (X_2), SLR (X_3), and the interaction between C_{IL} and SLR.

$$\text{yield of extraction}(\mu g_{fuco} \cdot g_{dry\ biomass}^{-1}) = -542 + 13.1(X_1) + 9.2(X_2) - 36{,}769.1(X_3) + 71.6(X_2 \cdot X_3) \quad (4)$$

In these assays, $R^2 = 0.98$ was achieved with an F-calculated value at 649 (~216-fold higher than the tabulated F), showing a high-predictive model at a 95% confidence level. The results depicted in Figure 3 evidence that the aqueous solution of $[N_{1,1,1,10}]Br$ at 400 mM in a system composed of 84% of aqueous solution of IL (and 16% of oil), with homogenization fixed in 30 min and the SLR at 0.017, provides the highest yield of extraction ($1836 \pm 36\ \mu g_{fuco} \cdot g_{dry\ biomass}^{-1}$). The accuracy and precision of the model were performed by a validation experimental test at the optimum operational conditions with a low deviation (2.21%) compared to the predicted results (Table S6 in the Supporting Information). Also, the predictive vs experimental data demonstrates a high confidence of the obtained results, guaranteeing the reproducibility of the process in a high-confidence level (Figure S4 and Table S6, respectively, in the Supporting Information).

The second response that was evaluated was the yield of extraction of chlorophyll. The main effects and interactions were estimated for the yield of extraction of chlorophyll, resulting in eq 5. An R^2 value of 0.78 was obtained, showing that the predictive model could be achieved. The F value was approximately 70-fold higher than the respective tabulated F. The pure error was acceptable to generate a predictive model for the yield of extraction chlorophylls using an aqueous solution of $[N_{1,1,1,10}]Br$, and the response surfaces are plotted in Figure 4.

$$\text{yield of extraction}(mg_{chl} \cdot g_{dry\ biomass}^{-1}) = -2.95 + 0.00003(X_1)^2 + 0.3146(X_2) - 0.00005(X_2)^2 + 0.12391(X_4) - 0.00172(X_4)^2 \quad (5)$$

By the analysis of the Pareto chart (Figure S7 in the Supporting Information), the SLR is not a significant variable in the extraction of chlorophylls, contrarily to what was seen for the extraction of fucoxanthin, where the combination of the SLR with the other independent variables positively influences the response. Figure 4 also shows that when the C_{IL} ranges between 250 and 450 mM at a time of extraction of 30−40 min, the ratio of volumes of IL and oil ($\%_{IL}$) is almost indifferent, i.e., more or less oil can be used, allowing the manipulation of this parameter according to the application of the extracted pigment.

In conclusion, based on the data provided in Figures 3 and 4, the best operational conditions to be further applied were the $\%_{IL}$ of $[N_{1,1,1,10}]Br$ (aq) = 84% (consequently 16% of oil), C_{IL} = 400 mM, SLR = 0.017 $g_{dry\ biomass} \cdot mL_{solvent}^{-1}$, and t up to 30 min. Considering these operational extraction conditions, the predictive model was validated with a low deviation (3.44%, Table S7 in the Supporting Information).

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These data are in agreement with the predicted vs observed graph and Pareto chart (Figures S6 and S7 in the Supporting Information, respectively), which showed the main influence of the C_{IL} and the lack of influence of the parameter SLR in the response, showing that the whole process was optimized under the expected predictions. The single-step approach using $[P_{4,4,4,14}]Cl$ was also optimized. A coefficient of determination (R^2) of 0.86 indicated a good agreement of the model eq 6 with the experimental results (Figure S8 in the Supporting Information). The influence exerted by three independent variables on the extraction yield of fucoxanthin during the assays is displayed in the Pareto chart presented in Figure S9 in the Supporting Information. At a 95% confidence level of significance, the linear effect of C_{IL} was the most significant, followed by the negative quadratic effect of C_{IL}, linear effect of the time, and linear effect of $\%_{IL}$. These effects could be observed in the response surface plots (Figure 5), which clearly illustrate the combined interaction of the extraction operational conditions optimized. As depicted in Figure 5, the best response on the extraction of fucoxanthin was obtained by using 40 min of homogenization, a C_{IL} of 350 mM, and the highest $\%_{IL}$ (84%). This model has a high predictive accuracy since the optimal conditions of fucoxanthin extraction were validated by a low relative deviation (1.61%, Table S8 in the Supporting Information).

