A review on microalgae cultivation and harvesting, and their biomass extraction processing using ionic liquids

Jia Sen Tan\textsuperscript{b}, Sze Ying Lee\textsuperscript{c}, Kit Wayne Chew\textsuperscript{d}, Man Kee Lam\textsuperscript{e,f}, Jun Wei Lim\textsuperscript{f,g}, Shih-Hsin Ho\textsuperscript{h}, and Pau Loke Show\textsuperscript{a}

\textsuperscript{a}Department of Chemical and Environmental Engineering, Faculty of Science and Engineering, University of Nottingham Malaysia, Semenyih, Malaysia; \textsuperscript{b}Department of Biotechnology, Faculty of Applied Science, UCSI University, Kuala Lumpur, Malaysia; \textsuperscript{c}Department of Chemical Engineering, Lee Kong Chian Faculty of Engineering and Science, Universiti Tunku Abdul Rahman, Sungai Long Campus, Kajang, Malaysia; \textsuperscript{d}School of Mathematical Sciences, Faculty of Science and Engineering, University of Nottingham Malaysia, Selangor, Malaysia; \textsuperscript{e}Chemical Engineering Department, Universiti Teknologi PETRONAS, Perak, Malaysia; \textsuperscript{f}Centre for Biofuel and Biochemical Research, Institute of Self-Sustainable Building, Universiti Teknologi PETRONAS, Seri Iskandar, Malaysia; \textsuperscript{g}Fundamental and Applied Sciences Department, Universiti Teknologi PETRONAS, Seri Iskandar, Malaysia; \textsuperscript{h}State Key Laboratory of Urban Water Resource and Environment, School of Environment, Harbin Institute of Technology, Harbin, China

ABSTRACT
The richness of high-value bio-compounds derived from microalgae has made microalgae a promising and sustainable source of useful product. The present work starts with a review on the usage of open pond and photobioreactor in culturing various microalgae strains, followed by an in-depth evaluation on the common harvesting techniques used to collect microalgae from culture medium. The harvesting methods discussed include filtration, centrifugation, flocculation, and flotation. Additionally, the advanced extraction technologies using ionic liquids as extractive solvents applied to extract high-value bio-compounds such as lipids, carbohydrates, proteins, and other bioactive compounds from microalgae biomass are summarized and discussed. However, more work needs to be done to fully utilize the potential of microalgae biomass for the application in large-scale production of biofuels, food additives, and nutritive supplements.

ARTICLE HISTORY
Received 20 September 2019
Revised 12 December 2019
Accepted 12 December 2019

KEYWORDS
Microalgae; cultivation; harvesting; extraction; ionic liquids; downstream processing

Introduction
Algae are a group of photosynthetic autotrophs which normally thrives in various types of water bodies such as lakes, rivers, and sea. They are accounted for producing atmospheric oxygen via photosynthesis, which is a process that converts water and carbon dioxide into carbohydrate using solar energy. Algae group is diverse and encompasses numerous different phyla with their own unique characteristics and properties ranging from prokaryotic single cellular cyanobacteria to more complex multicellular eukaryotic algae, as summarized in Table 1.

Under the group of algae, microalgae are unicellular microorganisms which thrive in both saltwater and freshwater environments. Despite the absence of complex structure and organs when compared to their plant cousins, microalgae are able to perform...
photosynthesis using sunlight, carbon dioxide and water owing to the presence of photosynthetic pigments such as chlorophylls in their cells. Microalgae species have provided a limitless opportunity for being utilized in different sectors for the benefits of mankind [1]. Human has cultivated microalgae such as *Spirulina* for their nutritive properties [2]. Besides, microalgae contain various valuable bioactive compounds which can be derived from their cells, including lipids, proteins, carbohydrates, carotenoids, and vitamins. These valuable bioactive compounds can be widely used in commercial applications.

However, many microalgae species still remain underutilized and numerous researchers have explored many different possibilities to fully utilize microalgae. Ionic liquids (ILs) have emerged among other candidates which appreciate the vast amount of precious bio-compounds that reside within microalgae. The tunable solvent properties of ILs enable them to extract a wide range of bio-compounds from microalgae biomass at a comparative raw material and energy utilization compared to the conventional method available. Therefore, besides evaluating the technologies being studied recently for microalgae cultivation and harvesting, this review focuses on the extraction of bio-compounds from microalgae using ILs as extractive solvents. To be more specific, the use of ILs as extractive solvents using different techniques such as microwave-assisted IL extraction, ultrasound-assisted IL extraction, and IL-based aqueous biphasic systems (ABS) are discussed, and the recent works are summarized.

**Microalgae cultivation**

Microalgae are capable to grow rapidly. Their high photosynthesis efficiency coupled with the ability to accumulate a large amount of bioproducts within their cells make them a suitable candidate to serve as industrial raw material [1]. Besides, cultivation of microalgae does not require fertile land, a large quantity of freshwater, and herbicides and pesticide when compared to the other crops and thus will not be competing for resources [3].
Furthermore, cultivation of microalgae can even be performed using wastewater such as domestic sewage water and palm oil milling effluents which can assist in bioremediation of wastewater [4,5]. Apart from wastewater treatment, cultivation of microalgae can also help with reduction of atmospheric carbon dioxide through photosynthesis, effectively contributing to the efforts of tackling greenhouse effect and global warming. Despite the benefits of microalgae cultivation, its developments are still plagued with various problems. For example, the low biomass production and the small size of cells when they are cultured in liquid medium render the harvesting process of microalgae very costly.

One of the ways to work around the shortcomings of usage of microalgae in the industry is by increasing their growth rate to compensate for their low cell density and difficulties in harvesting. Numerous equipment and technologies have been improved across the years to ramp up the production of microalgae. Although microalgae can be easily cultured in a highly controlled laboratory condition, however, it is still harder to ensure the high productivity of microalgae in large-scale production. An ideal microalgae culturing system should possess the characteristics, including: (1) adequate light source, (2) effective transfer of material across liquid-gas barrier, (3) simple operation procedure, (4) minimal contamination rate, (5) cheap overall building and production cost, and (6) high land efficiency [6]. In general, microalgae culturing system can be broadly classified into two categories, which are the open pond and photobioreactor. Each system has its pros and cons.

**Open pond**

Open pond cultivation has been one of the oldest and simplest ways to cultivate microalgae in large scale. Open pond is widely used in the industry due to its relatively cheaper construction, maintenance and operation cost. Other advantages of using open pond system include simplistic operation and maintenance, low energy demand, and ease to scale up [7]. There are few types of open pond, which includes natural water such as lakes and ponds, and artificial water bodies such as circular and raceway ponds. At some cases, a container such as a tank can also be used to culture microalgae [8]. Open pond system has the advantage of being the most cost-efficient cultivation system. However, despite the large cultivation area, cultivation of microalgae from natural water has relatively lower cell concentration and thus a highly efficient harvesting method is required [9]. In addition, issues such as rainwater runoff which affects the growth condition of microalgae such as salinity and pH, erosion of banks that resulting in leakage and increased water turbidity might significantly affect the productivity of microalgae in open ponds [10]. Another issue that presents in open pond cultivation system is the possibility of contamination by protozoa and bacteria which causes the products to be toxic and unusable. The current available way to circumcise this problem is by cultivating microalgae which are capable of surviving at extreme alkaline or saline condition since only a few contaminants are able to thrive under this condition [11,12]. Besides, due to the open nature of the system, it is harder to control certain growth parameters such as temperature and light intensity which might affect the growth rate of microalgae [11].

