1. Introduction

Microalgae, with their fast growth rate, high lipid contents, astonishing CO₂ biofixation capabilities and effective mitigation of pollutants including nitrogen, phosphorus and metals, are considered as a highly promising feedstock that potentially can address the challenges in global energy crisis, environmental protection, and climate change [1-3]. In addition, microalgae have also been developed as an emerging source of bioactive compounds, such as antioxidative pigments (e.g., astaxanthin and carotenoids) [4], polysaccharides (e.g., starch, cellulose, and algal glucose), proteins (e.g., animal and aquaculture feeds and human food supplements), and functional fatty acids (e.g., docosahexaenoic acid and eicosapentaenoic acid) [5,6], which are widely applied in the fields of nutraceuticals, pharmaceuticals, and cosmetics, and have attracted tremendous attentions in the recent decades. Microalgal biorefining, referring to the process of transforming microalgal biomass into such valuable products, is an important step in the commercialization of microalgae [6]. Generally, the framework of microalgal biorefining consists of cultivation, harvesting, extraction and products processing (Fig. 1), within which, energy-efficient extraction of the target compounds is a crucial step [6,7]. The techno-economic analysis revealed that the extraction cost accounted for around 30-40% of the overall microalgal biofuel production cost [8,9]. However, the rigid cell wall of microalgae makes it difficult to release their intracellular contents, which consequently hinders the commercialization of microalgal bio-products [10-12].

A pretreatment process for cell disruption is usually necessary to assist algal product extraction. Several pretreatment technologies have been developed, such as (1) physical approaches including milling, homogenization, ultrasonification, and microwave, (2) chemical methods including hydrothermal conversion, acid or alkaline treatment, supercritical fluid treatment, ionic liquid treatment, and oxidative treatment, and (3) biological methods such as enzymatic hydrolysis or cell lysis by algicidal bacteria [7,10,13-15]. Particularly, the use of ultrasound in...
microalgal biorefining has drawn increasing attention, due to its advantages such as high efficiency, mild operating conditions, low toxicity, and time saving [16,17]. Ultrasound is the mechanical acoustic wave with oscillating frequency higher than the upper limit of normal human hearing range, from roughly 20 kHz to 10 MHz (Fig. 2) [18]. The earlier studies on the disruptive effects of ultrasound for the breakage of unicellular microalgae could trace back to 1970 s [19]. Since then, a tremendous amount of work has been reported on effective microalgal cell disruption by ultrasound [20–23]. In this review, recent advances of ultrasound in microalgal cell disruption and product extraction are summarized. Through illustrating the mechanisms of ultrasound induced cell disruption, analyzing the effect of ultrasonic conditions on cell disruption, exploring combined utilization of ultrasound and other disruptive methods, and pointing out the challenges and further research directions, this paper aims to provide a deep and thorough presentation of ultrasound assisted cell disruption and product extraction of microalgae.

2. Microalgal cell disruption mechanisms

Acoustic cavitation is a well-recognized theory for ultrasonic cell disruption. Ultrasound waves generate molecular motions through a series of rarefaction and compression cycles. Ultrasonic energy is transferred into the liquid solution and causes pressure alteration. Negative pressure is produced during the rarefaction cycle, and once the negative pressure falls below the vapor pressure of the liquid, small gas or vapor-filled bubbles are created in the liquid. These bubbles grow in the succeeding rarefaction and compression cycles. When the bubbles reach a size that the ultrasonic energy is insufficient to keep the vapor inside, they collapse violently during a compression cycle, releasing large amounts of energy [14]. The powerful collapsing of these microbubbles during cavitation generates both mechanical/physical and chemical effects for cell rupture (Fig. 3), which were pertinently reported in previous researches regarding the conversion of biological materials to valuable products [24]. Surprisingly, very few in-depth studies have been conducted towards the mechanism of ultrasound assisted intracellular material extraction for microalgal biorefining. Instead, the mechanism of ultrasonic attack for algal cell inactivation has been investigated widely in the field of harmful algae control [25] and numerous high resolution microscopic evidences were presented to directly reveal ultrasonic impacts on cell wall morphology. This section is dedicated to the mechanism of cell disruption by ultrasonic treatment without detailed description of the cavitation mechanisms, but focusing mostly on its mechanical/physical and chemical effects, bubble dynamics, and the cell wall morphological vibration.

2.1. Mechanical/physical effects of ultrasonic waves

Intense shock waves, shear forces, microbubble resonance, local high temperature and pressure constitute the main mechanical/physical causes of ultrasonic breaking of microalgal cells. Firstly, the acoustic cavitation via mechanical effects can induce sufficient shear forces, which can directly rupture microalgal cells and cause cell fragmentation [26,27]. Secondly, under proper conditions, the size of oscillating bubbles in ultrasonic systems are comparable to that of most microalgal cells, hence they can effectively excite mechanical resonance and consequently induce cell disruption. As reported by Kurokawa et al. [28], at each best frequency for cell disruption, the microalgal cell radius roughly correlated with the bubble resonance radius, indicating the non-negligible effects of bubble oscillation. Thirdly, the cavitation bubbles explode rapidly during ultrasound treatment, which can lead to localized temperature and pressure rises of up to 5,000 K and 100 MPa, respectively [29]. From a relatively macro perspective, the overall sample temperature during ultrasonic treatment would also grow accordingly. González-Fernández et al. [30] reported that the temperature reached to as high as 85°C within 15 min when 100.7 MJ/kg of energy was supplied by ultrasound and suggested that thermal effects might had accounted for much of the observed cell disruption of Scenedesmus biomass. In the study by Parniakov et al. [31], ultrasound was carried out with or without temperature control for phenolic compounds and chlorophyll extraction from Nannochloropsis spp., and the phenolic yield in uncontrolled group approximately equaled the sum of those of temperature controlled group and hot water extraction group, reflecting the additive effect of ultrasound with heating.
2.2. Chemical effects of ultrasonic waves

The chemical effect of ultrasonic cavitation generally refers to the oxidation by free radicals, including hydroxyl radicals (OH), hydrogen radicals (H), perhydroxyl radicals (HO₂) and others [29,32]. Reactive hydroxyl and hydrogen radicals (OH and H) are generated from the decomposition of water vapor trapped in the collapsing bubbles due to the extremely high temperature and pressure formed in collapsing bubbles [25,26], and their recombination or reaction with other gaseous species in the bubble leads to the formation of active species such as HO₂, O and H₂O₃ [18,32]. It has been reported that more hydroxyl radicals were generated at high frequencies than at low frequencies (1120 kHz > 740 kHz > 20 kHz) [29]. Besides, the amount of free radicals increases with the sonication time [25,29,33]. Upon the generation of free radicals, cell membrane lipids can be oxidized and membrane permeability can be changed, leading to the release of intracellular substances [29]. Besides, the antioxidant enzymes in microalgal cells, including superoxide dismutase (SOD), catalase (CAT) and malondialdehyde (MDA) were found to respond correspondingly [25]. The SOD and CAT of *Anabaena flos-aquae* increased rapidly at first, then stayed constant or decreased after cell inactivation, indicating the collapsing of antioxidant system [25].

When considering the possible mechanical/physical and chemical effects of ultrasonication, a few studies have suggested that chemical effects may not be the main mechanism for microalgal cell disruption [28]. The addition of potent free radical scavenger did not cause differences in the removal efficiency of *Microcystis aeruginosa* by ultrasound, implying the insignificant role of free radical oxidation [34].

2.3. Acoustic cavitation and bubble dynamics

According to the description of Gogate [35], acoustic cavitation is defined as “Here the pressure variations in the liquid are effected using sound waves, usually ultrasound 16 kHz-100 MHz. The chemical changes taking place due to the cavitation induced by the passage of sound waves are commonly known as sonochemistry”. Based on Miano et al. [36], ultrasound has direct and indirect effects on the treated material. The direct effect can be compared to the sponge effect and inertial flux, while the indirect effect is related to microchannel formation, and both effects contribute to mass flow. Compression and rarefaction of ultrasound waves forms bubbles and results in bubble explosion. Bubbles are stretched until they reach velocity resonance. Their radius grows dozens of times of the initial size, and collapse occurs rapidly in the negative pressure region [37]. Cavitation bubbles can be generated close to the cell surface, and their sharp collapse near the cell surface occurs asymmetrically, which results in high-speed liquid jets that generate shock waves, contributing to the disruption of cell walls. The contact area between algal cells and the extractant is therefore increased and the mass transfer is promoted, leading to improved extraction efficiency [37,38]. As suggested by Adam et al. [46], microstreaming and heightened mass transfer produced by cavitation and bubble collapse are the two critical factors affecting the extraction yields. Besides, the voids formed in cell membranes due to ultrasound are believed to be important for mass flow and enhance the mass transfer phenomena. This phenomena can be improved when a reasonable amount of cavities are formed and connected with each other, so called microchannels [36]. The maximum radius to initial radius (Rₘₐₓ/R₀), number and collapse pressure of bubbles, which correlate directly with resonance effect, are depending on operation conditions such as ultrasound intensity, frequency, liquid properties and reactor design. Thus by manipulating the operation conditions, the intensity of bubble collapse can be controlled and performance of cell disruption can be monitored.