$$\text{yield of extraction}(\mu g_{fuco} \cdot g_{dry\ biomass}^{-1}) = -714.284 + 6.513(X_1) + 7.934(X_2) - 0.011(X_2)^2 + 8.527(X_4) \quad (6)$$

Regarding the extraction of chlorophyll using the $[P_{4,4,4,14}]Cl$, the model was not considered as predictive. This means that any change in the studied operational conditions is not statistically significant to improve the yield of extraction of chlorophyll. However, at the optimum conditions to extract fucoxanthin, the biomass residues at the end of the process are almost colorless, suggesting a complete extraction of the pigments, including chlorophylls (Figure S10 in the Supporting Information). After a careful analysis, the best operational conditions for each system as well as the results obtained are summarized in Table 2 and Figure S11 in the Supporting Information.

Most works focusing on the extraction of bioactive molecules from algae use multiple operations. As an example in a recent work using *Spirulina* sp.,[49] supercritical CO_2 was used to recover in separate steps carotenoids, chlorophylls, and phycocyanins. The process proposed here, while using a simple approach, allows the simultaneous extraction and purification of the two main pigments present in the alga studied, both of high interest and commercial value. In addition to the stability of carotenoids provided by ILs (e.g., fucoxanthin,[50] all isomers of lycopene,[51] and all isomers of carotene[52]), the oil fraction rich in hydrophobic compounds is usually more thermally stable than aqueous and ethanolic extracts. This guarantees the pigment stability, allowing its higher shelf time and thus increasing the range of possibilities for new products (e.g., as emulsifiers or supplements) or even loaded in the formulation of new biomaterials.[27,53] Considering the purity of the fractions, in comparison to the initial screening (Figure S3 of the Supporting Information), the spectra depicted in Figure S11 of the Supporting Information clearly show the purification of both pigments during the process, presenting spectra very similar to the pure pigments, as can be checked in the literature.[54,55] In the end, considering not only the final results obtained after the optimization but also the higher selectivity, the time saved, and the scalable potential, the single-step approach based on $[P_{4,4,4,14}]Cl$ was selected for complementary studies, envisioning the design of a complete process.

Table 2. Optimized Operational Conditions for the Systems Composed of Aqueous Solutions of $[N_{1,1,1,10}]Br$ and $[P_{4,4,4,14}]cl$ and Oil Plus the Respective Results in Terms of Yields of Extraction of Fucoxanthin and Chlorophyll[a]

parameters	$[N_{1,1,1,10}]Br$	$[P_{4,4,4,14}]Cl$
operational conditions		
$\%_{IL}$ (%)	84	84
C_{IL} (mM)	400	350
SLR ($g_{dry\ biomass} \cdot mL_{solvent}^{-1}$)	0.017	0.017
t (min)	30	40
results		
yield of extraction of fucoxanthin ($\mu g_{fuco} \cdot g_{dry\ biomass}^{-1}$)	1836 ± 54[a]	1956 ± 84[a]
yield of extraction of chlorophyll ($mg_{chl} \cdot g_{dry\ biomass}^{-1}$)	4.528 ± 0.079[b]	4.93 ± 0.22[a]
contamination of chlorophyll in the IL-rich phase ($mg \cdot L^{-1}$)	10.48 ± 0.40[b]	8.76 ± 0.42[a]

[a]Different letters represent statistically different values ($p < 0.05$). The analyses were carried out separately for each result to allow the comparison of systems based on different ILs.