Harvesting of microalgae from naturally occurring sources has been one of the oldest microalgae cultivation techniques known to man. The oldest record of microalgae harvesting was done by Aztec peoples where they harvest *Spirulina* from Lake Texcoco located at Mexico [13]. This cultivation method highly depends on naturally occurring water bodies to provide right condition and nutrients for the growth of microalgae. Currently, one of the largest commercial cultivation of microalgae in natural water is located at Hutt Lagoon, Australia which is capable of producing 6 tons of β-carotene every year form *Dunaliella* using its 700-hectare ponds [10].

Circular ponds are the first artificial pond to be used in large-scale cultivation of microalgae. This cultivation system got its name from its circular-shaped culture tank, and typically have the depth of 30–70 cm and width of 45 m along with a rotating agitator located at the center of the pond [14]. The rotating agitator is being used to ensure efficient mixing and prevent sedimentation of algae biomass [15]. However, the design of this cultivation system is limited by its size since bigger pond might introduce stronger water resistance,
and therefore causes strain on the mechanical parts of agitator [16]. Moreover, this design has disadvantages for high energy usage in the agitation process and high construction cost [13]. Currently, this cultivation system has been used in Japan and Taiwan to culture *Chlorella* for consumption [14,17].

Raceway pond is one of the most frequently used open pond types for the cultivation of microalgae. It consists of a series of closed loop channel around 30-cm deep and paddlewheel which enable recirculation of microalgae biomass to ensure equal distribution of nutrients and prevent sedimentation of microalgae biomass. Raceway pond has been perceived as one of the best open pond cultivation design available due to its energy efficiency, as a single paddlewheel is sufficient enough to properly agitate a 5-hectare raceway pond [18]. One of the successful raceway pond cultivation is by Sapphire Energy’s Columbus Algal Biomass Farm located at Columbus, United States, which has successfully produced 520 metric tonnes of dried microalgae biomass during 2 years of its operation without any technical issue [19].

**Photobioreactor**

Photobioreactor is a bioreactor system used to culture phototrophs such as microalgae in an enclosed system which does not allow direct exchange of material between the culture and environment. Photobioreactor is able to overcome several constraints faced commonly by open pond culture design. First, the size of bioreactor is more compact compared to open pond, therefore providing more efficient land usage. Second, the system provides a closed and highly controlled growth condition for the culture, thus able to produce a contamination free, single strain microalgae culture [20]. In addition, the highly controlled culture condition can also translate into higher nutrient and metabolic efficiency which results in higher biomass production per unit of substrate. However, the bottleneck of practical usage of photobioreactor is its limited scalability due to various design flaws, rendering it uneconomical to be used in large-scale production [21]. Moreover, highly controlled growth condition of photobioreactor always comes with high capital and operating costs.

Tubular photobioreactor is comprised of transparent long tubes made out of glass or transparent plastics which are arranged in horizontal, vertical, helix, or slanted orientation to maximize capture of sunlight [13,22]. The microalgal culture is circulated within the loop by the means of mechanical pump or air lift system [23]. However, the biggest drawback of tubular photobioreactor design is in its poor mass transfer across the system since the long tube used in the bioreactor design might result in the differences in concentration of substrate and product along the tubes. Commercial usage of large-scale tubular photobioreactor is being used to produce *Haematococcus* and *Chlorella* at Germany and Israel, respectively [24].

Vertical column bioreactor is constructed by a transparent vertical cylindrical tubing and a sparger which pumps in air bubbles to enable homogenization of the culture and allow transfer of carbon dioxide and oxygen between air and microalgae culture [25]. This culture system offers the best gas-liquid mass transfer efficiency compared to other system owing to the capability of the sparger used in this system to generate smaller bubbles which provide larger total surface area for more efficient transfer of substance [26]. In addition, the simplicity of the design allows it to have lower energy demand and simpler operating procedure. However, the cylindrical-shaped container does not provide adequate amount of light required by the microalgae to perform photosynthesis efficiently. Moreover, high construction cost and difficulties in cleaning the reactor make commercial usage of vertical column photobioreactor difficult [27]. Commercial usage of this photobioreactor design has yet to be found, however, numerous large-scale experimental reactor has been made including a 40-liter vertical column outdoor photobioreactor for the cultivation of *Chlorella zofingiensis* at Guangdong, China [28].

Flat-plate photobioreactor on the other hand is characterized by its rectangular-shaped compartment made out of transparent material with the depth between 1 and 5 cm. The culture inside the reactor is mixed via the means of recirculating airlift system [29]. This design supports the largest total surface area for illumination and low oxygen build up, thus achieving the highest photosynthetic
efficiency out of all photobioreactor design [30]. However, the aeration design of this photobioreactor causes stress damage to the microalgae cells [31]. Numerous innovative design has been implemented to further improve the efficiency of the flat-plate photobioreactor, such as twin layer flat-plate and plastic sheets photobioreactors [32]. Commercial usage of flat-plate photobioreactor has been used to produce astaxanthin by Algamo company located at Krkonoše, Czech Republic.

Harvesting of microalgae
Harvesting of microalgae is one of the main parts in microalgae processing. Several studies have suggested that it makes up 20–30% of the total production cost due to high energy demand and capital cost [33,34]. In general, all harvesting techniques aim to remove as much culture media from the microalgae biomass to facilitate next downstream processing such as extraction of bioactive compounds. Numerous harvesting methods have been used to collect biomass, including filtration, centrifugation, flocculation, and flotation [35]. For some circumstances, a combination of two or more techniques are employed to further increase harvesting efficiency. Table 2 presents several harvesting approaches and their respective target microalgae species, advantages, and disadvantages.

**Filtration**
Filtration process utilizes a semipermeable membrane which can retain microalgae on the membrane while allowing the liquid media to pass through, leaving the algae biomass behind to be collected [50]. This method can harvest high concentration of cell from the medium, and the varying pore size of the filter membrane enables the system to suit the need of different microalgae and are able to handle the more delicate species which are prone to damage due to shearing. However, this method is very prone to fouling and clogging and therefore requires frequent change of fresh filter or membrane that might contribute significantly to its processing cost [51]. In view of this constraint, filter membrane using cheap and easily accessible material has been developed. Bejor and his colleagues [52] have successfully developed a filter membrane made out of stretch cotton which can achieve harvesting efficiency of 66–93%.

| Harvesting technique | Microalgae species | Advantage | Disadvantage | Reference |
|----------------------|-------------------|-----------|--------------|-----------|
| Cross flow filtration| Chlorella sp.      | High energy efficiency | Prone to membrane fouling and shearing of fragile materials | [36]       |
| Axial vibration membrane filtration | Chlorella pyrenoidosa | Reduced membrane fouling | Require power-consuming pumping units | [37]       |
| Polyacrylonitrile-based membrane filtration | Scenedesmus and Phaeodactylum | Reduced membrane fouling | Require power-consuming pumping units | [38]       |
| Tilted membrane panel filtration | Wild microalgae strain | Reduction of membrane cost, and energy consumption | Membrane fouling | [39]       |
| Ultrafiltration | Dunaliella salina | Less cell shearing, low energy and chemical consumption | High capital cost | [40]       |
| Electro-flocculation | D. salina | Cost efficient and chemical free | High energy demand | [41]       |
| Plant bio-flocculation (Moringa oleifera) | Chlorella sp. | Cost efficient and limited toxicity | Contamination of microalgae products | [42]       |
| Microbial bio-flocculation | Desmodesmus brasiliensis | Cost efficient and biodegradable | Contamination of microalgae products | [43]       |
| Chemical flocculation | Chlorella sp. | Cost efficient and ease for up scale | Utilization of toxic chemicals | [44]       |
| Buoy-bead flotation | Chlorella vulgaris | Chemical free and high reusability | High cost | [45]       |
| Magnesium coagulation-dissolved air flotation | Chlorella zofingiensis | Does not utilize external coagulant and high recyclability of coagulant and biomass | Utilization of toxic chemicals | [46]       |
| Electrolytic flotation | C. vulgaris | Chemical free, low energy demand and can be used in continuous system | High operating and capital cost | [47]       |
| Foam flotation | C. vulgaris, Isochrysis galbana, and Tetraselmis suecica | Low cost and energy demand, highly scalable | High operating and capital cost | [48]       |
| Ozone flotation | Scenedesmus sp. | Increased microalgae bio-compound recovery | Require specialized ozone generation equipment onsite | [49]       |
**Centrifugation**

