Malaysian Journal of Analytical Sciences (MJAS)
Published by Malaysian Analytical Sciences Society

EXTRACTION SOLVENTS IN MICROALGAL LIPID EXTRACTION FOR BIOFUEL PRODUCTION: A REVIEW

(Pelarut Pengekstrakan dalam Pengekstrakan Lipid Mikroalgal untuk Penghasilan Bahan Api Bio: Satu Tinjauan)

Tan Yeong Hwang[1]*, Chai Mee Kin[1], Wong Ling Shing[2]

[1]College of Engineering,
University Tenaga Nasional, Jalan Ikram-Uniten, 43000 Kajang, Selangor, Malaysia
[2]Faculty of Health and Life Science, INTI International University,
Persiaran Perdana BBN Putra Nilai, 71800 Nilai, Negeri Sembilan, Malaysia

**Corresponding author: yeonghwang_113@hotmail.com*

Received: 28 July 2021; Accepted: 22 September 2021; Published: xx October 2021

Abstract

Oleaginous microalgae biomass has gained noteworthy attention as feedstock for biofuel production due to its fast growth rate and capability of growing in non-arable land with high lipid content. Among biofuels, biodiesel has been a prevailing area of interest to many researchers. Prior to transformation of microalgae lipid into biodiesel, a lipid extraction step needs to be performed to disrupt the microalgal cell walls in order to extract the lipid. Hence, selecting an appropriate extraction solvent is of the utmost importance to ensure the efficient extraction of desired lipid content which can then be transformed into high quality biodiesel. Conventional organic solvents such as chloroform, dichloromethane and methanol are usually used in lipid extraction due to its high extraction efficiency. However, toxicity and environmental issues related to these solvents are of major concerns. Hence, many recent studies have focused on the use of green solvents such as bio-based solvents, supercritical carbon dioxide and ionic liquids. This review discusses the use of conventional organic solvents and green solvents in microalgae lipid extraction. Advantages and shortcomings of these solvents are also discussed. In addition, the future perspective for extraction solvents used in lipid extraction is also discussed.

Keywords: extraction solvent, microalgae lipid extraction, green solvent, biofuel

Abstrak

Biojisim mikroalga berminyak dinilai sebagai bahan mentah yang terkenal untuk penghasilan bahan api bio kerana kadar pertumbuhannya yang cepat dan keupayaan bertumbuh di dalam tanah yang tidak sesuai untuk pertanian dengan kandungan lipid yang tinggi. Antara bahan api bio, biodiesel telah menarik minat ramai penyelidik. Sebelum transformasi mikroalgae ke dalam biodiesel, langkah pengekstrakan lipid perlu dilakukan untuk melemahkan dinding sel mikroalga untuk mengekstrak lipid. Oleh itu, memilih pelarut pengekstrakan yang sesuai adalah sangat penting untuk pengekstrakan kandungan lipid yang dikehendaki yang boleh diubah menjadi biodiesel berkualiti tinggi. Pelarut organik konvensional seperti kloroform, diklorometana dan metanol biasanya digunakan dalam pengekstrakan lipid kerana kecekapan pengekstrakan yang tinggi. Namum, ketoksikan dan isu-isu alam sekitar pelarut-pelarut ini adalah kebimbangan utama. Oleh itu, banyak kajian baru-baru ini telah memberi tumpuan kepada penggunaan pelarut hijau seperti pelarut berasaskan bio, karbon dioksida superkritikal dan cecair ionik. Tinjauan ini membincangkan pelarut organik konvensional dan pelarut hijau yang digunakan dalam pengekstrakan lipid microalga. Kelebihan dan kelemahan pelarut-pelarut tersebut juga dibincangkan. Di samping itu, perspektif masa depan untuk pelarut-pelarut pengekstrakan yang digunakan dalam pengekstrakan lipid juga dibincangkan.

Kata kunci: pelarut pengekstrakan, pengekstrakan lipid mikroalga, pelarut hijau, bahan api bio

Introduction

The increasing demand for sustainable energy resources has spurred the intensive research into biofuels derived from microalgae. Several types of biofuels can be transformed from microalgae biomass such as biodiesel, biogas and biochar [1]. Among them, biodiesel has gained massive interest from many researchers [2]. Compared to plant-based feedstock, microalgae have higher photosynthetic efficiency and a rapid growth rate [3]. The lipid content of microalgae can be up to 80% and their fatty acid profiles are similar to plant-based biodiesel but with low sulphur content and particulate matter [4-5]. In addition, many microalgae can thrive well in non-arable lands such as saline water [6] and wastewater [7]. Hence, microalgae do not compete for the land required for producing food. For biodiesel production, a lipid extraction step is essentially performed to rupture the microalgal cell wall and separate the desired lipids from the complex. Several parameters have been identified as factors that impact the extraction efficiency of lipids from microalgae biomass including types of extraction solvents [8-9], cell disruption techniques [10], biomass-to-solvent ratio [11], extraction time [12], extraction temperature [13] and solvent mixture ratio [14].