IL Recovery. Aiming to decrease the environmental and economic impacts of the process, it is imperative to define a strategy to recover and reuse the IL, that is, the costliest solvent used. The aqueous IL-rich (bottom) phase was separated from the oil-rich (top) phase. Then, the IL content was measured in the IL-rich phase and in the respective bottom phase of the control represented by the system where no biomass was used. The same amount of IL initially added to the system (oil + IL + water) was quantified in the IL-rich phase, meaning that the oil-rich phase is free of IL, which consequently indicates that the chlorophyll-based extract is also free of IL.

Regarding the aqueous IL-rich phase, a back-extraction was applied to remove the fucoxanthin from the aqueous phase of $[P_{4,4,4,14}]Cl$, thus allowing the IL recovery. Toluene, ethyl acetate, and diethyl ether were chosen due to their immiscibility with water and approved industrial application despite the need for explosive atmosphere-certified facilities (ATEX) and subsequent capital expenditure involved. Each organic solvent was individually added to the aqueous phase of IL, these mixtures being homogenized and centrifuged to allow the phase separation and the pigment partition. The results are presented in Table 3.

Table 3. Results Obtained for the Pigment Partition to the Organic Phase and the % of the IL Recovered after Back-Extraction for Each Organic Solvent Tested

System composed of IL-rich phase + organic solvent	Complete pigment extraction to the organic phase	% IL remaining in the aqueous phase
Diethyl ether	No	
Ethyl acetate	Yes	30%
Toluene	Yes	82%

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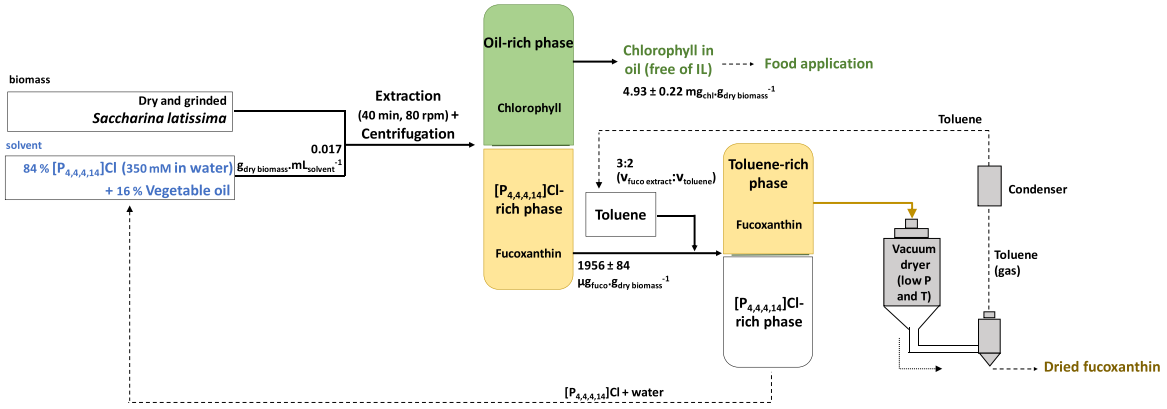

Figure 6. Conceptual process diagram proposed for the recovery of chlorophyll and fucoxanthin from *S. latissima* (Linnaeus). Dashed lines are a suggested process but have not been tested in this work.

All systems were able to form two immiscible phases. In the case of diethyl ether, the pigment partitioned equally between the two phases, meaning that the organic phase (diethyl ether-rich phase) was not able to recover the total content of the pigment. On the other hand, in the systems composed of ethyl acetate and toluene, all pigment content has partitioned to the organic phase, leaving the aqueous phase completely clear. For the systems with the complete partition of pigments for the organic phase, i.e., ethyl acetate and toluene, the presence of IL was identified and further quantified by its content in chloride ion using an ion selective electrode. According to the results, 30 (= 105 mM) and 82% (= 287 mM) of the initial amount of IL within the system (350 mM) remained in the aqueous phase for systems composed of ethyl acetate and toluene, respectively. This means that when fucoxanthin partitions toward the ethyl acetate phase, around 70% of the IL also partitions to the ethyl acetate phase. However, the highest recovery results were obtained using toluene, which proved to be a good candidate to recover the IL content (more than 80% of the initial amount of IL remained in the aqueous phase and can be reused).