Centrifugation operation separates microalgal cells from the culture media based on each component’s density and particle size using centrifugal force [53]. This technique has high cell harvesting efficiency, but the process is time consuming and energy intensive [54,55]. Moreover, high gravitational force used in centrifugation might cause cellular damage making it unfavorable for certain applications since the sensitive nutrients might be lost [56,57]. Several types of centrifugal systems have been used in the industry; these include disk stack centrifuges, perforated basket centrifuges, imperforated basket centrifuges, decanters, and hydro-cyclones [35].

**Flocculation**

Flocculation is a process where free floating unicellular microalgal cells aggregate together to form a larger particle known as floc by the addition of flocculating agent to remove the surface charge of cells [58]. Flocculating agents can be grouped into two major types, namely chemical flocculants and bio-flocculants. Low cost and highly available chemical flocculants such as iron and aluminum salts have been used widely in industry [59]. The study carried out by Chatsungnoen and Chistt [60] has demonstrated that the metal salts such as aluminum sulfate and iron chloride are able to remove 95% of the microalgal biomass at a standard condition. However, the chemicals are not eco-friendly due to their high toxicity, and they must be removed by additional treatment processes which add to the production cost [61].

Bio-flocculants on the other hand are much safer and eco-friendly when compared to their chemical counter parts. They are also cheaper to be used, and typically there is no pretreatment required before further downstream processing of microalgae and recycling of culture media [62,63]. Most of the bio-flocculants used are biopolymers such as acrylic acid and chitosan which exist naturally or produce artificially [64]. It is reported that chitosan at a lower dosage is capable of reaching 90% cell recovery when compared to chemical flocculants such as aluminum sulfate which requires higher concentration to achieve same results [65].

**Flotation**

Flotation utilizes small bubbles which attach on microalgal cells to promote the floating of cells on the surface of the culture media for easy harvesting [66]. The advantages associated with the flotation system include relatively high harvesting efficiency, easy operating procedure, and high processing throughput at low cost [67].

There are 3 main types of flotation systems which generate air bubbles required by using different mechanisms. Dissolved air flotation system generates air bubbles by the means of saturating the culture with compressed air and then discharging the culture at atmospheric condition. This method has been widely used in wastewater treatment but is hindered by high cost due to power consumption and usage of chemicals [68]. Dispersed air flotation on the other hand uses a sparger to generate air bubble which in turn have lower energy demand when compared to dissolved air flotation [69]. The third method is electro-flotation, which applies electrolysis operation to generate microbubbles from its electrode to trap free floating microalgae [70]. Apart from harvesting, this method also allows simultaneous cell disruption operation when alternating current is being used [71]. However, the respective system is extremely energy consuming and frequent replacement of electrode is required due to fouling which might then bring up the production cost [35].

**Extraction of bioproducts from microalgae using ionic liquids**

Microalgae are known to contain vast amount of high-value phytochemicals. However, since most of the compounds are locked within the rigid cell walls, the extraction of bioactive compounds from microalgae involves cellular disruption to release the intracellular contents so that it could be accessed and purified. Numerous ways have been devised to extract the bioactive compounds from microalgae biomass. Typical conventional extraction method can be broadly classified into four groups which are: (1) mechanical extraction which utilizes shear forces, electrical pulses, waves or heat to disrupt cellular structure [72], (2) chemical extraction which uses different kinds
of solvents such as polar or nonpolar organic solvents, supercritical carbon dioxide and ILs to extract the intracellular compounds [73], (3) physical extraction which applies the microwave and ultrasound operations [74], and last (4) enzymatic lysis which employs enzymes such as trypsin to digest the hard cell wall of microalgae [75]. Among all the extraction methods, ILs have emerged as a promising green solvent for the extraction of microalgae bio-compounds, and different approaches using ILs have been examined.

**Ionic liquids as extractive solvents using different approaches**

ILs are described as molten organic salts which has melting point below 100°C [76,77]. The properties of ILs vary greatly depending on their molecular size, functional groups attached, and also the type of cation and anion pairs. Nevertheless, several common characteristics have made IL as an ideal candidate for green applications. First, the nonvolatile nature makes ILs ideal when compared to other organic solvents such as ethanol and methanol which are extremely combustible. Second, some ILs have low viscosity which is a plus for any IL that is intended to be used for large-scale industrial application since low fluid viscosity facilitates pumping and agitation operations and reduce the power consumption [78]. Additionally, the low vapor pressure of ILs makes them relatively safe to use especially when they have low emission rate and consequently reduce exposure toward operators. The negligible vapor pressure of ILs also translate into less air pollution since there is lesser ILs will be liberated into the atmosphere. Furthermore, ILs can be specifically tailored-made to suit specific conditions and to solubilize specific compounds by altering their functional groups or selection of appropriate cation and anion pairs. Moreover, ILs have high thermal and chemical stability, high conductivity, and remarkable solubilizing capacity for various organic and inorganic compounds [79,80].

Amongst the commercially available ILs, ILs from imidazolium cation family are widely investigated for their potential to extract bioproducts from microalgae. Imidazolium cation has 5 membered, heterocyclic structure with a pair of nitrogen at the 1st and 3rd position in the ring [81]. Its peculiar structure allows it to be used for various applications. Apart from imidazolium-based ILs, cholinium-based ILs that are highly recognized for their marginally toxicity have been applied for the microalgae extraction studies [82]. However, there is still lack of study in this field using other ILs such as pyridinium- and phosphonium-based ILs.

Several extraction methods using ILs have been studied. Although direct dissolution of microalgae biomass to extract their intracellular compounds is within the realm of possibility, most of the time additional external force is used to aid the extraction process [83]. Microwave, ultrasound, electrical pulse field, and bead milling can be coupled with the use of ILs to generate additional force to intensify the extraction process. These methods are mainly developed to work around the tough cell wall of microalgae that is being a hindrance to the full utilization of microalgae-derived compounds.

**Microwave-assisted ionic liquid extraction**

Microwave employs high-frequency waves to cause vibration of water molecule within the microalgae biomass, which in turn increase the temperature and pressure generated from the evaporation of water resulting in the rupture of cell wall [74,84]. The ruptured microalgae cell releases all its intracellular contents and these contents are freely available to be extracted using ILs. Recent work conducted by Wahidin et al. [85] reported that by using 1-ethyl-3-methylimidazolium methyl sulfate as extractive solvent and combined with microwave heating, it is possible to convert directly *Nannochloropsis* sp. biomass to biodiesel in a single step *in situ* transesterification process. The approach has achieved efficient biodiesel production per unit of microalgae biomass [85].