At constant ultrasonic intensity and initial cavity size, the maximum size of a bubble decreases with increasing frequency, but collapsing of bubbles become very rapid thereby causing increase in the collapse pressure generated [40]. Hao et al. [37] suggested that the bubbles cannot grow big enough even if the cavitation bubbles are formed, they will not collapse due to too short expansion and compression times of
acoustic waves with the increase in ultrasound frequency. Yamamoto et al. [41] found that bubble radius decreased from 160 to 3.3 μm as ultrasonic frequency increased from 20 to 1146 kHz by using Minnaert’s formula. The resonance radius of 3.3 μm is very close to that of *Chlamydomonas concordia* of 3–6 μm and could effectively excite mechanical resonance and induce cell disruption. Formation of hydroxyl radicals were also found to be correlated with ultrasonic frequency [29]. Compared with lower frequency, more gas nuclei could reach the resonance size more quickly and generates greater collapse pressure at higher frequency [40]. However, decline in high frequency ultrasound energy was observed in ethanol, and cavitation bubbles were found to be more likely formed at a lower frequency of 20 kHz [37].

Higher ultrasonic intensity could induce stronger cavitation effect. As the ultrasonic power input increases, the stretching effect of ultrasound on bubble formation is enhanced in the positive pressure region [37]. The increase in the maximum size of the bubble/cavity was found to be about 30% at the optimum intensity and after that intensity the increase is less than 10% [40]. The increasing $R_{\text{max}}/R_0$ ratio of the bubble as a result of the increased intensity of ultrasound could result in more cavitationally active volume [18,42]. Besides, bubble wall pressure at the collapse point of the bubble was decreased with an increase in the ultrasound intensity. Thus the overall effect of the increased intensity results in larger maximum size of bubble, lower pressure pulse generated, increased bubble lifetime, increasing number of cavitation events and cumulative collapse pressure [18,40]. As reported by Wang and Yuan [18] that with an increasing power intensity from 100 to 250 W, the $R_{\text{max}}/R_0$ ratio, number of collapsing bubbles ($N_{\text{bubble}}$), $\text{H}_2\text{O}_2$ production rates ($\text{rH}_2\text{O}_2$) were all increased, while collapse pressure ($P_{\text{collapse}}$) decreased (Table 1). Hence, cumulative collapse pressure, representing the results of multiplying number of collapsing bubbles and collapse pressure of each bubble, was used to quantify cell disruption. And the strong correlation of modeling and experimental results suggested that cumulative collapse pressure computed from the developed model could be used as an indicator to reflect the cell disruption [18].

Solvents can be beneficial in penetrating the cell wall and membrane of microalgae, however, a high portion of solvents in the system might decrease the extraction efficiency due to less gas dissolved, thus resulting in decreasing cavitation [35,40]. Vapor pressure of the solvent is also an influencing factor. It has been found that vapor content in cavities formed in solvents with higher vapor pressure were higher thereby lowering the energy released during the collapse [35]. However, number of cavities were observed to increase due to lowering of cavitation threshold using solvents with higher vapor pressure [40]. The temperature of the system can affect the size of acoustic bubbles by influencing the expansion resistance and solvent viscosity. Bubble radius increases with increasing temperature due to decreasing pressure of cavitation threshold at higher operation temperatures [37]. The type of the sonoreactor plays an important role in formation and collapsing of the bubbles, for example, using horn or probe system could cause increasing intensity of irradiation by decreasing the area of irradiation surface at a constant power input. In this case, collapse pressure could decrease as a result of increasing intensity but the number of cavities generated remained the same, thus decreasing the overall cavitational effects [40].

### 2.4. Morphological alteration induced by ultrasound

The collapsing of microbubbles on the surface of microalgal cells generate diversified effects on the deformation of their surface morphology (Fig. 4).

#### 2.4.1. Fragmentation

The rupture of microalgal cells by ultrasound treatment was observed in most cases. Fragmentation, with the broken appearance and reduction in particle sizes, revealed the robust destructive force of ultrasound (Fig. 4A-D) [43–45]. Once the cells are disintegrated into small pieces, the intracellular constituents release readily. Meanwhile, the increase in surface area of cell particles caused by fragmentation could facilitate mass transfer, therefore increase the extraction yield [43,46].

#### 2.4.2. Perforation

The sonoporation effect of ultrasound is well recognized for biological applications [46], and more formation in microalgal cell wall treated by ultrasound has been noticed by some researchers [47]. For instance, the cell surface areas of *Chlorella vulgaris* increased significantly, and the microstructure exhibited more interspaces and holes after ultrasonic treatment than that by conventional agitated bead extraction [47]. Irregular structure filled with cavities on the trilaminar sheath of *Haeomatococcus pluvialis* cell wall was also resulted from ultrasound (Fig. 4E–F) [48]. The increase in the porosity of microalgal cells may lead to increased permeability of cell membrane, thus would enhance the release of intracellular components or the penetration of solvent into microalgal cells [48].

#### 2.4.3. Swelling or shrink

Some researchers have noticed the swelling or shrink of microalgal cells when treated by ultrasound. For example, when low frequency (50/60 Hz) of ultrasound was used to assist solvent extraction of lipids, carbohydrates, and proteins from *Chlorella vulgaris*, *Nannochloropsis oculata* and *Scenedesmus obliquus*, no cell disruption was observed. Instead, slight swelling of cells was noticed under SEM, which may prompt the solvent flow through the cell membrane, and subsequently facilitate solvent extraction of intracellular contents [49]. In another study by Huang et al. [50] using ultrasonic probe for *Chlorella vulgaris* disruption, the cell shape remained normal within 5 min of treatment, and then turned to be greatly shrinking after 60 min (Fig. 4G-H) [50]. The release of intercellular matrix was probably the main cause for them to shrink [29]. Also, as reported by Peng et al. [25], wrinkles on cell surface and a small number of broken fragments were observed in *Anaabaena flos-aquae* cells treated by ultrasound, indicating damaged cell structure caused by ultrasonic treatment.

#### 2.5. Detailed mechanisms and effects of ultrasound on different microalgae species

The quality and yield of intracellular compounds highly depend on the microalgal cell disruption method. One important factor for consideration to design an effective disruption method is the rigidity and structure of the microalgal cell wall. Several microalgal species including *Nannochloropsis* sp., *Chlorella* sp. and *Chlamydomonas* sp. have

### Table 1

| Power intensity of ultrasonic processor (W) | Actual power dissipated in the given volume (W) | $R_{\text{max}}/R_0$ | $\text{rH}_2\text{O}_2$ (mol l$^{-1}$ s$^{-1}$) | $N_{\text{bubble}}$ | $N_{\text{bubble}}$ (l$^{-1}$ s$^{-1}$) | $P_{\text{collapse}}$ (Pa) |
|-------------------------------------------|-----------------------------------------------|----------------------|-----------------------------------------------|---------------------|-------------------------------------|------------------------|
| 100                                       | 9.70                                          | 10                   | $2.46 \times 10^5$                            | $7.80 \times 10^{12}$ | $3.15 \times 10^5$                      | $6.59 \times 10^{10}$   |
| 150                                       | 15.73                                         | 10                   | $4.21 \times 10^5$                            | $8.27 \times 10^{12}$ | $5.09 \times 10^5$                      | $6.07 \times 10^{10}$   |
| 200                                       | 22.35                                         | 10                   | $6.05 \times 10^5$                            | $8.49 \times 10^{12}$ | $7.13 \times 10^5$                      | $5.72 \times 10^{10}$   |
| 250                                       | 27.78                                         | 10                   | $7.51 \times 10^5$                            | $9.02 \times 10^{12}$ | $8.32 \times 10^5$                      | $5.51 \times 10^{10}$   |
been widely cultured and applied as sustainable sources for bioproducts production. These microalgal species vary in size, cell wall structure and rigidity. The detailed mechanism of ultrasound over the microalgal species has been summarized in Table 2. And discussions on the detailed mechanism and influences of ultrasound treatment on disrupting the varieties of microalgal species are as following.

2.5.1. Nannochloropsis sp.

The microalgae Nannochloropsis contains a high lipid content of above 30% of dry biomass [22]. Ultrasound treatment could efficiently change the microstructure and morphology of Nannochloropsis oculata cells. Adam et al. [39] observed damaged parietal system and smaller cells after ultrasound assisted extraction, which suggested that ultrasound could change the external structure of cell surface. The high local temperature and pressure caused by collapsing bubbles could lead to the breaking of Nannochloropsis cells into small fragments, and resulted in release of oil into the liquid phase [22]. However, the direct effects of ultrasound waves on Nannochloropsis cells might be small. For example, the wavelength of high frequency ultrasound of 4.3 MHz in water was estimated to be 350 μm, which is 270 times greater than the radius of Nannochloropsis sp, since the size of algal cells is too small compared to the wavelength of ultrasound. Thus the authors concluded that cavitation is the essential part, rather than the direct effects of ultrasound, that is required in ultrasonic algae disruption [28].