Final Conceptual Process. Figure 6 represents the final conceptual process proposed to recover the pigments from *S. latissima* (Linnaeus) based on the results obtained in this work. This final process is composed of a (i) single-step approach to simultaneously extract and separate fucoxanthin and chlorophylls, at this point the chlorophyll-rich phase (oil phase) being ready for further use in food applications, for example, since it is free of IL; (ii) a back-extraction using toluene allowing the IL recovery; and lastly, (iii) a vacuum drier that is proposed as a method to recover fucoxanthin[56] from the toluene phase at low pressures and temperatures (35 °C) to avoid the carotenoid degradation, being thus used in and allowing the recovery and reuse of the organic solvent, closing the recycle loops of the process. Since the recovery of the pigments from toluene was not carried out experimentally, there is no detailed information about toluene loss or possible contamination of the product with toluene, although the use of this type of technique to eliminate the organic solvent is normally recognized as very efficient.[57]

As an example of pigments in oil for food application, different products are available in the market such as "liquid chlorophyll super concentrated" used as food supplements in glycerin. Following the same rationale, but with another pigment, a mayonnaise-like food using carotenoid-rich oil obtained from an Amazonian fruit was developed with enhanced biological properties.[5] Additionally, the analysis of the fucoxanthin powder was not performed; however, it may contain trace elements of IL and/or toluene, this analysis being needed before application.

The scale-up trials shall also include fresh material either from *S. latissima* (Linnaeus) or from other industrially relevant biomass as a source of fucoxanthin and chlorophylls.

Life Cycle Assessment. The results obtained using the ReCiPe 2016 Midpoint method at the Hierarchist perspective[58] to translate environmental emissions and resource extraction into environmental impacts are presented in Table 4 and Figure 7.

Table 4. Life Cycle Assessment Results for the Recovery of Chlorophyll and Fucoxanthin from 0.2 g of Dry Biomass of *S. latissima* (Linnaeus)

impact category	no IL reuse	IL reuse
global warming (g CO_2 eq)	35.5	31.4
ozone formation, human health (g NO_x eq)	0.0924	0.0828
terrestrial acidification (g SO_2 eq)	0.181	0.166
fossil resource scarcity (*g oil eq*)	15.3	13.3

The impact categories selected for analysis comprise the global warming (equivalent to the carbon footprint), photochemical ozone formation (effects on human health), terrestrial acidification, and fossil resource scarcity. The main

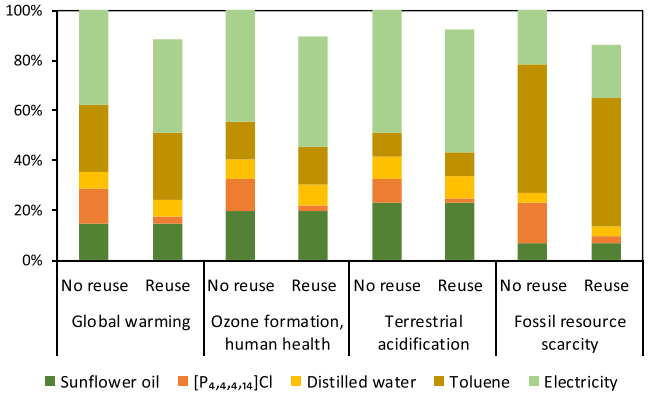

Figure 7. Relative contribution of the inputs for the life cycle assessment results.