**Ultrasound-assisted ionic liquid extraction**

Ultrasound induces formation of cavity within microalgae cells, and when the cavity collapses it generates large amount of force which causes the shearing of microalgae cell wall [86]. This method has a high potential for microalgae biodiesel production since it is able to achieve cell disruption without the energy intensive dewatering procedure [87]. Ultrasonic treatment of 25kHz on *Spirulina platensis* suspended in 1:1 ratio ILs mixture of
2-hydroxyethylammonium acetate and 2-hydroxyethylammonium formate has been studied, and experimental results demonstrated that the ILs mixture solution has higher extraction efficiency of phycocyanin, allophycocyanin, and phycoerythrin when compared to conventional sodium phosphate buffer and 1-butyl-3-methylimidazolium chloride [88].

Ionic liquid-based aqueous biphasic systems
Beside of being applied in microwave- and ultrasound-assisted ILs extraction, ILs are studied as phase-forming components in ABS for the separation of bioproducts derived from microalgae biomass. ABS can be formed by mixing two different water-soluble ILs which are not immiscible with each other [89,90]. The bioproducts from microalgae can be separated into different aqueous phases based on their physical properties and chemical affinity. It is reported that the ABS formed by 1-butyl-3-methylimidazolium dibutylphosphate and tributylmethylphosphonium methyl sulfate was able to extract protein, fatty acid and carbohydrate from *Neochloris oleoabundans* at 80%, 68%, and 77%, respectively. This low energy demanding method has shown better yield compared to their organic solvent counterparts [91].

**Bioproducts derived from microalgae using ionic liquid technology**
Microalgae-derived bioproducts can be categorized into four major groups, namely lipids, carbohydrates, proteins, and bioactive compounds. Table 3 summarizes the recent studies utilizing ILs to extract these bioproducts from microalgae.

**Lipids**
Microalgae have been known for their richness in lipid content, where the lipid content of the dried microalgae biomass is around 20–50%, however with specific optimized culturing technique the lipid content of microalgae can be risen to 80% [101]. In general, there are two major applications for microalgae-derived lipids, which are biodiesel and food supplement. Microalgae biodiesel is considered as the green fuel for the future owing to its carbon neutrality and the utilization of feedstock which does not compete with our current food supply for resources [101–103]. Several published works have conceptualized the use of ILs to extract lipid from microalgae for biodiesel production. In a study conducted by Zhou and his colleagues [87], 1-butyl-3-methylimidazolium methyl sulfate was able to extract the total lipid of 16.04% from dried *N. oleoabundans* biomass when the ratio of

### Table 3. Extraction studies of bioproducts from microalgae using ILs as extractive solvents.

| Bioproduct          | Microalgae species                  | IL                                         | Reference |
|---------------------|-------------------------------------|--------------------------------------------|-----------|
| Lipid               | *N. oleoabundans*                   | 1-butyl-3-methylimidazolium methyl sulfate | [87]      |
| Lipid               | *N. oculata*                        | Tetrakis(hydroxymethyl)phosphonium chloride | [92]      |
| Lipid               | *Chlorella, Chlorococcum sp.* and *Nannochloropsis oculata* | Butyrolactam hexanoate | [93,94] |
| Lipid               | *C. vulgaris*                       | 1-butyl-3-methylimidazolium trifluoromethanesulfonate | [104] |
| Lipid               | *Chlorella sorokiniana, Nannochloropsis salina* and *Galdieria sulphuraria* | 1-butyl-3-methylimidazolium hydrogen sulfate | [95]      |
| Lipid               | *C. vulgaris*                       | 1-ethyl-3-methyl imidazolium ethyl sulfate, 1-ethyl-3-methyl imidazolium thiocyanate, 1-ethyl-3-methyl imidazolium hydrogen sulfate, 1-ethyl-3-methylimidazolium ethylsulfate | [96] |
| Fatty acid methyl ester | *C. vulgaris*                   | 1-ethyl-3-methylimidazolium ethylsulfate | [97]      |
| DHA                 | *Thraustochytrium sp.*              | 1-ethyl-3-methylimidazolium ethylsulfate, tetrabutylphosphonium propionate | [106] |
| DHA                 | *Aurantiopycnchryum sp.*            | Iron(III) chloride hexahydrate, 1-Ethyl-3-methylimidazolium acetate mixture | [98]      |
| Carbohydrate and lipid | *C. vulgaris* and *S. platensis* | Choline L-argininate | [111] |
| Glucan, arabinan and protein | *I. galbana*                     | 1-methyl-3-octylimidazolium chloride | [99]      |
| Protein             | *C. vulgaris*                       | Cholinium 2-hydroxy-3-morpholinopropanesulfonate | [86] |
| C-phycoerythrin     | *S. platensis*                      | 1-octyl-3-methylimidazolium bromide | [100] |
| Astaxanthin         | *H. pluvialis*                      | 1-ethyl-3-methylimidazolium di-butylphosphate | [118] |
IL: methanol used was 1:1. The extraction efficiency can be further increased to beyond 20% when the IL: methanol mixture utilized was at the ratio of 1:3 and 1:7 under the optimized extraction condition.

*C. vulgaris* is one of the most studied microalgal strains available for biodiesel production. It was reported that 1-butyl-3-methylimidazolium trifluoromethanesulfonate achieved lipid extraction efficiencies of 12.5% and 19% for commercial and cultivated dried *C. vulgaris* biomass, respectively, which were much higher when compared to conventional Bligh and Dyer’s method with extraction efficiency achieved at 10.6% and 11.1% [104,105]. In addition to that, the fatty acid profile of the crude lipid extract from ILs extraction showed that it was rich in palmitic, linoleic, and palmitoleic fatty acids which can undergo a cheap and fast transesterification process with the presence of an acid or alkaline catalysis to produce biodiesel [104]. On the other hand, lipids extracted from microalgae can also be used as food additives to supply additional nutritive factors in human food. The study on the extraction of DHA from *Thraustochytrium* sp. biomass reported 1-ethyl-3-methylimidazolium ethylsulfate and tetrabutylphosphonium propionate, with the use of IL: methanol at 1:10 and 1:4 ratio, respectively, achieved lipid extraction yield of 91% [106].

**Carbohydrates**

Microalgae cells are packed with different complex carbohydrates such as cellulose, agarose, starch, and glycogen which can be readily used as feedstock to produce bioethanol [3]. Bioethanol produced from microalgae carbohydrates can be used directly by currently available internal combustion engine without significant modification. In addition, high octane rating and oxygen content of bioethanol fuel translate well into higher engine performance and reduced emission rate [107]. ILs are suitable solvents to extract carbohydrate from microalgae since the tough crystalline structure of cellulose microfibril can be solubilized using ILs to release starch granules stored in cells [108]. Several research studies reported that the usage of ILs is in fact the most energy efficient and least time-consuming method to extract carbohydrate from microalgae biomass in the absence of acid, alkali, and catalyst [109]. Besides, it is reported that the carbohydrate-rich fraction which remains after ILs extraction is suitable to be used as feedstock to produce butanol via fermentation without any further pretreatment [110]. Another study reported the inexpensive choline amino acid-based IL is capable to extract total sugar content of 71 and 21%, respectively, from *C. vulgaris* and *S. platensis* [111].