Fig. 4. Impacts of ultrasonic treatment on microalgal cell wall morphology. (A) Chlorella sp. without treatment, (B) ruptured Chlorella sp. after ultrasonic treatment, adapted from Ma et al. [43]; (C) Chlamydomonas mexicana without treatment, (D) ruptured Chlamydomonas mexicana after ultrasonic treatment, adapted from Eldalatony et al. [44]; (E) Haematococcus pluvialis without treatment, (F) deformed Haematococcus pluvialis with an irregular structure filled with cavities on the cell wall after ultrasonic treatment, adapted from Khoo et al. [48]; (G) Chlorella vulgaris without treatment, (H) Shrunken Chlorella vulgaris after ultrasonic treatment, adapted from Huang et al. [50].
Table 2
Detailed ultrasonic cell disruption mechanism over the microalgal species.

| Microalgae strains   | Detailed cell disruption mechanism                                                                 | Morphological alteration                                                                 | References |
|----------------------|----------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------|------------|
| *Nannochloropsis* sp.| High shear forces and acoustic cavitation are likely the causes for ruptured cells                | Ruptured cell debris was aggregated                                                     | [27]       |
| *Nannochloropsis* oculata | Physical effect of cavitation caused external structure alteration of cells surface             | Smaller cells with damaged parietal system                                                | [39]       |
| *Chlorella vulgaris* | Both physical and chemical attack caused the cells damage                                           | Shrunked cells shrivelled with many punctures at the surface                             | [50]       |
| *Chlorella* pyrenoidosa | High-speed and violent liquid stream force leading to the point puncturing effect was the main reason for cell rupture | Defective cell with clear edge                                                          | [51]       |
| *Scenedesmus quadricauda* | Intense shock wave originated from collapsing of cavitating microbubbles caused cell rupture    | –                                                                                        | [19]       |
| *Microcystis aeruginosa* | Thermal effects during sonication might account for much of cell disruption                     | –                                                                                        | [30]       |
| *Microcystis aeruginosa* | The mechanical effects of ultrasound mainly caused local damage to the intracellular structure of algal cells | Algal cells showed wrinkles                                                               | [29]       |
| *Microcystis aeruginosa* | At low ultrasonic frequencies, mechanical effects, i.e. high shear forces mainly lead to the direct rupture of cells. At high ultrasonic frequencies, chemical attacks from free radicals also contributed to the weakness of cell walls | –                                                                                        | [36]       |
| *Haematococcus pluvialis* | Solvent vapor pressure tightly related to bubble collapses influences astaxanthin extraction efficiency | –                                                                                        | [52]       |

2.5.2. *Chlorella* sp.

*Chlorella vulgaris* is a microalgal species that contains bioactive compounds such as lipids, proteins, vitamins, polysaccharides, and pigments. It is a green eukaryotic microalga which belongs to the family of *Chlorellaceae* and genus of *Chlorella* [50]. The cell wall of *Chlorella vulgaris* is rigid thus disruption usually requires more energy [47]. Generation of free radicals and implosion of microbubbles inside and outside algal cells may contribute to the rupture of *Chlorella vulgaris* cell walls, and application of solvents such as chloroform and dichloromethane can also weaken the cell wall [53]. The specific surface area of microalgae cells increased from 3.831 to 11.923 m²/g after ultrasonic extraction, suggesting cell wall destruction and fragmentation [47]. As reported by Huang et al. [50], the round shape of *Chlorella vulgaris* changed to irregular under exposure to ultrasonic energy, and release of cellular components such as protein, lipid, nucleic acid and chlorophyll increased the UV absorbance in the liquid phase. And as mentioned above, the free radicals generated during ultrasonication might cause degradation and oxidation of polyunsaturated fatty acids and other antioxidant compounds, but according to the study of Ma et al. [54], a low sonication power of 50 W and a short duration of 10 min didn’t result in oxidation of PUFA due to the protection effect of carotenoids.

2.5.3. *Chlamydomonas* sp.

According to the observation of Bigelow et al. [33], as sonication duration became longer (from 5 to 10 s to 3–9 min), most *Chlamydomonas reinhardtii* cells remain intact at first but began to break apart. Release of small amount of chlorophyll in moderate exposure duration of 15–45 s indicated that cells have begun to lyse but the membrane fragments were still in large pieces, and after long exposure of 3–9 min complete cell fragmentation was almost achieved. Since chlorophyll locates in chloroplast, release of chlorophyll also suggests that ultrasound could disrupt both cellular and intracellular structure of *Chlamydomonas reinhardtii*. Sonication process may assist not only disruption of cell and organelle, but also in the dispersion of cell debris of *Chlamydomonas reinhardtii*, thus improved release lipids from broken cells into the aqueous environment [55].

2.5.4. Other species

As revealed by scanning electron microscopy analysis, ultrasonication treated *Microcystinum reinseri*, *Scenedesmus obliquus* and *Triropsoma aequale* samples showed ruptured cell wall, while the untreated samples had smooth cell surfaces [44]. When combined with solvent extraction, ultrasonication helped with solvent penetration into cell wall and membrane, and prompted solvent extraction from *Scenedesmus obliquus* [49]. Though release of target compounds usually comes with disintegrated cells, even if the cells were not fully disrupted, the maximum extractability of lipids from *Schizochytrium limacinum* was achieved, suggesting that in addition to cell disruption, ultrasonication may increase the permeability of microalgae cells [55]. The intense shock wave generated by microbubbles collapse can easily produce microscopic cracks and damage the solid surface of *Scenedesmus quadricauda*. It was suggested that cell surface could also act as a host for new cavitation events since nucleus can localize onto cell surface [19]. The size of microalga cells has great influence on sonication efficiency. Natarajan et al. [56] compared the sonication efficiency on *Tetraselmis suecica* and *Chlorella* sp. and found that under the same ultrasonic conditions, *Chlorella* sp. required more energy due to their rigid cell wall structure and smaller size.

3. Ultrasound operation parameters for effective cell disruption

3.1. Effects of ultrasonic parameters

Regardless of the specific cause or causes of cell disruption under ultrasonic treatment, many factors could impact on the final result, and the overall cavitation effects can be influenced by various parameters such as ultrasound frequency, intensity and treatment time. In the past decades, multiple researches have been performed to investigate the influence of those parameters and optimize the extraction process for a better cell disruption and higher extraction yield, as well as lowering the cost and energy consumption of ultrasound-assisted extraction. Detailed research advances of the three parameters that have been studied the most in literature are elaborated below.

3.1.1. Frequency

Since ultrasound is a mechanical wave, its frequency is one important characteristic that can influence the acoustic cavitation [46]. Frequency of ultrasound indicates the number of acoustic cycles per unit time, and higher frequency gives a greater number of acoustic cycles during a given period [35]. Both low (less than100 kHz) and high frequencies (100 kHz-20 MHz) have proven to be effective for microalgal cell disruption [57]. As revealed by the authors' previous study,
chlorophyll $a$ fluorescence density (an indirect indicator of cell disruption) and the lipid fluorescence density of Scenedesmus dimorphus and Nannochloropsis oculata increased significantly under both high frequency (3.2 MHz) and low frequency (20 kHz) ultrasonic treatment, revealing efficient cell disruption and lipid releasing caused by ultrasonic treatment [57].

Conventionally, low ultrasound frequencies in the range of 20–40 kHz were most commonly used in the process of microalgal bioproducts extraction [48,55,58–62]. Specially, for suspensive microalgae with thin cell walls, low-frequency ultrasound is highly effective towards cell disruption. Chlamydomonas reinhardtii with hydroxyproline-rich glycoprotein based cell walls showed high sensitivity to low frequency ultrasound that >80% of the cells were ruptured after sonication at 35 kHz for 5 min [61], and almost all cells were ruptured after 18 min at the same frequency of 35 kHz [63]. Similarly, complete cell disruption of microalgae Ankistrodesmus fusiiforms was achieved with ultrasonic processing at 20 kHz for 60 min [64].

On the other hand, ultrasound with high frequencies of about 1.0–5.0 MHz was also used in microagal pretreatment [28,41,57]. From the perspective of ultrasonic sonochemistry, high frequencies of ultrasound are believed to be beneficial for cavitation effects, and subsequently for microalgal cell disruption. This can be attributed to the fact that at higher frequencies, the cavity sizes become smaller, thus fewer acoustic cycles are needed for the achievement of the requisite resonant size [35]. That is, the collapse of the cavity would be accelerated at high frequency, which therefore leads to an increase in the magnitude of the collapse pressure [35]. Besides, the resonance radii of microbubbles decreased with the increase in ultrasonic frequency, and were several $\mu m$ at high frequencies of over 1 MHz [28,41], which are on the same order of the radii of most microalgae, thus the oscillation of these microbubbles near microalgal cells can be an excitation source for their mechanical resonance, hence inducing shape vibration of the cells [41].

Several studies have investigated the disruptive effects of a series of different frequencies on microagal suspensions and revealed the beneficial effects of high frequencies. For instance, within the applied frequency range, the maximum reductions in cell numbers of both Chlamydomonas concordia and Dunaliella salina were obtained at the highest frequency of 1.146 MHz [41]. The remarkable disruptive effect of high ultrasonic frequency is more evident in the treatment of highly resistant microalgae. As reported by Kurokawa et al. [28], ultrasonic treatment at 4.3 MHz, which was highest at all frequencies investigated, resulted in approximately 90% cell disruption on Nannochloropsis sp. at 10 min. While, no cell disruption was observed for Nannochloropsis oculata even after treatment for 180 min at low frequency of 50/60 Hz [49], indicating that for microalgae with robust cell wall, high frequency ultrasonic treatment would be more desired.

However, it should be noted that higher frequency of ultrasound does not always bring better effects. The threshold intensity or power to attain cavitation increases with frequency increase, consequently at the same operating intensity of ultrasound, the cumulative effect of cavitation would be reduced by the lower number of cavitation events [40]. Some supporting evidences showed that for Chaetoceros gracilis and Chaetoceros calcitrans, high frequency of 4.3 MHz treatment resulted much lower disruption efficiency, and the most effective cell disruption of them were achieved at 2.2 and 3.3 MHz, respectively [28]. Similarly, the order of efficiency for Microcystis aeruginosa reduction was 20 less than 1146 less than 864 less than 560 kHz [21]. However, although the concept of optimal frequency of ultrasound is well established, there is still no standard guideline to follow for setting up frequency. According to the previous research of the authors’ group, the combination of high and low frequency treatments could be even more effective than single frequency treatment [57]. The microalgal species of Scenedesmus dimorphus and Nannochloropsis oculata were sequentially treated by two separate ultrasonic devices with high frequency (3.2 MHz) and low frequency (20 kHz) respectively, leading to increased lipid recovery, probably due to the combined benefits of the high and low frequency treatments [57].