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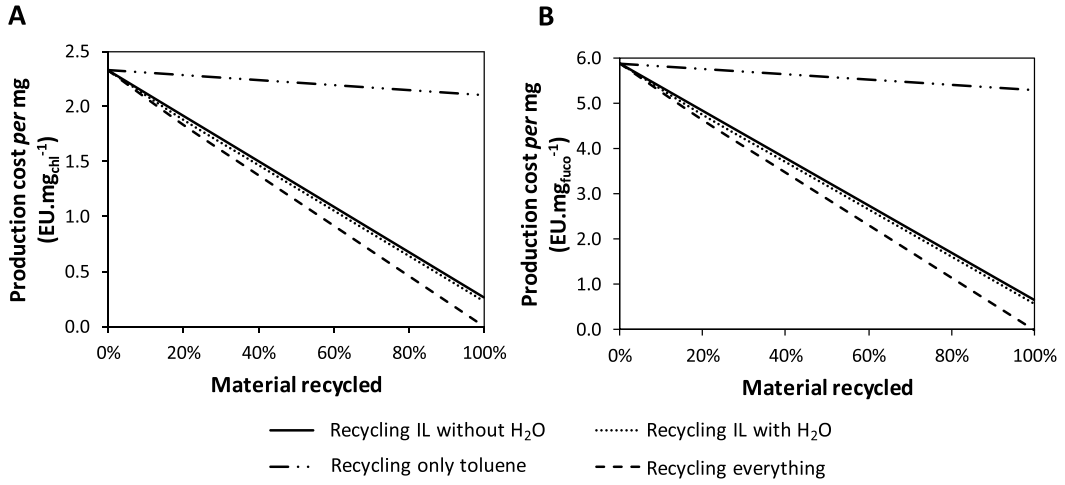

Figure 8. Results for the economic evaluation of the production process for (A) chlorophyll and (B) fucoxanthin. For both graphs, all lines start from a single data point (no recycling of any of the solvents) and spread across the graphs depending on the percentage of materials recycled and the recycling scenario.