**Proteins**

Microalgae are one of the protein-rich crops, where their protein yield is said to be comparable to the protein yield of conventional protein sources such as soy, milk, and animal meat. *Spirulina* has been cultivated and marketed in the form of tablets and pills as protein supplements for human consumption [2]. The crude extract of *T. suecica* was reported to contain proteins with emulsification, foaming, and gelation properties which may open up a variety of application options in food industry [112]. Another type of protein that can be extracted from microalgae is R-phycoerythrin, which is a type of phycobiliprotein that is usually used as fluoresce dye in diagnostic and research field [113]. In the study, cholinium-based IL was able to extract 46.5% of R-phycoerythrin from *Gracilaria* sp., which was significantly higher when compared to conventional method [114]. Besides, extraction study of Rubisco, which is an abundant microalgae enzyme, revealed that the IL-based ABS investigated was 3–4 times more efficient in separating complex plant proteins compared to the polyethylene glycol-based extraction [91]. The results have shown the potential of ILs as extractive solvent to separate and purify complex proteins such as enzymes from microalgae for industrial applications. Nevertheless, the functional usage of microalgae proteins extracted using ILs remains largely unexplored at large.

**Bioactive compounds**

Although many researches on microalgae have mainly centered around the biofuel production, microalgae metabolites have a variety of functions. Metabolites such as carotenoids are well-known for their antioxidant properties. Examples of such carotenoids which are widely used in pharmaceutical and food industries as nutritive supplements and coloring agents are β-carotene and astaxanthin that are mainly
extracted from *D. salina* and *Haematococcus pluvialis*, respectively [115,116]. The use of 1-ethyl-3-methylimidazolium dibutylphosphate for the extraction of astaxanthin from *H. pluvialis* certainly aids the industry in fulfilling the need of the market via vastly improving the extraction yield at a lower energy demand and production cost which in turn makes the product to be more competitive even with the presence of artificially synthesized astaxanthin in the market [117,118]. Other microalgae metabolites of interest include tocopherols, phenolics compounds, flavonoids, and vitamins that have been previously found in microalgae extracts of different species and may be potentially extracted using ILs [119–121]. These compounds have various therapeutics functions such as anti-inflammatory, antimicrobial, anti-viral, antioxidant, and even anticancer effects [122,123].

**Challenges of commercialization of ionic liquids for the extraction of bioproducts from microalgae**

Despite the various studies that emphasize on the benefits of ILs for the extraction of microalgae derived bio-compounds, ILs are still to this date not being used commercially. This may be attributed to the toxicity and non-biodegradability of some traditional ILs such as imidazolium-based ILs, which may pose as an environmental risk when they are not properly treated prior to discharge to the environment. In fact, several toxicology studies have reported that conventional ILs are able to disrupt cell membrane and increase reactive oxygen species production in various organisms such as bacteria, fungus, plants, and animal cell lines *in vitro* and ultimately lead to death. Moreover, due to the complexity of ILs’ molecular structure, they tend to be much harder to be synthesized, and therefore contribute to high production cost and are generally 5–20 times more expensive than conventional solvents. On-going efforts need to be continued to lower down the production cost of ILs, and also optimize their usage.

**Conclusions**

It is well established that microalgae have tremendous potential as a source of biofuel, food and high value bio-compounds. However, the limitations in productivity of microalgae and the drawbacks of bioprocessing technologies render the fully utilization of microalgae biomass to be impractical. Therefore, more work needs to be done to further improve the existing technology. For instance, more advanced culturing technique should be developed to increase the productivity of microalgae, and novel biotechnology such as gene editing can be attempted to increase the output of bioactive compounds from the microalgae strain. Besides, the ILs studied should be examined for their toxicity before application. In addition, computer simulation can be used to further explore the combination of various cation and anion of ILs to maximize the extraction capacity of desired compounds from microalgae.

**Highlights**

- Microalgae cultivation using open ponds (circular and raceway ponds)
- Different designs of photobioreactors: tubular, vertical column, and flat-plate
- Harvesting techniques: filtration, centrifugation, flocculation, and flotation
- Extraction of bioproducts from microalgae using ionic liquid as extractive solvent
- Bioproducts extracted: lipids, carbohydrates, proteins and bioactive compounds

**Disclosure statement**

No potential conflict of interest was reported by the authors.

**Funding**

The authors gratefully acknowledge the support of Universiti Tunku Abdul Rahman (UTAR) research grant [Grant number: IPSR/RMC/UTARRF/2019-C1/L05].

**ORCID**

Jun Wei Lim | http://orcid.org/0000-0003-0158-8822
Pau Loke Show | http://orcid.org/0000-0002-0913-5409

**References**

[1] Randrianarison G, Ashraf MA. Microalgae: a potential plant for energy production. Geol Ecol Landscapes. 2017;1(2):104–120.
[2] Grahl S, Strack M, Weinrich R, et al. Consumer-oriented product development: the conceptualization of novel food products based on spirulina (arthrospira platensis) and resulting consumer expectations. J Food Qual. 2018;2018:1–11.

[3] Khan MI, Shin JH, Kim JD. The promising future of microalgae: current status, challenges, and optimization of a sustainable and renewable industry for biofuels, feed, and other products. Microb Cell Fact. 2018;17(1):36.

[4] Selmani N, Mirghani ME, Alam MZ. Study the growth of microalgae in palm oil mill effluent wastewater. In: IOP Conference series: earth and environmental science.; Putrajaya, Malaysia: IOP Publishing; 2013.

[5] Posadas E, Alcántara C, García-Encina PA, et al. Microalgae-based biofuels and bioproducts. In: Gonzalez-Fernandez C, Muñoz R, editors. Handbook of microalgae cultivation in wastewater. Woodhead Publishing; 2017. p. 67–91. DOI:10.1016/B978-0-08-101023-5.00003-0

[6] Jegathese SJP, Farid M. Microalgae as a renewable source of energy: A niche opportunity. J Renewable Energy. 2014;2014:1–10.

[7] Costa JAV, de Morais MG. An open pond system for microalgal cultivation. In: Pandey A, Lee D-J, Chisti Y, Soccol CR, editors. Biofuels from Algae. Elsevier; 2014. p. 1–22. DOI:10.1016/B978-0-44-459558-4.00001-2

[8] Sun Z, Liu J, Zhou Z-G. Algae for biofuels: an emerging feedstock. In: Luque R, Lin CSK, Wilson K, Clark J, editors. Handbook of biofuels production. In: Mohan SV, Rohit MV, Subhash GV, et al. Biofuels from algae. In: Pandey A, Chang J-S, Soccol CR, Lee D-J, Chisti Y, editors. Algal oils as biodiesel. Elsevier; 2019. p. 287–323. DOI:10.1016/B978-0-44-4452114-9.00002-0

[9] Torzillo G, Zittelli GC. Tubular photobioreactors. In: Algal biorefineries. Switzerland: Springer International Publishing; 2015. p. 187–212.

[10] Costa JAV, de Morais MG. An open pond system for microalgal cultivation. In: Pandey A, Lee D-J, Chisti Y, Soccol CR, editors. Biofuels from Algae. Elsevier; 2014. p. 1–22. DOI:10.1016/B978-0-44-459558-4.00001-2

[11] Stark M, O’Gara I. An introduction to photosynthetic microalgae. Disruptive Sci Technol. 2012;1(2):65–67.

[12] Ugwu CU, Aoyagi H. Designs, operation and applications. Biotechnology. 2012;11(3):127–132

[13] Hamed I. The evolution and versatility of microalgal biotechnology: a review. Compr Rev Food Sci Food Saf. 2016;15(6):1104–1123

[14] Shen Y, Yuan W, J. Pei Z, et al. Microalgae mass production methods. Trans ASABE. 2009;52(4):1275–1287.