Microalgae are covered with rigid cell wall which impedes the extraction efficiency. Therefore, several cell disruption techniques such as the use of microwave, ultrasonics, osmotic shock and high-pressure homogenization are employed to break the cell wall. Then, the extraction solvent gains entry into the cell and extracts out the desired components. Therefore, selecting an appropriate extraction solvent is of utmost importance to ensure the efficient extraction of the desired lipid content which can then be transformed into high-quality biodiesel. This paper reviews the conventional extraction solvents and green solvents used in microalgal lipid extraction. In addition, future perspectives of extraction solvents are also discussed.

Conventional extraction solvents for microalgal lipid extraction

Based on the polarity, lipids in microalgae can be classified into polar and nonpolar (or neutral) lipids. With respect to different factors such as cultivation conditions and microalgae species, the lipids profiles can vary [18-19]. Polar lipids such as phospholipids and glycolipids are main constituents of the cell or organelle membrane whereas non-polar lipids such as triglycerides (TAGs) are important energy reserves [18-19]. In common extraction procedures (Figure 1), after the microalgae biomass is harvested and dried, extraction solvent will be added to the biomass. The mixture is then shaken for a certain period of time followed by separation into organic and aqueous phases. Organic phase contains extracted lipids whereas aqueous phase contains polar molecules such as proteins and carbohydrates. Finally, the organic phase will be collected followed by vacuum evaporation and transesterification.

As a rule of thumb, the extraction solvent should be able to penetrate the cell membrane and should be highly specific to the desired lipids to enable the dissolving of these lipids into the solvent while minimizing the co-extraction of undesired contaminants. Besides that, the ideal extraction solvent should also have properties of being insoluble in water and a low boiling point to ease the evaporation of the extraction solvent from lipids [20]. Various types of organic solvents such as chloroform, diethyl ether, acetone, hexane and methylene chloride [21-24] have been investigated to extract lipids from microalgae.

Nevertheless, using a single solvent alone might not be strong enough to break the strong interaction between neutral lipids and other biomolecules in the cell membrane [25-26]. To overcome this interaction, a nonpolar solvent is often employed simultaneously with a polar solvent. Polar solvents can break the lipid-protein interaction by forming strong hydrogen bonds with neutral lipid in the complex [27]. However, the use of a polar solvents might result in the co-extraction of undesired polar biomolecules [28]. Previous studies [29-30] have indicated that total lipid extraction can be increased by using polar and nonpolar solvent mixtures, however, it does not necessary increase the quantity of extracted esterifiable lipid. In many cases, the type and ratio of nonpolar and polar solvents must be determined to ensure that the extracted lipid profile is suitable for high-quality biodiesel production.

Various solvent systems have been proposed as potential extracting solvents such as chloroform/methanol [32-33], chloroform/ethanol [21], dichloromethane/methanol [34], hexane/ methanol [35] and hexane/ethanol [14]. Among these solvent systems, chloroform/methanol and n-hexane/methanol mixtures are mostly applied in lipid extraction processes due to their high extraction efficiency and easy recovery [36-37]. However, these organic solvents exhibit toxicity, high volatility and are hazardous to both the operator and the environment. The summary of microalgae lipid extraction efficiency using various conventional solvents is summarized in Table 1.