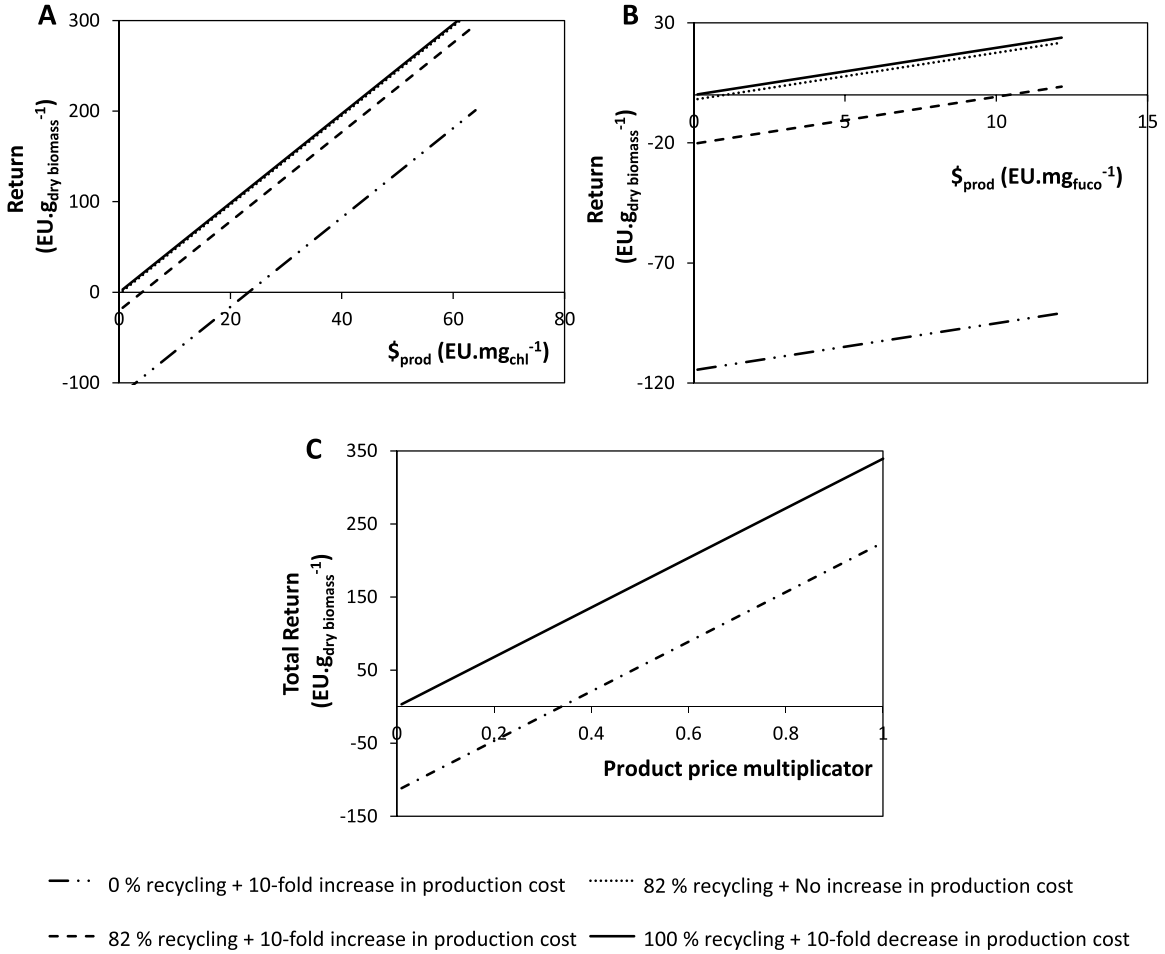

Figure 9. Return analysis of (A) chlorophyll, (B) fucoxanthin, and of (C) both products. $\$_{prod}$ (market price) of each pigment is based on Sigma-Aldrich values. In (A), the solid line almost overlaps the dashed line. In (C), the *x* axis was changed to the product price multiplier (0.01, 0.1, and 1×).

contribution to the impacts other than fossil resource scarcity is the electricity consumption by the equipment, which amounts to 38−49% of the total impacts. For fossil resource scarcity, toluene has the major role not only due to the use of fossil-based energy during its production but also because it is produced from naphtha. The reuse of the IL leads to a reduction of the environmental impacts in the order of 8−14% due to savings of fresh IL.

Economic Analysis. After assessing the environmental impact of the overall process proposed in this work, the

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economic footprint was evaluated, envisioning a more complete analysis for the potential scale up of the process. The results from the calculation of production costs per mg of each pigment are depicted in Figure 8. The complete collection of results for all scenarios (production costs per mg of each pigment with and without recycling of solvents with all recycling scenarios and percentages) are included in Table S9 in the Supporting Information.

The production cost (capital expenditure, operational expenditure, i.e. materials/consumables, labor, and others) for the process depicted in Figure 6 is 2.33 and 5.86 EU per mg for chlorophyll and fucoxanthin, respectively. After including the recycling scenarios, the production costs decrease (Figure 8). Results for chlorophyll and fucoxanthin show that recycling toluene, even at 100%, has the lowest impact on the production costs. For a deeper interpretation, even recycling only 20% of the ILs grants a lower production cost. Preliminary recycling results presented in previous sections showed that it was possible to recover up to 82% of IL. If it is possible to re-use this amount, then this will provide a complete production cost lower than 0.64 and 1.61 EU per mg for chlorophyll and fucoxanthin, respectively. As a framework, recycling 100% of IL, water, and toluene will further decrease the production costs providing the minimum cost for this process.

For the return, eqs 2 and 3 provided a deep insight on the impact that the production costs can have on the profit. For this part of the analysis, the variables studied (not fixed values) were the percentage of the recycled material (0−100%), the product market price (base price and 10- and 100-fold decrease), and the multiplier of production costs (10-fold increase and decrease). Full results are included in the Supporting Information (Tables S10 and S11), while the most relevant results are depicted in Figure 9.

For the calculation of production costs, all operation units were considered for both products even if, to get each product, only a smaller set of unit operations was needed. This is because the design presented in Figure 6 generates chlorophyll and fucoxanthin together, so both paths are going to be completed each batch. This indicates that both products have the same behaviors seen in Figure 8A,B. Although they behave similarly, their absolute values are different. Contrarily, the results for return consider different product prices, so the potential profit can be substantially different. This differential behavior can be seen when contrasting Figure 9A,B; this is captured by the slopes of the lines depicted here. Moreover, the relevance of Figure 9 is that both lines show the boundaries of the analysis. For both graphs, the top line shows the best scenario; this is 100% of the material is recycled and production costs are decreased 10-fold, while the bottom line is the worst scenario, where no material is recycled and costs increased 10 times. Both graphs show in the x axis the product market prices with a 10- and 100-fold decrease. Additionally, Figure 9C shows a potentially more real scenario. As both products are generated simultaneously, production costs are shared, so the profit will actually be higher overall, as shown in this graph.