[15] Show P, Tang M, Nagarajan D, et al. A holistic approach to managing microalgae for biofuel applications. Int J Mol Sci. 2017;18(1):215.

[16] Lee Y-K. Microalgal mass culture systems and methods: their limitation and potential. J Appl Phycol. 2001;13(4):307–315.

[17] Lee Y-K. Commercial production of microalgae in the Asia-Pacific rim. J Appl Phycol. 1997;9(5):403–411.

[18] Rogers JN, Rosenberg JN, Guzman BJ, et al. A critical analysis of paddlewheel-driven raceway ponds for algal biofuel production at commercial scales. Algal Res. 2014;4:76–88.

[19] White RL, Ryan RA. Long-term cultivation of algae in open-raceway ponds: lessons from the field. Ind Biotechnol. 2015;11(4):213–220.

[20] Posen C. Design principles of photo-bioreactors for cultivation of microalgae. Eng Life Sci. 2009;9(3):165–177.

[21] Gupta PL, Lee S-M, Choi H-J. A mini review: photobioreactors for large scale algal cultivation. World J Microbiol Biotechnol. 2015;31(9):1409–1417.

[22] Mishra AK, Kaushik MS, Tiwari D. Nitrogenase and hydrogenase: enzymes for nitrogen fixation and hydrogen production in cyanobacteria. In: Mishra AK, Tiwari DN, A.N. Rai AN, editors. Cyanobacteria. Academic Press; 2019. p. 173–191. DOI:10.1016/B978-0-12-14667-5.00008-8

[23] Xu Z. Biological production of hydrogen from renewable resources. In: Yang S-T, editor. Bioprocessing for value-added products from renewable resources. Elsevier; 2007. p. 527–557. DOI:10.1016/B978-0-44-4452114-9.00002-0

[24] Tonelli P, Akbari S, Molina M, et al. Development of the flat-plate photobioreactor for carbon dioxide removal using phototrophic microalgae. J Biotechnol. 2013;32(2):225–232.

[25] Mohan SV, Rohit MV, Subhash GV, et al. Biofuels from algae. In: Pandey A, Chang J-S, Soccol CR, Lee D-J, Chisti Y, editors. Algal oils as biodiesel. Elsevier; 2019. p. 287–323. DOI:10.1016/B978-0-44-4452114-9.00002-0

[26] Al-Hadad H, Al-Jasser A, Al-Mulhim F, et al. Development of a novel flat-plate photobioreactor system for carbon dioxide removal using phototrophic microalgae. J Biotechnol. 2013;32(2):225–232.

[27] Huang Q, Jiang F, Wang L, et al. Design of photobioreactors for mass cultivation of photosynthetic organisms. Engineering. 2017;3(3):318–329.

[28] Huo S, Wang Z, Zhu S, et al. Biomass accumulation of Chlorella zofingiensis G1 cultures grown outdoors in photobioreactors. Front Energy Res. 2018;6:49.

[29] Tamburic B, Zemichael FW, Crudge P, et al. Design of a novel flat-plate photobioreactor system for green algal hydrogen production. Int J Hydrogen Energy. 2011;36(11):6578–6591.

[30] Yan C, Zhang Q, Xue S, et al. A novel low-cost thin-film flat plate photobioreactor for microalgae cultivation. Biotechnol Bioprocess Eng. 2016;21(1):103–109.

[31] Sierra E, Acien FG, Fernandez JM, et al. Characterization of a flat plate photobioreactor for the production of microalgae. Chem Eng J. 2008;138(1–3):136–147.

[32] Vo HN, Ngo HH, Guo W, et al. A critical review on designs and applications of microalgae-based photobioreactors for pollutants treatment. SciTotal Environ. 2018;651:1549–1568.

[33] Uduman N, Qi Y, Danquah MK, et al. Dewatering of microalgal cultures: a major bottleneck to algae-based fuels. J Renewable Sustainable Energy. 2010;2(1):012701.
[34] Barros AI, Gonçalves AL, Simões M, et al. Harvesting techniques applied to microalgae: a review. Renew Sust Energ Rev. 2015;41:1489–1500.

[35] Singh G, Patidar S. Microalgae harvesting techniques: a review. J Environ Manage. 2018;217:499–508.

[36] Giménez JB, Bouzas A, Carrere H, et al. Assessment of cross-flow filtration as microalgal harvesting technique prior to anaerobic digestion: evaluation of biomass integrity and energy demand. Bioresour Technol. 2018;269:188–194.

[37] Zhao F, Chu H, Zhang Y, et al. Increasing the vibration frequency to mitigate reversible and irreversible membrane fouling using an axial vibration membrane in microalgae harvesting. J Membr Sci. 2017;529:215–223.

[38] Marbella L, Mulier M, Vandamme D, et al. Polyacrylonitrile membranes for microalgae filtration: influence of porosity, surface charge and microalgae species on membrane fouling. Algal Res. 2016;19:128–137.

[39] Eliseus A, Bilad MR, Nordin NAHM, et al. Tilted membrane panel: a new module concept to maximize the impact of air bubbles for membrane fouling control in microalgal harvesting. Bioresour Technol. 2017;241:661–668.

[40] Wenten I, Steven S, Dwiputra A, et al. From lab to full-scale ultrafiltration in microalgae harvesting. In Journal of Physics: Conference Series. 2017. Bandung, Indonesia: IOP Publishing.

[41] Zenouzi A, Ghabadian B, Hejazi MA, et al. Harvesting of microalgae Dunaliella salina using electroflocculation. Journal of Agricultural Science and Technology. 2013;15(5):879–887.

[42] Hamid SHA, Lananan F, Khatoon H, et al. A study of coagulating protein of Moringa oleifera in microalgae bio-flocculation. Int Biodeterior Biodegrad. 2016;113:310–317.

[43] Ndikubwimana T, Zeng X, Murwanashyaka T, et al. Harvesting of freshwater microalgae with microbial bioflocculant: a pilot-scale study. Biotechnol Biofuels. 2016;9(1):47.

[44] Kim D-Y, Lee K, Lee J, et al. Acidified-flocculation process for harvesting of microalgae: coagulant reutilization and metal-free-microalgae recovery. Bioresour Technol. 2017;239:190–196.

[45] Xu K, Zou X, Wen H, et al. Buoy-bead flotation harvesting of the microalgae Chlorella vulgaris using surface-layered polymeric microspheres: A novel approach. Bioresour Technol. 2018;267:341–346.

[46] Zhang X, Wang L, Sommerfeld M, et al. Harvesting microalgal biomass using magnesium coagulation-dissolved air flotation. Biomass Bioenergy. 2016;93:43–49.

[47] Luo S, Griffith R, Li W, et al. A continuous flocculants-free electrolytic flotation system for microalgae harvesting. Bioresour Technol. 2017;238:439–449.

[48] Alkarawi MA, Caldwell GS, Lee JG. Continuous harvesting of microalgae biomass using foam flotation. Algal Res. 2018;36:125–138.

[49] Oliveira GA, Carissimi E, Monje-Ramírez I, et al. Comparison between coagulation-flocculation and ozone-flocculation for Scenedesmus microalgal biomolecule recovery and nutrient removal from wastewater in a high-rate algal pond. Bioresour Technol. 2018;259:334–342.

[50] Al Hattab M, Ghaly A, Hammoud A. Microalgae harvesting methods for industrial production of biodiesel: critical review and comparative analysis. J Fundam Renewable Energy Appl. 2015;5(2):1000154.

[51] Milledge JJ, Heaven S. A review of the harvesting of micro-algae for biofuel production. Rev Environ Sci Biotechnol. 2013;12(2):165–178.