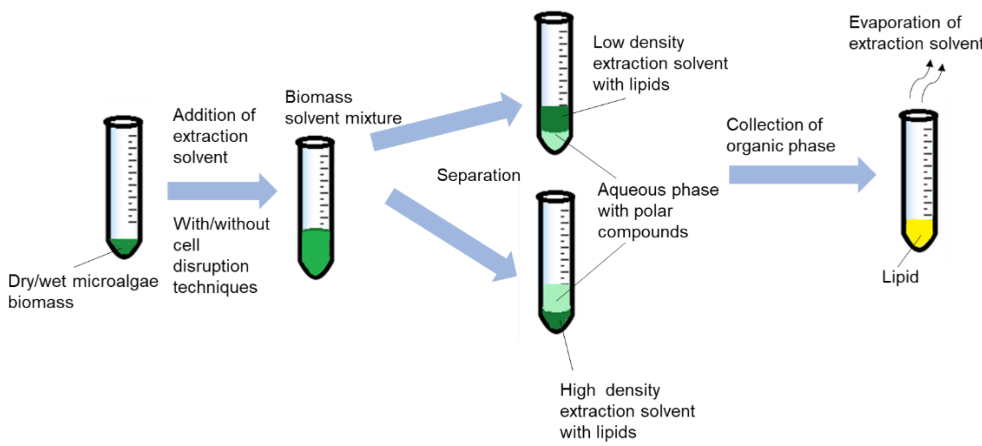

Figure 1. Common method for microalgae lipid extraction

Table 1. Microalgae lipid extraction efficiency of conventional solvents

Extraction Solvent (Volume/Volume)	**Microalgae**	**Cell Disruption Techniques**	**Lipid Extracted (%)**	**Ref**
Chl: Met (2:1)	*Oedogonium*	Soxhlet extraction	14	[32]
Chl: Met (2:1)	*Oedogonium*	Bligh and Dyer	11.5	[32]
Chl: Met (2:1)	*Chlorella vulgaris* UTEX 395	Accelerated solvent extraction	25.1	[33]
Chl: Met (2:1)	*Chlorella sorokiniana*	Accelerated solvent extraction	25.8	[33]
Chl: Met (2:1)	*Nannochloropsis gaditana*	Accelerated solvent extraction	29.8	[33]
Chl: Met (2:1)	*Chlorella vulgaris*	Chemical extraction	25.33	[31]

Table 1 (cont'd). Microalgae lipid extraction efficiency of conventional solvents

Extraction Solvent (Volume/Volume)	Microalgae	Cell Disruption Techniques	Lipid Extracted (%)	Ref
Chl: Met (1:2)	*Chlorella vulgaris*	Chemical extraction	38.57	[31]
Chl: Eth (2:1)	*Chlorella sp.*	Soxhlet extraction	11.76	[21]
Chl: Met (2:1)	*Scenedesmus obliquus*	Microwave-assisted extraction	19.25	[34]
Chl: Eth (1:1)	*Scenedesmus obliquus*	Microwave-assisted extraction	10.08*	[34]
Chl: Met (2:1)	*Tetraselmis sp. KCTC12429BP*	Chemical extraction	5.51	[35]
Chl: Met: Water (5:10: 4)	*Acutodesmus obliquus*	Bligh and Dyer	7.41	[14]
DCM: Met (2:1)	*Scenedesmus obliquus*	Microwave-assisted extraction	19.00	[34]
Hex: Met (1:1)	*Tetraselmis sp. KCTC12429BP*	Chemical extraction	5.16	[35]
Hex: Met (1:2)	*Chlorella vulgaris*	Chemical extraction	25.17	[31]
Hex: Eth (1:2)	*Acutodesmus obliquus*	Soxhlet extraction	6.82	[14]
Hex: Eth (1:2)	*Acutodesmus obliquus*	Ultrasonic-assisted extraction	4.28	[14]

Chl: chloroform; DCM: dichloromethane; Eth: Ethanol; Hex: n-Hexane; Met: Methanol;
*The lipid was extracted from wet microalgae biomass.

Green solvents for microalgal lipid extraction

With the rising concern of environmental pollution, health impacts and the development of green chemistry, finding suitable green solvents to replace petroleum-based solvents has become a major task of chemists. An ideal green solvent should meet the twelve criteria proposed in the principles of green chemistry [38]. Different solvents such as bio-based solvents, ionic liquid, supercritical carbon dioxide and switchable solvents have been classified as green solvent. The advantages and disadvantages of these solvents are summarized in Table 2.

Bio-based solvents are derived from agricultural biomass such as lignocellulosic biomass [39] and fruit peels [40]. The research evinced that 2-methyltetrahydrofuran [39], terpenes [40], ethyl lactate and ethyl acetate [41] possess similar characteristics to those in chloroform and n-hexane therefore they can be used to extract neutral lipids efficiently with high-quality lipid profiles. Compared to chloroform and hexane, terpenes are less hazardous due to its higher flash point and low toxicity [39-41]. Moreover, due to similar characteristics, the extraction process using bio-based solvents is as easy as conventional solvent extraction methods. However, the application of bio-based solvents for lipid extraction is not extensively evaluated probably due to the limited choice of bio-based solvents. Furthermore, the prices for some bio-based solvents are very expensive which is a limiting for economical industrial-scale extraction [39].