With the help of the boundaries shown in Figure 9, it is possible to determine that most combinations of other scenarios will be found in between both lines. Figure 9A shows that a chlorophyll market price above 20 EU per mg is required to have all scenarios with a positive return. If this condition is met, then recycling is not needed to have a positive outcome. As the chlorophyll price decreases, either the recycling percentage needs to increase or production costs to decrease (or a combination of both). For fucoxanthin, the results seem to be different. There is a wide range of scenarios where it is not possible to have a positive outcome. Considering the base product price for fucoxanthin, a positive return is possible even without recycling, but if production costs increase 10-fold, then a recycling of 80% of the material is needed for a positive return (1.16 EU·$g_{dry\ biomass}^{-1}$). Meanwhile, for analyzing the product price, if it decreases 10-fold while production costs stay at the base level, then a recycling of 100% will be required (0.23 EU·$g_{dry\ biomass}^{-1}$). If the worst cases for the production cost (10-fold increase) and product price (10-fold decrease) are considered, then a 100% recycling is needed to have a positive outcome (0.19 EU·$g_{dry\ biomass}^{-1}$). As a contrast for the results for fucoxanthin, Figure 9C shows that when both products are considered simultaneously, the low return that fucoxanthin provides is greatly improved by the return obtained from chlorophyll. Moreover, the combined effect (total return) increased the range of possible scenarios that can grant a positive Return to the final process.

The experimental results showed that possibly 82% of the material can be recycled. This scenario is considered in both graphs (Figure 9A,B) while maintaining the base production costs and product price. Overall, it is possible to obtain a positive return while maintaining realistic values for the rest of the variables. This serves as a platform to base further developments in this area. In addition, it should also be considered that the remaining biomass has potential to be applied, either as a final product as feed or fertilizer or as a matrix to extract other biomolecules, which again will improve the value of the biomass in a biorefinery (multi-product) chain.

CONCLUSIONS

In this work, a single-step approach to extract and separate chlorophyll from fucoxanthin was proposed. A mixture composed of an aqueous solution of a tensioactive IL and a vegetable oil was used together with dry biomass in the preparation of a liquid−liquid extraction system, the oily phase rich in chlorophyll and the IL phase mainly composed of fucoxanthin. After selecting the best systems to extract the pigments from the brown algae ($[N_{1,1,1,10}]Br$ and $[P_{4,4,4,14}]Cl$), the operational process conditions were optimized, statistically analyzed, and validated. The best performance were achieved for systems with $[P_{4,4,4,14}]Cl$ with $\%_{IL}$ = 84% ($\%_{oil}$ = 16%), C_{IL} = 350 mM, SLR = 0.017 $g_{dry\ biomass}$·$mL_{solvent}^{-1}$, and t = 40 min leading to yields of extraction of 4.93 ± 0.22 mg_{chl}·$g_{dry\ biomass}^{-1}$ and 1955.7 ± 84.4 μg_{fuco}·$g_{dry\ biomass}^{-1}$. Besides the recovery of two different pigments with a high commercial value in a single- step approach, a recovery of up to 82% (= 287 mM) of the IL from the fucoxanthin phase was also achieved. In the end, this work provides an optimized, simple, and efficient process to extract and purify the hydrophobic pigments from *S. latissima* (Linnaeus). The scale up of this process being straightforward, its industrial potential is envisioned, which is supported by both environmental and economic analyses.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acssuschemeng.0c09110.

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Molecular structures of ILs and their respective critical micelle concentration, calibration curves, detailed values, and calculations for economic analysis; photographs and UV−vis spectra of the extracts obtained in the screening of solvents; detailed analyses of the central composite rotatable designs; photographs of the proposed final systems; and details on life cycle assessment (PDF)

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Notes

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