[52] Bejor ES, Mota C, Ogarekpe NM, et al. Low-cost harvesting of microalgae biomass from water. Int J Dev Sustain. 2013;2(1):1–11.

[53] Soomro RR, Ndikubwimana T, Zeng X, et al. Development of a two-stage microalgae dewatering process—a life cycle assessment approach. Front Plant Sci. 2016;7:113.

[54] Dassey AJ, Theegala CS. Harvesting economics and strategies using centrifugation for cost effective separation of microalgae cells for biodiesel applications. Bioresour Technol. 2013;128:241–245.

[55] Rawat I, Ranjith Kumar R, Mutanda T, et al. Biodiesel from microalgae: a critical evaluation from laboratory to large scale production. Appl Energy. 2013;103:444–467.

[56] Heasman M, Diemar J, O’connor W, et al. Development of extended shelf-life microalgae concentrate diets harvested by centrifugation for bivalve molluscs—a summary. Aquacult Res. 2000;31:637–659.

[57] Knuckey RM, Brown MR, Robert R, et al. Production of microalgal concentrates by flocculation and their assessment as aquaculture feeds. Aquacult Eng. 2006;35(3):300–313.

[58] Muylaert K, Bastiaens L, Vandamme D, et al. Microalgae-based biofuels and bioproducts. In: Gonzalez-Fernandez C, Muñoz R, editors. Harvesting of microalgae: overview of process options and their strengths and drawbacks. Woodhead Publishing; 2017. p. 113–132. DOI:10.1016/B978-0-08-101023-5.00005-4

[59] Bracharz F, Helmdach D, Aschenbrenner I, et al. Harvest of the oleaginous microalgae Scenedesmus obtusiusculus by flocculation from culture based on natural water sources. Front Bioeng Biotechnol. 2018;6:200.

[60] Chatsungnoen T, Chisti Y. Harvesting microalgae by flocculation and their assessment as aquaculture feeds. Aquacult Eng. 2006;35(3):300–313.

[61] Rinanti A, Purwadi R. Harvesting of freshwater microalgae biomass by Scenedesmus sp. as bioflocculant. in IOP Conference Series: Earth and Environmental Science. 2018. Jakarta, Indonesia: IOP Publishing.

[62] Salim S, Bosma R, Vermuë MH, et al. Harvesting of microalgae by bio-flocculation. J Appl Phycol. 2011;23(5):849–855.

[63] Nguyen TDP, Le TVA, Show PL, et al. Bioflocculation formation of microalgae-bacteria in enhancing
microalgae harvesting and nutrient removal from wastewater effluent. Bioresour Technol. 2019;272:34–39.
[64] Pugazhendhi A, Shobana S, Bakonyi P, et al. A review on chemical mechanism of microalgae flocculation via polymers. Biotechnol Reports. 2019;21:e00302.
[65] Zhu L, Li Z, Hiltunen E. Microalgae Chlorella vulgaris biomass harvesting by natural flocculant: effects on biomass sedimentation, spent medium recycling and lipid extraction. Biotechnol Biofuels. 2018;11(1):183.
[66] Laamanen CA, Ross GM, Scott JA. Floation harvesting of microalgae. Renew Sust Energ Rev. 2016;58:75–86.
[67] Nidikubwimana T, Chang J, Xiao Z, et al. Flotation: A promising microalgae harvesting and dewatering technology for biofuels production. Biotechnol J. 2016;11(3):315–326.
[68] Niaghi M, Mahdavi MA, Gheslaghi R. Optimization of dissolved air flotation technique in harvesting microalgae from treated wastewater without flocculants addition. J Renewable Sustainable Energy. 2015;7(1):013130.
[69] Alhattab M, Brooks MS-L. Dispersed air flotation and foam fractionation for the recovery of microalgae in the production of biodiesel. Sep Sci Technol. 2017;52(12):2002–2016.
[70] Baierle F, John DK, Souza MP, et al. Biomass from microalgae separation by electroflotation with iron and aluminium spiral electrodes. Chem Eng J. 2015;267:274–281.
[71] de Carvalho Neto RG, Do Nascimento JGDS, Costa MC, et al. Microalgae harvesting and cell disruption: a preliminary evaluation of the technology electrofloation by alternating current. Water Sci Technol. 2014;70(2):315–320.
[72] Lee SY, Cho JM, Chang YK, et al. Cell disruption and lipid extraction for microalgal biorefineries: A review. Bioresour Technol. 2017;244:1317–1328.
[73] Mubarak M, Shaija A, Suchithra T. A review on the extraction of lipid from microalgae for biodiesel production. Algal Res. 2015;7:117–123.
[74] Kapoore R, Butler T, Pandhal J, et al. Microwave-assisted extraction for microalgae: from biofuels to biorefinery. Biology (Basel). 2018;7(1):18.
[75] Ranjith Kumar R, Hanumantha Rao P, Arumugam M. Lipid extraction methods from microalgae: a comprehensive review. Front Energy Res. 2015;2:61.
[76] Ghandi K. A review of ionic liquids, their limits and applications. Green Sustainable Chem. 2014;4(01):44–53.
[77] Ratti R. Ionic liquids: synthesis and applications in catalysis. Adv Chem. 2014;2014:16.
[78] Wilkes JS. Properties of ionic liquid solvents for catalysis. J Mol Catal A Chem. 2004;214(1):11–17.
[79] Tolesa LD, Gupta BS, Lee M-J. The chemistry of ammonium-based ionic liquids in depolymerization process of lignin. J Mol Liq. 2017;248:227–234.
[80] Toledo Hijo AA, Maximo GJ, Costa MC, et al. Applications of ionic liquids in the food and bioproducts industries. ACS Sustain Chem Eng. 2016;4(10):5347–5369.

[81] Wang B, Qin L, Mu T, et al. Are ionic liquids chemically stable? Chem Rev. 2017;117(10):7113–7131.
[82] Lee SY, Vicente FA, E Silva FA, et al. Evaluating self-buffering ionic liquids for biotechnological applications. ACS Sustain Chem Eng. 2015;3(12):3420–3428.
[83] Fujita K, Kobayashi D, Nakamura N, et al. Direct dissolution of wet and saliferous marine microalgae by polar ionic liquids without heating. Enzyme Microb Technol. 2013;52(3):199–202.
[84] Chew KW, Chia SR, Lee SY, et al. Enhanced microalgal protein extraction and purification using sustainable microwave-assisted multiphase partitioning technique. Chem Eng J. 2019;367:1–8.
[85] Wahidin S, Idris A, Yusof NM, et al. Optimization of the ionic liquid-microwave assisted one-step biodiesel production process from wet microalgal biomass. Energy Convers Manag. 2018;171:1397–1404.
[86] Lee SY, Show PL, Ling TC, et al. Single-step disruption and protein recovery from Chlorella vulgaris using ultrasonication and ionic liquid buffer aqueous solutions as extractive solvents. Biochem Eng J. 2017;124:26–35.
[87] Zhou W, Wang Z, Alam M, et al. Repeated utilization of ionic liquid to extract lipid from algal biomass. Int J Polym Sci. 2019;2019:1–7.
[88] Rodrigues RDP, de Castro FC, Santiago-Aguirau RSD, et al. Ultrasound-assisted extraction of phycobiliproteins from Spirulina (Arthrospira) platensis using protic ionic liquids as solvent. Algal Res. 2018;31:454–462.
[89] Shukla SK, Pandey S, Pandey S. Applications of ionic liquids in biphasic separation: aqueous biphasic systems and liquid–liquid equilibria. J Chromatogr A. 2018;1559:44–61.
[90] Lee SY, Khoiroh I, Ooi CW, et al. Recent advances in protein extraction using ionic liquid-based aqueous two-phase systems. Sep Purif Rev. 2017;46(4):291–304.
[91] Desai RK, Streefland M, Wijffels RH, et al. Extraction and stability of selected proteins in ionic liquid based aqueous two phase systems. Green Chem. 2014;16 (5):2670–2679.
[92] Zhang R, Parniakov O, Grimi N, et al. Emerging techniques for cell disruption and extraction of valuable bio-molecules of microalgae Nannochloropsis sp. Bioprocess Biosyst Eng. 2019;42(2):173–186.
[93] Shankar M, Chhotaray PK, Gardas RL, et al. Application of carboxylate protic ionic liquids in simultaneous microalgal pretreatment and lipid recovery from marine Nannochloropsis sp. and Chlorella sp. Biomass Bioenergy. 2019;123:14–24.
[94] Shankar M, Chhotaray PK, Agrawal A, et al. Protonic ionic liquid-assisted cell disruption and lipid extraction from fresh water Chlorella and Chlorellaococccum microalgae. Algal Res. 2017;25:228–236.
[95] Pan J, Muppaneni T, Sun Y, et al. Microwave-assisted extraction of lipids from microalgae using an ionic liquid solvent [BMIM][HSO4]. Fuel. 2016;178:49–55.
[96] Choi S-A, Oh Y-K, Jeong M-J, et al. Effects of ionic liquid mixtures on lipid extraction from Chlorella vulgaris. Renewable Energy. 2014;65:169–174.