3.1.2. Ultrasonic intensity or power input

Ultrasonic intensity is defined as the power dissipated per unit of surface area of the sonotrode and its unit is usually W/cm². This parameter reflects the pressure amplitude of ultrasound waves [46]. Molecules of the liquid phase are temporarily dislodged from their original position while they are exposed to sound waves. When the intensity of the waves is sufficiently high, the attraction forces among liquid phase molecules will be exceeded by dislodging force and cavities in the liquid will be generated [46]. High energy intensity would provide violent shearing forces, high pressure and temperature, and hence effectively disrupt the cells [17].

Many researchers used power input of the sonochemical reactor directly to represent ultrasonic intensity, but for more accurate estimation of the acoustic power dissipated into the system, calorimetry method has been widely applied to determine the acoustic power or intensity during microagal cell disruption [25,28,29,48,51,61,63]. In this method, temperature was recorded continuously during sonication to reflect the ultrasound power dissipated through the system. Tan et al. [63] has reported a strong linear relationship between sonication time and temperature ($r^2 = 0.99, p < 0.01$) during ultrasound treatment, suggesting monitoring temperature could provide estimation of sonication power input. The acoustic power and intensity can be computed using the equations below [28]:

$$\text{Acoustic power} = \frac{mC_p\Delta T}{\Delta t}$$

(1)

$$\text{Acoustic intensity} = \frac{\text{Acoustic power}}{\text{volume or area}}$$

(2)

where $C_p$ is the specific heat capacity of the solvent, $m$ is the mass of solvent, $\Delta T$ is the increase of temperature of the solvent, and $\Delta t$ is ultrasonic irradiation time.

Generally, the range of ultrasound used for microalgae cell disruption/ removal in sonochemical reactors is 1–300 W/cm². When a high-power ultrasonic flow system was applied for cell disruption of two algal strains Scenedesmus dimorphus and Nannochloropsis oculata, the cell disruption effects of different ultrasound intensity (0.8, 1.36 and 1.76 kW) were evaluated and compared. And the result revealed that the concentration of cell debris of both algal strains showed an increasing trend at stronger ultrasonic energy input [65]. Cell breakage rate of Chlorella sp. also increased when ultrasonic power input was raised from 600 to 1000 W [23].

Typically the cavitational yield increases with increasing power input unless the ultrasound power reaches a particular limit. The increase in the maximum size of the bubble/cavity is about 30% at the optimum intensity and after that intensity the increase is less than 10% [40]. It is because the ratio of maximum radius to initial radius increases as the intensity of ultrasound increases, which results in more cavitationally active volume [42]. Conversely, with an increase in the intensity of the ultrasound, the bubble wall pressure at the collapse point of the cavity decreases as the energy associated with the bubble may be taken up by the compressible liquid medium [40]. It also cannot be ignored that higher power input requires more energy and is not always desirable in practical uses. Recently, more studies have focused on optimizing power input for algal cell disruption. For example, for ultrasonic-assisted extraction of lipids from fresh Nannochloropsis oculata cells, ultrasonic power of 300, 450, 650, 850, and 1000 W was used to estimate the optimum conditions for oil extraction through response surface methodology (RSM). Although the maximum oil recovery was achieved at 1000 W, the effect of ultrasonic power was not directly significant on the oil recovery rate ($p = 0.9415$) in this design model [39]. Thus, the influence of ultrasonic power input on target compound recovery is usually dependent on other related parameters such as sonochemical reactors, the corresponding operating parameters, as well as the type of cell treated. For example, the power input of Wang et al. [66] was set...
from 132 to 468 W with a center point of 300 W to obtain crude lipids from *Nannochloropsis oculata*. It was found that the influence of power input on lipid recovery was dependent on sonication duration and algal cell concentration. A shorter sonication duration required stronger power input while power input had no major influence at too high cell concentrations. In another study, concentration of proteins and carbohydrates in the microalgae supernatant, used as cell disruption indicator, was found to increase when ultrasonic energy intensity increased from 0.1 to 0.4 kWh/L, but decreased when it reached 0.5 kWh/L, implying an optimum energy dosage existed. This could be explained by the excessive interactions among cavitations instead of their interaction with the cells [67].

Several studies have been performed to understand the relationship between ultrasound intensity and other extraction parameters. Greenly and Tester [60] studied the extraction kinetics and found that high intensity (2.5 W/mL) and low frequency (20 kHz) ultrasound offered the most significant cell disruption at the initial 2–5 s of sonication. A maximum power input of 2.2 kW was applied on *Sischochitrium limacinum* and *Chlamydomonas reinhardtii* [53]. In both algal species, increasing energy input resulted in an increased lipid extraction. However, they also found that greater cell disruption under stronger energy input didn’t result in greater lipid extractability, which was explained that maximum extractability could be reached before the cells were fully disintegrated. Ultrasound intensity and ethanol concentration showed significant influence on carotenoid extraction from *Heterochorea luteoviridis*, and 50% intensity (corresponds to 25 W/cm² of dissipated power and 435 kJ/kg) resulted in the highest carotenoid yield [62]. A decrease in extraction yield was observed at 100% ultrasound intensity (corresponds to 50 W/cm² of dissipated power and 217.5 kJ/kg) probably due to the formation and accumulation of radicals during cavitation process. Concentration of algal biomass could also influence the effectiveness of ultrasound treatment. The process becomes more energy efficient at higher algal biomass concentration because it is more effective in wave contact with solid matter such as microalgae cells, as for all released components, the highest yields (94 ± 4.7% proteins, 40 ± 2.8% carbohydrates, and 26 ± 0.9% lipids) were achieved at the highest biomass concentration of 75 g/L *Desmodesmus* sp. cells [58]. However, they also found that ultrasonic intensity had a negative effect on the yield of released biocomponents and concluded that the highest yield could be achieved at the low-power/long-time ultrasound pretreatment (ultrasonic intensity of 0.32 W/mL). Increasing ultrasonic power from 80 to 180 W facilitated the release of lipids from *Spirulina* sp., however, this increase was not significant, with weight yield enhanced from 1.1% to 1.6% [68]. Thus, the effect of ultrasound intensity or power input on the extractability of a target component is complex and requires more investigations into the detailed extraction procedure.

### 3.1.3. Treatment time

The ultrasound assisted extraction process has been known for its fast operation, usually in minutes instead of hours compared to conventional methods [39]. Generally, increasing the sonication treatment time increases the content of released components as cell disruption takes time, and complete cell fragmentation and release of cellular component could only be induced after sufficient exposure duration [33]. For example, as reported by Adam et al. [39], the oil yield of *Nannochloropsis oculata* was enhanced with increasing treatment duration. In addition, it was reported that the highest ester yield was 99.9% after 120 min of treatment at 180 W. However, 30, 60, and 120 min treatments didn’t show significant differences, suggesting that short sonication time was enough to achieve suitable yield [68]. Cell disruption rate was also found to be dependent on the microalgae species treated, and *Chlorella pyrenoidosa* was highly resistant to sonication, with the cell rupture of only 7.5% after treating for 60 min [61]. The initial few seconds of sonication caused the most significant cell disruption in *Chlamydomonas reinhardtii* and *Thalassiosira pseudonana*, with the disruptive effect slowing down as time progressed. The time that intact cells were halved was 2 s and 1 min for the two strains, respectively. Attenuation of the sound wave by the local field of cavitating bubbles was mostly likely the reason of the slowing down of cell breakage after the initial few seconds [60]. However, a long sonication exposure period could result in release of free radical, oxidation of lipids and accumulation of heat generated. Some researchers also investigated lipid extractability as a function of exposure time and concluded that complete cell lysis was not necessary to extract lipids from *Chlamydomonas reinhardtii* since this algae has a vulnerable cell wall [33]. Besides, sonication time is directly related and proportional to energy consumption [51], thus a short-period ultrasound process is always preferred.

### 3.2. Effect of liquid phase properties

Direct extraction from wet microalgal biomass has drawn growing interests and shown promising effects on lipid extraction from algal suspension (biomass concentration 250 mg/L) [67]. Ultrasonic treatment on dilute algal slurries might not be desirable because most of the ultrasonic energy is wasted on heating up the solution rather than breaking the cells. Nevertheless, it was reported that the extraction yield decreased with increasing solid concentration due to higher viscosity and slower speed of sound [27]. Similar results were also reported by Greenly and Tester [60] that higher concentrations of *Isochrysis* disintegrated slower as the concentration of cell increased from 0.5 to 7.5 wt%. The influence of liquid phase may also depend on other parameters such as the viscosity limitation of the system since mixing microalgal suspension with high viscosity requires more than acoustic streaming. For example, the maximum protein and chlorophyll releases from *Chlamydomonas reinhardtii* were found at a microalgae concentration of 25 g/L, while the protein and chlorophyll contents in the extract supernatant halved as the concentration reached 50 g/L. It was suggested that the reduction in cell lysis was due to the limitation of their ultrasound transducer system [33]. On contrary, statistically no significant differences in cell disruption among various microalgal concentrations were observed when a flow system was applied to assist the dispersion of microalgae through the focal region [33].

Besides the concentration and viscosity of the treated microalgal suspensions, solvents used for extraction could also influence the extraction process. Solvent choice is driven by solubility of target components, viscosity, surface tension, and vapor pressure of the solvent, usually a solvent with low vapor pressure is preferred since the collapse of cavitation bubble can be more intense [46]. Solvents also contribute to weakening algal cell wall, thus solvents used for ultrasonic assisted extraction is crucial for enhancing lipid yield. Ultrasound also improves solvent flow through cell membrane and promotes solvent extraction (Ferreira et al., 2016). The Bligh and Dyer method using methanol, chloroform, and water with ultrasound resulted in the highest oil yield from *Chlorella vulgaris*, which could be attributed to the disruption of cell wall by ultrasonic cavitation and high selectivity of lipids toward chloroform-methanol-water system [53]. The highest oil and sugar extraction yields from *Chlorella vulgaris* were obtained by using a binary mixture of n-hexane and isopropanol together with ultrasonic treatment, while only small amounts of pigments were extracted in absence of ultrasound [49].

### 3.3. Effects of sonochemical reactor design

Currently, the common reactor designs for generating acoustic cavitation can be categorized as ultrasonic horn, bath, and flow cell [58, 5]. An ultrasonic horn is usually operated at a fixed frequency, and the rated power is dissipated through a specific horn area. It can be operated in continuous mode. However, this results in temperature rise and might cause destruction of target compounds, therefore, cooling is usually required during or after operation. The ultrasonic bath is a water bath with transducers attached at the bottom of the reactor to provide
Fig. 5. Diagram of an ultrasonic horn reactor (A and B, reproduced from Wang and Yuan [104]) and an ultrasonic flow cell reactor (C, reproduced from Wang and Yuan [65]). (A) represents the diagram of the ultrasonic nozzle spraying system and (B) displays the spraying nozzle setup.
acoustic energy. It also operates at a fixed frequency with a rated output power, and usually batch mode is operated. The flow cell consists of several sets of transducers mounted on a big vessel. The transducers might operate at the same or different frequencies with specific power rating for each set, and can operate in batch or continuous mode [69,70].

An ultrasonic processor equipped with a 3/8-inch diameter tip horn was inserted 3.5 cm into a 45 mL cell suspension to investigate cell rupture effects. It was reported that the most significant cell disruption occurred during the initial seconds of sonication, and the concentration of cells didn’t show a significant influence on Isochrysis disintegration [60]. They also suggested that with a simple flow system to aid with driving algal cells through focal regions, the effect of solid concentration on cell disruption could be circumvented [33].

An integrated sugaring-out assisted liquid biphasic flotation and ultrasound system was applied to extract proteins from Chlorella vulgaris. The aqueous sugar solution with microalgae biomass formed the bottom phase and acetonitrile for protein extraction was the top phase in the liquid biphasic system. The maximum protein separation efficiency and phase and biomass bottom phase. It was also found that placing the horn and a cavitating tube (21.5 kHz), were investigated. The cavitating tube liquid biphasic system. The maximum protein separation efficiency and phase and acetonitrile for protein extraction was the top phase in the factory by comparing to other species in microalgal biorefining. On the rating for each set, and can operate in batch or continuous mode might operate at the same or different frequencies with specific power, and usually batch mode is operated. The flow cell consists of two high-power (100 W) devices, an immersion horn (19.5 kHz) and a cavitating tube was preferred [71].

The biological and physical properties of microalgae, particularly the cell wall structure and cell size, are critical factors affecting the cell disruption efficacy in ultrasonic processing (Table 3). In terms of cell wall structure, microalgae with cellulose carbohydrate-based cell wall typically show more resistance against ultrasound than cells with cell wall mainly composed of hydroxproline-rich glycoproteins [61]. The cell disruption efficiencies of Chlorella and Nannochloropsis species were only around 50–60% when low frequency of ultrasound was used [60,72], as they both possess a thick and strong cell wall with cellulose and hemicellulose as the major component (Table 3). Moreover, only 7.5% of cell disruption was achieved for Chlorella Pyrenoidosa, though the suspension was treated for as long as 60 min [61]. When high power ultrasound was applied, a much higher rupture efficiency of around 84% towards Chlorella vulgaris was obtained [50], which was still unsatisfactory by comparing to other species in microalgal biofrefining. On the contrary, a large proportion of Chlamydomonas cells can be ruptured by ultrasonic treatment, and even complete disruption was observed within a short time, demonstrating the susceptibility of proteinaceous cell wall [63].

In addition, the cell breakage rate is tightly related to the cell size. Three Microcystis aeruginosa strains with similar biological characteristics and cells shapes, but difference cell sizes were used to investigate the effect of cell size on ultrasonic disruption. And the results reveal that cell disruption was positive correlated to cell size, indicating that under the same ultrasonic conditions, a decrease in cell diameter could reduce cell disruption efficiency of ultrasonication [61]. The disruption efficiency

### Table 3

| Microalgae strains | Cell size | Optimal ultrasonic conditions | Performance | References |
|--------------------|-----------|-------------------------------|-------------|------------|
| Chlorella sp.       | 3–8 μm in diameter | 20 kHz, 1080 W, 60 min | 83.8% cell disruption | [50] |
| Chlorella vulgaris   |            | 20 kHz, 20 min | 56.4% cell disruption | [72] |
| Chlorella pyrenoidosa|          | 35 kHz, 0.043 W mL⁻¹, 360 min | 52.2% cell disruption | [63] |
| Nannochloropsis sp. | About 2–4 μm in diameter | Phenolic extraction yield was ~ 2 times higher than the control | Less than 60% cell disruption | [66] |
| Haematococcus pluvialis | 5–50 μm | 20 kHz, 2 min | 11.79% astaxanthin extractability | [74] |
| Chlamydomonas sp.   | 10–12 μm in diameter | 24 kHz, 400 W, 30 min | The maximum oil extraction yield was 22% (g g⁻¹), a 1.7-fold increase compared to control | [75] |
| Scenedesmus dimorphus| Bean shaped | 35 kHz, 0.043 W mL⁻¹, 18 min | Almost complete cell disruption | [63] |
| Scenedesmus reinhardti | Bean shaped | 35 kHz, 0.043 W mL⁻¹, 30 min | Almost complete cell disruption | [61] |

(continued on next page)
of *Ankistrodesmus fusiformis*, whose length was over 20 μm and width 1–6 μm, approached 100% within 20 min, also demonstrating the high efficiency of ultrasonic process upon large-size algae. In fact, the ultrasonic cell disruption is generally a comprehensive outcome by the effects of both cell wall structure and cell size. Compared to *Tetraselmis suecica*, whose average diameter was almost 10 μm, *Chlorella* sp. was found to have significantly lower cell disruption efficiency by ultrasonication, as *Chlorella* sp. have a very rigid three layered cell wall structure, as well as a much smaller cell diameter of about 2 μm [56]. However, *Haematococcus pluvialis*, with its well-known hydrolysis-resistant outer layer [76], were highly resistant to ultrasonic treatment that the astaxanthin extractability was only 11.79% after ultrasonic treatment [74], though the cells generally range from 10 to 20 μm in diameter.

Overall, for the microalgal species showing great potentials in bio-refining, the ease of cell rupture by ultrasonic attack can be summarized in the following order: *Haematococcus pluvialis* ≈ *Chlorella* ≈ *Nannochloropsis* ≈ *Thalassiosira* ≈ *Chlamydomonas reinhardtii* ≈ * Isochrysis galbana* (lack a cell wall) ≈ *Porphyridium cruentum* (lack a well-defined cell wall) ≈ *Arthrospira platensis* (Fig. 6) [60,65,77].

In order to obtain the best disruption and extraction performance, a number of studies conducted optimization of ultrasonic conditions over varieties of microalgal species and mostly multiple parameters including ultrasonic frequency, intensity, cell concentration, treatment duration, temperature were investigated by single factor experiment or response surface methodology [58,78]. As for *Haematococcus pluvialis* (representing the microalgal group that is most difficult to rupture), *Zou* et al. [82] optimized extraction solvent, liquid-to-solid ratio, temperature and time for astaxanthin extraction, results revealed that the astaxanthin yield by optimal ultrasonic treatment was around 1.5 times of conventional extraction [82]. As for *Nannochloropsis*, parameters including ultrasonic power, extraction time and biomass dry weight content were optimized through response surface methodology for lipids extraction from *Nannochloropsis oculata*, and the biomass dry weight content, representing liquid viscosity was supposed to be the most significant factor for ultrasound assisted lipid recovery [39]. As for *Chlorella*, lutein extraction from *Chlorella vulgaris* was improved by optimizing extraction temperature, time and solvent concentration, and the lutein yield with the optimal ultrasound treatment with or without enzymatic treatment was 1.8–2.0 times of conventional extraction [47]. Similarly, the effects of four parameters including ultrasonic power, extraction time, flow rate and algal cell concentration, on carbohydrate extraction from *Chlorella* sp. were investigated, and the glucose yield under optimal ultrasonic conditions was much higher (3-folds) compared to conventional solvent extraction [23]. Overall, these evidences suggested that diverse factors during ultrasonic treatment jointly affect the disruption and extraction performance regardless of the strain species. And in spite of the difficulties of ultrasonic cell disruption for some microalgal strains, ultrasonication provides better results than conventional extraction, and the ultrasonic performance can be further enhanced through conditions optimization.

### 4. Synergistic integration of ultrasound and other cell disruption methods

Apart from ultrasound treatment, a variety of disruption methods have been investigated in the field of microalgal bio-refining, and each of them has its own strengths and limitations [11,13,14]. Combined utilizations of these diverse methods have been frequently adopted for the purpose of enhanced disruption efficiency. The benefits of hybrid techniques were highlighted by some previous reviews [10,11]. Here, the integration of ultrasound with other disruptive methods for microalgal biorefining is summarized, with the focus being on the extraction performance regardless of the strain species. And in spite of the difficulties of ultrasonic cell disruption for some microalgal strains, ultrasonication provides better results than conventional extraction, and the ultrasonic performance can be further enhanced through conditions optimization.