[97] Orr VC, Plechkova NV, Seddon KR, et al. Disruption and wet extraction of the microalgae Chlorella vulgaris using room-temperature ionic liquids. ACS Sustain Chem Eng. 2015;4(2):591–600.

[98] Choi S-A, Jung J-Y, Kim K, et al. Effects of molten-salt/ionic-liquid mixture on extraction of docosahexaenoic acid (DHA)-rich lipids from Aurantiocytium sp. KRS101. Bioprocess Biosyst Eng. 2014;37(11):2199–2204.

[99] Santos JOH, Trigo JP, Maricato É, et al. Fractionation of isochrysis galbana proteins, arabinans, and glucans using ionic-liquid-based aqueous biphasic systems. ACS Sustain Chem Eng. 2018;6(11):14042–14053.

[100] Chang Y-K, Show P-L, Lan JC-W, et al. Isolation of C-phycocyanin from Spirulina platensis microalgae using Ionic liquid based aqueous two-phase system. Bioresearch Technol. 2018;270:320–327.

[101] Chisti Y. Biodiesel from microalgae. Biotechnol Adv. 2007;25(3):294–306.

[102] Mata TM, Martins AA, Caetano NS. Microalgae for biodiesel production and other applications: a review. Renew Sust Energ Rev. 2010;14(1):217–232.

[103] Kings AJ, Raj RE, Miriam LRM, et al. Cultivation, extraction and optimization of biodiesel production from potential microalgae Euglena sanguinea using eco-friendly natural catalyst. Energy Convers Manag. 2017;141:224–235.

[104] Kim Y-H, Choi Y-K, Park J, et al. Ionic liquid-mediated extraction of lipids from algal biomass. Bioresearch Technol. 2012;109:312–315.

[105] Breil C, Abert Vian M, Zemb T, et al. “Bligh and Dyer” and Folch methods for solid–liquid–liquid extraction of lipids from microorganisms. Comprehension of solvation mechanisms and towards substitution with alternative solvents. Int J Mol Sci. 2017;18(4):708.

[106] Zhang Y, Ward V, Dennis D, et al. Efficient extraction of a docosahexaenoic acid (DHA)-rich lipid fraction from Thraustochytrium sp. using ionic liquids. Materials. 2018;11(10):1986.

[107] Quader M, Ahmed S Bioenergy with carbon capture and storage (BECCS): future prospects of carbon-negative technologies. In: Rasul MG, Azad AK, Sharma SC, editors. Clean energy for sustainable development. Academic Press; 2017. p. 91–140. DOI:10.1016/B978-0-12-805423-9.00004-1

[108] Simas-Rodrigues C, Villela HDM, Martins AP, et al. Microalgae for economic applications: advantages and perspectives for bioethanol. J Exp Bot. 2015;66(14):4097–4108.

[109] Teixeira RE. Energy-efficient extraction of fuel and chemical feedstocks from algae. Green Chem. 2012;14(2):419–427.

[110] Gao K, Orr V, Rehmann L. Butanol fermentation from microalgae-derived carbohydrates after ionic liquid extraction. Bioresour Technol. 2016;206:77–85.

[111] To TQ, Procter K, Simmons BA, et al. Low cost ionic liquid–water mixtures for effective extraction of carbohydrate and lipid from algae. Faraday Discuss. 2017;206:93–112.

[112] Garcia ES, van Leeuwen JJA, Safi C, et al. Techno-functional properties of crude extracts from the green microalgae tetraselmis suecica. J Agric Food Chem. 2018;66(29):7831–7838.

[113] Sekar S, Chandramohan M. Phycobiliproteins as a commodity: trends in applied research, patents and commercialization. J Appl Physiol. 2008;20(2):113–136.

[114] Martins M, Vieira FA, Correia I, et al. Recovery of phycobiliproteins from the red macroalgae Gracilaria sp. using ionic liquid aqueous solutions. Green Chem. 2016;18(15):4287–4296.

[115] Bhalamurugan GL, Valerie O, Mark L. Valuable bio-products obtained from microalgal biomass and their commercial applications: A review. Environ Eng Res. 2018;23(3):229–241.

[116] Khoo KS, Lee SY, Ooi CW, et al. Recent advances in biorefinery of astaxanthin from Haematococcus pluvialis. Bioresource technology. 2019;288:121606. DOI:10.1016/j.biortech.2019.121606

[117] Miazek K, Kratky I, Sulc R, et al. Effect of organic solvents on microalgae growth, metabolism and industrial bioprocess extraction: a review. Int J Mol Sci. 2017;18(7):1429.

[118] Desai RK, Streefland M, Wijffels RH, et al. Novel astaxanthin extraction from Haematococcus pluvialis using cell permeabilising ionic liquids. Green Chem. 2016;18(5):1261–1267.

[119] Safafar H, van Wagener J, Møller P, et al. Carotenoids, phenolic compounds and tocopherols contribute to the antioxidative properties of some microalgae species grown on industrial wastewater. Mar Drugs. 2015;13(12):7339–7356.

[120] Jerez-Martel I, García-Poza S, Rodriguez-Martel G, et al. Phenolic profile and antioxidant activity of crude extracts from microalgae and cyanobacteria strains. J Food Qual. 2017;2017:1–8.

[121] Martinez-Francés E, Escudero-Ohate C. Cyanobacteria and microalgae in the production of valuable bioactive compounds. Microalgal Biotechnol. 2018. p. 105.

[122] de Morais MG, Vaz BD, de Morais EG, et al. Biologically active metabolites synthesized by microalgae. Biomed Res Int. 2015;2015. DOI:10.1155/2015/835761

[123] Sosa-Hernández JE, Romero-Castillo K, Parra-Arroyo L, et al. Mexican microalgae biodiversity and state-of-the-art extraction strategies to meet sustainable circular economy challenges: high-value compounds and their applied perspectives. Mar Drugs. 2019;17(3):174.