### 4.1. With chemical methods

In most studies regarding ultrasound assisted extraction of target products from microalgal cells, the ultrasonic pretreatment was jointly used with different solvent systems, which generally resulted in better product yields comparing to those using ultrasound (solvent-free) or solvent extraction alone [89]. By producing cavitation in the solvent through passaging ultrasonic waves, ultrasound is able to allow greater penetration of the solvent into microalgal biomass, subsequently promote the interaction of solvent with intracellular components, and then

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**Table 3 (continued)**

| Microalgae strains | Cell size, form and cell wall structure features | Optimal ultrasonic conditions | Performance references |
|-------------------|------------------------------------------------|-----------------------------|------------------------|
| *Ankistrodesmus fusiformis* | 1–6 μm in length and width 22–60 μm | 20 kHz, 130 W, 60 min | Complete cell disruption (100%) [64] |
| *Tetraselmis suecica* | 5–15 μm in diameter | 20 kHz, 500–1000 W | Nearly complete cell disruption [56] |

*Fig. 6. The ease of cell rupture by ultrasonic treatment. Microalgal species shown here are with great potentials in bio-refining and their main valuable components are listed. The cell size and cell wall construction are key factors for cell rupture efficiency by ultrasonic treatment. (PUFAs: polyunsaturated fatty acids).*
the extraction efficacy.

As for pigment retrieval, mild organic solvents such as ethanol, methanol, acetone and ethyl acetate are effective and preferred in microalgal biorefining [90]. The ultrasonic treatment in the presence of ethanol significantly promoted the carotenoids extraction yield to 80% [62]. Similarly, remarkable increases in the chlorophyll yield were also observed under ultrasonic extraction than conventional methods using the same solvent of ethanol [31,80]. Nevertheless, it seemed that comparing to other physical–chemical methods, ultrasound assisted extraction brought little benefits towards astaxanthin production from Haematococcus pluvialis, due to the presence of thick and hydrolysis-resistant cell wall [12,74].

As inspired by their great potential in biofuel production, the lipid extraction from oleaginous microalgae has been intensively studied in recent decades [7]. Two well-established solvent-based lipid extraction methods, Bligh and Dryer, and Soxhlet methods, were applied in most studies [67]. Additionally, a few researchers also adopted Folch, Chen, and Hara and Radin methods [53]. Among all these methods, Bligh and Dryer method using the solvent mixture of methanol and chloroform, outperformed the others in terms of lipid yield and operation time. Moreover, coupled utilization of ultrasound treatment and Bligh and Dryer method led to the highest lipid extraction efficiency that around 2-folds higher lipid extraction from Scenedesmus sp. was attained compared to using Bligh and Dryer method alone [91].

Apart from conventional organic solvent systems, the joint effects of ultrasound with other innovative chemical disruptive techniques such as

| Table 4 | Combined utilization of ultrasound and other techniques for cell disruption and products extraction in microalgal biorefining. |
|----------------|-------------------------------------------------------------------------------------------------------------------------------|
| Disruption strategy | Microagal strains | Target products | Performance | References |
| Ultrasound + Ozone | Sequential mode: First ozonation, followed by ultrasound | Mixed microagal culture, dominated by Chlorococcales | Carbohydrate | [83] |
| Lipids | The extraction yields of lipids and carbohydrates reached 59% and 81%, respectively, comparing to the extraction of 22% of total lipids and 47% of total carbohydrates by sole ozonation. | | | |
| Ultrasound + H2O2 | In one pot: H2O2 was added into microagal suspensions, which were sonicated then | Chlorella pyrenoidosa | Protein | The cell lysis efficiency of combined treatment was approximately 2 fold of the ultrasound alone treatment. | [51] |
| Ultrasound + microwave | In one pot: Transesterification of microalgae was employed in a ultrasound-microwave synergistic extraction apparatus | Chlorella vulgaris | Lipids | The highest fatty acid methyl ester (FAME) yield of 93.07 ± 2.39% was achieved under ultrasound-microwave reactor while only 63.49 ± 4.69% and 58.12 ± 2.84% of FAME yield were offered by ultrasound and microwave irradiation, respectively. | [84] |
| Ultrasound + homogenization | Circulation: Microagal solution circulated continuously between the connected homogenizer and sonicator | Dunaliella tertiolecta | Lipids | The lipid extraction rate was 49.97% when ultrasound and microwave were used in combination, higher than that of using ultrasound (45.94%), but lower than that of using microwave (57.02%). | [85] |
| Protein | For diluted suspension, synergetic behavior was displayed by the combined procedure during the extraction of ionic component, but was less effective or negative for carbohydrates and proteins extraction, respectively. | | | [86] |
| Carbohydrate | For diluted suspension, synergetic behavior was displayed by the combined procedure during the extraction of ionic component, but was less effective or negative for carbohydrates and proteins extraction, respectively. | | | |
| Pigment | The addition of either abrasive materials increased carbohydrate release by 1.6 fold. | | | [87] |
| Ultrasound + abrasives | In one pot: Silicon milling beads or river filter sand were mixed with dried microagal biomass | Algae from a local waste treatment facility, mainly Cladophora | Carbohydrate | Additon of powder silica did not improve the extraction of oil. | [53] |
| Protein | The addition of either abrasive materials increased carbohydrate release by 1.6 fold. | Chlorella vulgaris | Lipids | | |
| Ultrasound + mechanical agitation | Sequential mode: first ultrasound, then mechanical agitation | Spirulina platensis | Carbohydrate | | [88] |
| Protein | Sequential mode: first ultrasound, then enzymatic lysis | Neochloris oleoabundans | Lipids | The lutein yield of 3.36 mg g⁻¹ was obtained by ultrasound extraction with enzymatic pretreatment, much higher than that by ultrasound treatment alone (3.16 mg g⁻¹). | [45] |
| Protein | Sequential mode: first ultrasound, then enzymatic lysis | Chlorella vulgaris | Lutein | Both the disruption degree and the lipid yield of combined processes were higher than that of any other sole process. | [47] |
| Protein | Combined treatment enhanced the release of total reducing sugar and dissolved protein up to 53% and 7% compared to ultrasound alone (14 and 2.3%), respectively. | Chlamydomonas mexicana | Carbohydrate | [44] |
supercritical fluid extraction, ionic liquid extraction, ozonation, alkaline/acid treatment, and H$_2$O$_2$ oxidation, were also investigated [81,89,92]. The release of both carbohydrates and lipids were dramatically enhanced by the combined utilization of ozonation and ultrasound, that the extraction yields of lipids and carbohydrates were about 2-folds of those extracted by ozonation alone [83]. Comparing to the sole application of ultrasound [67], the lipid extraction yield by the combined strategy also increased from 46.5% to 59%. The study conducted by Duan et al. [51] also revealed that H$_2$O$_2$-aided ultrasound treatment gained a higher cell lysis efficiency (~2-folds) than that of ultrasound alone.

By reviewing these hybrid techniques tested so far, it can be concluded that ultrasound combined with either conventional solvent extraction or innovative chemical methods largely facilitates the retrieve of valuable intracellular components. Further, the overall energy consumption, operation duration, and chemical dosage are reduced by their integration, and hence providing powerful solutions to commercial extraction for microalgal biorefining scale-up.

4.2. With mechanical/physical methods

Several studies have compared the disruption effects of various mechanical/physical treatments for efficiently extracting products from microalgae cells [22,71,93–96], out of which, ultrasound, microwave and high pressure homogenization were distinguished from others by their effective cell rupturing abilities. However, these approaches share common drawbacks of high energy input and overheating [5,97]. Alliance of these powerful techniques may be able to overcome these weaknesses. The disruption treatment of microalgae by ultrasound in conjunction with microwave or homogenization was performed either simultaneously in one pot, or in sequential mode, or in circulation mode (Table 4). Nonetheless, the extraction performance varied among different operation modes. Under the optimal sequential treatment by ultrasound and microwave, the lipid productivity from Dunaliella tertiolecta was even lower than that obtained by microwave assisted extraction alone, though lower powers and operating times of both ultrasonic and microwave treatment were used in the combined method [85]. While, simultaneous irradiation of ultrasound and microwave was regarded one of the most promising hybrid techniques for fast and efficient extractions. For instance, the highest FAME yield of 93.07 ± 2.39% was achieved under ultrasound-microwave reactor while only 63.49 ± 4.69% and 58.12 ± 2.84% of FAME yield were offered by ultrasound and microwave irradiation separately, demonstrating the prominent synergistic effect of simultaneous ultrasound-microwave technology for effective biodiesel production [84].

The combined utilization of ultrasound with abrasives, or mechanical agitation was also attempted in some studies. Specifically, in alignment with the concept of microalgal biorefining, simultaneously extraction of valuable products, such as protein and carbohydrate was concerned in these studies, and the recovery yields by combined methods were improved to some extent [87,88]. For example, the carbohydrate release increased 1.6 fold by the addition of either abrasive silicon milling beads or river filter sand [87]. Under the optimized conditions of sequential ultrasound and agitation treatment, over 75% protein was recovered from Spirulina platensis cells, which was much higher than most reported results [88]. In spite of the favorable performance, the extraction of all target products in the biorefining process might not be balanced. In terms of simultaneous extraction of protein and carbohydrate, the carbohydrate extraction capability of combined sonication/mechanical agitation was much lower than that of protein, suggesting that the priorities of each product has to been determined first and the combination strategy used should be adjusted accordingly [88]. In addition, Woods et al. [87] demonstrated that the protein release was compromised by abrasive materials addition in the ultrasound system. The presence of silica powder even reduced the effect of ultrasound, leading to a lower extraction of oil [53]. Hence, further investigation for comprehensive evaluation on the aiding efficiency of those hybrid techniques will be essential and different optimal operation conditions should be used based on the priorities of the products.

It is worth mentioning that the integration of ultrasound with other mechanical/physical methods is typically carried out in a solvent system or followed by solvent extraction for enhanced product recovery. As aforementioned, the preferred solvents vary depending on the intracellular components that are interested, and this pattern is consistent with that found in the hybrid system of ultrasound with other mechanical/physical methods. Taking lipid extraction as an example, when chloroform/methanol was used as the solvent after circulated ultrasound and homogenization treatment, the lipid yields were 1.5 to 2 folds of those using hexane with increased reaction times, indicating the superior cell damage ability of this polar solvent [73].

4.3. With enzymatic methods

Enzymatic hydrolysis, characterized by mild operation conditions, low energy requirement, high specificity and less damage to the target product, is an environmentally friendly method for cell breakage that has been excessively studied in the field of microalgal biorefining. Despite certain benefits, the high cost of enzymatic treatment hinders its application at commercial scale. Besides, for the achievement of efficient disruption, relatively long processing time is needed for enzymatic hydrolysis, comparing to mechanical/physical/chemical techniques. The combination of enzymatic hydrolysis with other techniques has been studied, aiming to overcome their individual shortcomings and provide better extraction yield, thus improve the overall effectiveness.

Based on the cell wall composition, different enzymes such as protease, cellulase, driselase, lysozyme, viscozyme, and glucosidase were used for maximizing compound production [11]. Generally, for microalgae with carbohydrate-rich cell walls, such as Chlorella, Nannochloropsis and Dunaliella, a series of hydrolytic enzymes were used, including cellulase, amylase, xylanase, viscozyme, amyloglucosidase, and et al., according to the major compositions of the cell walls [98,99]. While, for those with protein-rich cell walls, such as Chlamydomonas, the use of endopeptidases and protease was more preferred [100]. Moreover, as most microalgae cell wall possess complex structures with diverse components, enzymatic cocktails were often used in order for maximizing the disruption efficiency.

The treatment by ultrasound in combination with these enzymes for cell lysis was mostly carried out sequentially. Moreover, unlike those focused mainly on lipid extraction with ultrasound plus chemical or mechanical/physical methods, diverse compounds were targeted by the combination of ultrasound and enzymatic treatments in the research of Eldalatony et al. [44] (Table 4). The product yields generally increased upon the combined treatments [44,45,47]. In a recent study, the authors declared that enzymatic pre-treatment before ultrasound allowed fractionation of neutral glycrides and polar glycolipid, which may be used for different aims [101]. However, when looking into their data carefully, it is apparent that though the lipid composition of obtained fractions was altered by the sequential treatment of cellulase/viscozyme and ultrasound, the concentration effects of certain lipid species were insufficient and further optimization of the operation conditions will be necessary. Overall, this research provided interesting insights of the synergistic effects about lipid fractionation by ultrasound with enzymatic treatment.

5. Techniques for measuring microalgal cell disruption by ultrasound

After cell disruption, an accurate and reliable monitoring of the cell rupture degree is essential for process optimization, and then the microalgae biorefining development. A variety of estimation methods have been established that can be roughly divided into two categories, one is direct measurement of cell disintegration or fragmentation of
intact cells [60], and the other one is indirect evaluation by examining the release of intracellular components [55,63]. Table 5 summarizes the advantages and disadvantages of these generally used estimation techniques.

5.1. Direct estimation methods

Mostly, intact cell density enumerated using a hemocytometer under a microscope or optical density (OD) measurement of cell suspension are widely used as the quantitative parameters for cellular disintegration [22,23,26,28,56,60,61]. Among all the estimation techniques, direct cell counting is considered the most reliable as it directly provides the ratio of intact cells and fragmented cells [50,60]. However, implementation of this technique is time consuming and highly dependent on the operator’s judgement. Instead, the measurement of microalgal suspension OD value is often adopted for monitoring cell density, and thereby used to quantify cell disruption degree. Under most circumstances, absorbance at the wavelength around 680 nm was employed ascribing to the peak absorbance of the chlorophyll-protein complex in microalgal cells [61,63]. Nonetheless, after ultrasonic treatment, microalgal cell debris and the released intracellular components may cause interference on light scattering or refraction, therefore affect the absorbance spectra features. To ensure the accuracy of quantitative results, a few studies have scanned the absorbance spectra of microalgal suspension and some plotted cell reduction as a function of the peak absorbance to select the optimal measuring wavelength [63]. As revealed by the absorbance spectra features, the correlation between cell reduction and OD values at the specific absorption peak varied for different microalgal species. Instead of the absorbance value, the absorbance peak height could also be used as an indicator that the peak height at 680 nm was applied for cell reduction calculation of Chaetoceros gracilis, Chaetoceros calcitrans and Nannochloropsis sp. after ultrasonication [29].

In addition, some researchers also applied scanning electron microscope (SEM) or other high resolution microscopy to monitor the morphological changes of microalgal cells after ultrasonic treatment [22,27,43,48,56,62]. Based on these micrographs, microalgal cell rupture features of shrinkages, creases or debris aggregation caused by the external forces were easily observed. As these microscopic inspections are on essence a qualitative evaluation towards the degree of microalgal cell fragmentation, they are typically used in combination with other estimation techniques.

Determination of cell size distribution was used as another direct estimation technique for monitoring cell disruption [73,102]. Following ultrasonic treatment, the cell size reduction of Scenedesmus dimorphus and Nannochloropsis oculata was readily reflected by flow cytometry, indicating efficient rupture of cells [65]. By filtration of microalgal suspensions through filters with specific diameter spores, the Chlorella sp. cell size distribution was also calculated and the variation of the distribution of cell fragments was used to indicate the degree of cell rupture [43]. However, tiny particles may pass into the filtrate, or not been detected by flow cytometry, subsequently causing inaccuracy to the results [65].

5.2. Indirect estimation methods

Measuring the release of intracellular components can largely reflect the extent of cell disruption [55,67], and are likely to provide accurate and reliable results comparable to those obtained by direct cell counting [63]. Particularly, the ease of extracting target compounds is tightly related to the efficacy of cell wall breakage in microalgal biofinerung. Many studies have employed the product yield as the determining factor for the optimization of ultrasound assisted cell disruption process [55,62,71,103].

Regarding ultrasonic pretreatment for biodiesel production from microalgal biomass, the lipid content in extracts revealed by weighing method or Nile red stained lipid florescence density, and fatty acid methyl ester profile were used to indicate the cell rupture extent [43,55,65]. As for pigments recovery, such as carotenoid, astaxanthin and chlorophyll, chromatography or spectrophotometry were used for pigment quantification, and then indicating cell disruption [47,52,55,74]. The release of intracellular protein measured by the classical Bradford assay was also broadly adopted for cell lysis evaluation [51].

The majority of researches utilize multiple estimation techniques for microalgal cell disruption monitoring. In the previous studies of the authors’ group, both cell size distribution and Nile red stained lipid fluorescence density were applied for evaluating the effectiveness of ultrasound treatment [57,65,104]. Similarly, the ultrasonic facilitation on lipid extraction from Chlorella sp. was revealed through the size distribution of cell fragment, lipid extraction yield, SEM observation of ruptured microalgal cells and fatty acids content analysis [43]. Comparing to the evaluation using a single method, a combination of

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Table 5

Comparison of different estimation methods for monitoring cell disruption by ultrasound in microalgal bioengineering.

| Estimation methods                      | Advantages                                                                 | Disadvantages                                                                 |
|-----------------------------------------|---------------------------------------------------------------------------|------------------------------------------------------------------------------|
| Direct counting of intact cells         | Unambiguous and the most reliable                                         | Time-consuming                                                               |
| Useful when comparing across a variety of species types | Simple and convenient to operate with spectrophotometer                   | OD values highly rely on the feature of microalgal strains: ① The optimal measurement wavelength for each microalgal strain has been determined separately; ② The correlation between cell reduction and OD values vary for different microalgae |
| Less effective                          |                                                                           |                                                                              |
| Tedious                                 |                                                                           |                                                                              |
| Optical density (OD) measurement of cell suspension | Simple and convenient to operate with spectrophotometer | OD values highly rely on the feature of microalgal strains: ① The optimal measurement wavelength for each microalgal strain has been determined separately; ② The correlation between cell reduction and OD values vary for different microalgae |
| Rapid acquisition of results           |                                                                           |                                                                              |
| Less reliable: The absorbance spectra of microalgal suspensions become complicated after cell disruption, leading to over- or under-estimation of cell reduction | Simple and convenient to operate with flow cytometer | Tiny particles less than 1 μm may not be detected |
| Determination of particle size distribution | The morphological rupture can be observed intuitively | Unable to measure cell disruption quantitatively |
| Scanning electron microscopy or other high resolution microscopy observation | Accurate and reliable                                                      |                                                                              |
| Determination of the release of intracellular components (pigments) | The intracellular pigments may be unstable during ultrasound treatment |                                                                              |
| Simple and rapid to operate by measuring the OD values of supernatants | Valid and suitable in the field of microalgal biofinerung: Can directly reveal the effects of cell disruption on target products extraction | If the target products from various microalga are different, it will be difficult to compare the cell disruption efficiency across a variety of species types |
various estimation techniques would integrate the advantages of each method and hence provide a much more comprehensive recognition to the cell disruption. As reported in the authors’ previous research, after short exposure to ultrasonication, the cell concentration of either *Scenedesmus dimorphus* or *Nannochloropsis oculata* did not vary significantly from control. However, both their chlorophyll a fluorescence density and Nile red stained lipid fluorescence density increased significantly, indicating effective cell disruption caused by ultrasonic treatment. Hence, combined utilization of these estimation techniques probably provides more realistic results [57].

Moreover, as the ultimate goal of ultrasonic cell disruption in microalgal biorefining is to assist with the extraction of target products, the release of the components of interest would be a vitally important indicator. Therefore, depending on the different biorefining aims, estimation of the corresponding target product release together with other cell disruption measurement techniques should be the optimal evaluation strategy for microalgal cell rupture induced by ultrasound treatment or even other mechanical cell wall breaking techniques.

6. Challenges in utilizing ultrasound for microalgal biorefining

6.1. Ultrasound energy input and destruction of target compounds

Ultrasound assisted extraction has been widely investigated at laboratory scale and is considered a sustainable “green” process [53]. An important issue for consideration of applying this technology at industrial scale is to upscale this process without sacrificing performance and quality in product recovery. Meanwhile, cost and environmental impacts should also be considered before upsizing [39]. Excessive ultrasound energy applied in the extraction procedure due to high ultrasound frequency, high intensity, or long treatment time, is not desirable in upscaling because of the energy wasted and degradation of target bioactive compounds caused by heat and free radicals [11]. As considerable heat could be generated during ultrasonic processing, the sample temperature should be regulated, otherwise proteins and other intracellular metabolites could be damaged or destroyed [11]. Besides, release of enzymes from cell debris caused by excess ultrasound energy could also decrease the yield of target compounds. For example, disintegration of part of diacylglycerides to monoacylglycerides by sonication was implied, suggesting that intracellular enzymes might be released into the solution and caused damage on the chain structure of lipids [67]. Additionally, for biodiesel production, the concentration of linolenic acid (C18:3) in lipid should not exceed 12% as a high concentration of unsaturated fatty acid may decrease the oxidation stability of the biofuel and lead to its rancidity. As reported, the concentration of linolenic acid in lipids extracted from *Scenedesmus* sp. through ultrasound was greater (ranging from 8.84% to 15.3%) than the specified limit (12%), suggesting the potential risk of utilizing ultrasound in biofuel production from this strain [105]. Depolymerization of carbohydrates was observed at long duration of ultrasound treatment, though the content of total phenolic compounds improved as treatment duration increased [31], indicating that the influence of ultrasonic energy is also dependent on the target compounds to be harvested.

Using excessive energy to disrupt microalgal cells could not only increase operation costs but also impair the quality of the extracted oil [35] due to the formation of free radicals [33,43]. Free hydroxyl ions and hydrogen atoms are formed immediately through water molecule ionization. These highly active free electrons would break the chemical bonds of the lipids, oxidize the unsaturated lipids, and result in increased short chain and saturated lipids [66]. It should be noted that very reactive hydroxyl radicals (H·, OH·) can be generated during sonication and these radicals react with most biomolecules. At higher ultrasonic frequencies, a larger proportion of free radicals are produced from the ultrasonic degradation of water, which chemically attack and weaken the cyanobacteria cell walls [26]. Compound degradation can easily occur when employing ultrasound assisted extraction [52].

Careful control of ultrasound condition is necessary to maximize the target product extraction at the lowest operational cost and to prevent the destruction of product quality. A shorter period of time might help on this regard. As the sonication time increases, decrease in products yield could be due to the damaging/degrading effect of ultrasonic wave. Similarly, a higher power of ultrasound, significant amounts of sound energy could be transformed to heat to damage or degrade the proteins [59]. In a recent study using ultrasound for lipid extraction from *Chlorella vulgaris*, significantly increased lipid and FAME yield was obtained without any effect on the FAME profile, suggesting the absence of polyunsaturated fatty acids oxidation. Low ultrasound power for short treatment time and the anti-oxidative carotenoids that protect the polyunsaturated fatty acids from free radical damages were speculated the two main reasons for the unaffected FAME profile [54]. Based on the discussion above, we may conclude that a low frequency, short treatment time, low power input ultrasonic process with high recovery efficiency is preferred to reduce the cost and adverse impacts on environment when upscaling is to be considered.

6.2. System up-scaling and process design

The design of an efficient ultrasound-assisted large-scale extraction system is necessary for the up-scaling of ultrasonic process. The major factor to be considered is the large quantity of microalgae biomass to be treated since the ultrasound reactors used at laboratory scale are restricted to small volumes. One solution is to use a continuous system that can handle a large amount of cell suspension in a restrictive volume of reactor, ultrasound power is then more concentrated. The other solution is to combine an agitation system, e.g., pumping system, in order to stir the mixture and make sure the whole cell suspension could get access to ultrasonic disruption [46]. Manufacturing large size sonication equipment, safety protocols, and the intensive energy input are also limiting the application of ultrasound. For instance, if solvents were applied in the extraction process, ultrasonication can induce high temperature at local spots, which might cause explosion if the heat could not be dissipated in time [17]. To reduce heat accumulation, a cooling jacket with cooling water could be applied to accelerate heat dissipation [51]. Besides, ultrasonication treatment of some microalgal species is not desirable due to its low cell disruption efficiency [11]. A pretreatment process such as enzymatic [44,47], alkali heat [106], and peroxide [51] may be able to reduce ultrasound treatment duration, energy input and cost, and enhance ultrasound performance for these “tough” microalgal cells.

7. Future perspectives

Quality control of target products by ultrasonic extraction requires considerable research for the fulfillment of practical industrial production. As discussed above, although formation of free radicals might aid in cell disruption, they can also negatively affect the antioxidant compounds extracted, such as unsaturated fatty acids and carotenoids, which are very susceptible to lipid antioxidation through the free radical mechanism [55,62]. Whereas, from the perspective of biofuel production, increased proportion of neutral lipids after ultrasonic treatment suggested a positive effect on the quality of recovered lipids [58]. Besides, the ultrasound extracted lipids from *Scenedesmus obliquus* are rich in saturated fatty acids (79.38%), but low in unsaturated fatty acids (19.12%), which are also suitable for biodiesel production [78]. Therefore, apart from extraction yield, knowledge in product properties and proper ultrasonic conditions is essential for further industrial processing. Strong consideration should be given to the primary product in the optimization of ultrasonic conditions.

As the production of a single intracellular metabolite from microalgae generally contains multiple energy intensive processing stages [107], microalgal biorefining, comprising the conversion of microalgal biomass into a variety of marketable chemicals, has emerged as a
promising approach to address the economic gap between the current costs of microalgal processing and profitable/competitive microalgal commercialization [6]. Nevertheless, by utilizing ultrasound in microalgal processing, most of the studies have focused on the extraction of a particular compound, mainly lipids. Very few studies have explored the ultrasonic disruption for protein, carotenoids, or other high value products from microalgae [89]. The information of ultrasonic treatment towards the extraction of multiple substances either simultaneously or sequentially is even scarcer [31,58]. Hence, in order for the development of an energy efficient ultrasonic process for commercial microalgal biofining, precise and detailed knowledge on the overall recovery performance to diverse products is still needed.

The integration of cell harvesting and ultrasonic disruption, or ultrasonic assisted extraction and separation can further reduce energy consumption, and make ultrasonic processing of microalgae more viable. As suggested by Krebbl et al. [108], the dissolved air flotation used for harvesting can generate microbubbles, which would enhance the cell disruption efficiency by ultrasound. Thus, energy saving benefit would be brought by the integration of dissolved air flotation and ultrasound disruption. Likewise, high protein production and separation efficiency was attained by integrating sugaring-out assisted liquid biphasic flotation and ultrasound, providing a cost-effective, energy-efficient solution for biomolecule extraction from microalgae [59]. These limited studies have demonstrated the potential of process integration for microalgal biofining. More investigations regarding novel protocols integrating multiple operations are still needed.

8. Conclusions
Ultrasound has been applied as a highly-effective, safe, and “green” cell disruption technology in microalgal biofining. Intense shock waves, shear forces, microbubble resonance, local high temperature and pressure, and generation of free radicals induced by ultrasound are the well-recognized theories for morphological alteration and cell disruption of microalgal cells. The adoption of suitable ultrasonic frequency, intensity, and duration is essential for effective cell disruption. The effects of ultrasonic parameters on cell disruption might also correlate with the microalgae species, properties of target compounds, liquid phase properties, and design of sonication reactor. Ultrasound could also be used together with microwave, abrasives, enzymes, solvents, mechanical agitation and so on, which could significantly enhance cell lysis and extraction yield, and reduce processing time and energy cost, thus providing a powerful solution to process and reactor design. The measurement of target product release together with other evaluation techniques is likely to provide thorough and in-depth reflection of cell disruption degree. The biggest challenge and barrier for utilizing ultrasound in microalgal biofining is to scale up without sacrificing its efficiency was attained by integrating sugaring-out assisted liquid biphasic flotation and ultrasound, providing a cost-effective, energy-efficient solution for biomolecule extraction from microalgae [59].

a) determine the order of priorities of each target product in microalgal biofining, and clearly understand the thermal, oxidative, physical and biological stabilities of the target products.

b) comprehensively analyze the ultrastructure and major components of the cell wall of interested microalgal strains.

c) evaluate the disruption effectiveness based on product yield and quality.

d) give sufficient consideration to cost, environmental impacts, product quality, excessive energy consumption, operator, and process design before putting ultrasonication into commercial microalgal biofining.

Declarations.

Ethics approval and consent to participate. Not applicable.

Consent for publication. Not applicable.

Availability of data and materials.
All data generated or analyzed during this study are included in this published article.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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