Ionic liquids (ILs) are defined as salt solution constituting organic cations and smaller inorganic or organic anions which they are maintained as liquids at temperatures between 0 and 140 °C [42]. ILs are considered as "designer solvents" due to their ability to accommodate different types of anions and cations [42]. In other words, polarity and properties of ILs can be specifically tailored to achieve the desired extraction efficiency. In addition, ILs have some common characteristics which render them as ideal green solvents for lipid extraction including non-volatile properties, low viscosity and low vapor pressure [43]. Many studies have shown that lipid extraction process and recovery efficiency using ILs has resulted in high yield of lipids (>70%) [44-46]. Nevertheless, ILs exhibit high viscosity in low temperature therefore lipid extraction is mostly processed with co-solvent addition or at the temperatures ranging from 100 to 120 °C [44]. To date, most studies have focused on investigation of imidazolium-based ionic liquids for lipid extraction. Wahidin et al. [46] reported that 1-ethyl-3-methylimmidazolium methyl sulphate with methanol can be simultaneously used for lipid extraction and direct transesterification of wet microalgae which reduces energy consumption. Although ILs are considered as green solvents, their "greenness" of synthesis process is still questionable and the environmental impact generated by these solvents needs to be investigated [47]. Several studies showed that some cations and anions in ILs are poorly biodegradable and toxic to organisms [47-49], hence, cations and anions of ILs should be chosen carefully with this consideration in mind.

Another promising green solvent called deep eutectic solvent (DES) has been emerged as an alternative to overcome the disadvantages of ILs. DESs generally consist of a hydrogen-bond acceptor and a hydrogen-bond donor compound that often interacts through the formation of hydrogen bonds to form eutectic mixtures [50]. DES has similar characteristics to ILs, which is highly desirable including low vapor pressure, non-flammable, and low volatility. Furthermore, the price of DES is lower than ILs [50] and some DES are reported to be nontoxic [51]. There are limited studies that have investigated the effectiveness of DES on lipid extraction from microalgae [52-55]. Tommasi et al. [53] reported that although DES formed by choline chloride and oxalic acid extracted lower lipid content from *Phaeodactylum tricornutum* compared to conventional solvents, the selectivity of desired fatty acids was better. Nevertheless, DES possess several disadvantages such as high viscous and difficulty of separating the target components from DES [55]. Obluchinskaya et al. [56] pointed out that highly viscous nature of DES may complicate the extraction and separation process. This problem can be overcome by combining with mechanical methods, adding extra water into the mixture, increasing the temperature [53, 56].

Several studies also indicated that some DES are toxic or become toxic at certain concentrations [57-58]. Hence, other alternatives such as supercritical carbon dioxide and switchable solvents should be considered and are gaining wide interest as green solvents. Although these solvents have been extensively used in environmental, food, industrial, pharmaceutical and biological research, there are limited studies on lipid extraction from microalgae using these solvents [59-60]. It should be noted that although these solvents are suggested as potential green solvents, there is no clear evidence indicating their "greenness" in term of biodegradability, biocompatibility and toxicity. Table 3 shows the summary of extraction efficiency for microalgae lipid extraction using various green solvents.

Table 2. Advantages and disadvantages of extraction solvent for lipid extraction

Solvents	**Advantages**	**Disadvantages**	**Ref**
Petroleum-based solvent (Conventional solvent)	• Low cost • High extraction efficiency	• Highly toxic to environment and health	[36]
Bio-based solvent	• higher flash point • low toxicity	• High production cost • Some are highly viscous • Less available choice	[36]

Table 2 (cont'd). Advantages and disadvantages of extraction solvent for lipid extraction

Solvents	Advantages	Disadvantages	Ref
Ionic liquid	• Properties can be tailored • Has varieties of cations and anions	• High cost • Some are toxic to environment and health	[43, 47]
Deep eutectic solvent (DES)	• Low cost • Properties are designable • Has different components which may be derived from biological resources • Some of them are nontoxic	• Some are toxic or become toxic at high concentration • highly viscous • Difficult to separate target components and DES	[50, 55-57]
Supercritical carbon dioxide	• Non-toxic solvent • Easy recyclable	• High processing cost for its infrastructure and operation	[59]
Switchable solvent	• Polarity can be changed abruptly using CO_2 • Simple process	• Tertiary amine which are usually used as extraction solvent are environmentally toxic	[59, 60]

Table 3. Green solvent employed for microalgae lipid extraction

Extraction Solvent	Microalgae	Cell Disruption Techniques	Lipid Extracted (%)	Ref
Bio-based solvent				
2-MeTHF: isoamyl alcohol (2:1, v/v)	*Chlorella pyrenoidosa*	Chemical extraction	99.6 mg/g	[39]
d-limonene	*Chlorella vulgaris*	Soxhlet method	1.29	[40]
α-pinene	*Chlorella vulgaris*	Soxhlet method	0.91	[40]
ρ-cymene	*Chlorella vulgaris*	Soxhlet method	1.52	[40]
Ethyl lactate	*Nannochloropsis sp.*	Soxhlet method	31.1	[41]
2-MeTHF	*Nannochloropsis sp.*	Soxhlet method	19.1	[41]
Ionic liquid				
Butyrolactam hexanoate	*Chlorella sp.*	Chemical extraction	70.90 mg/g*	[45]
Butyrolactam hexanoate	*Chlorococcum sp.*	Chemical extraction	19.86 mg/g*	[45]
[EMIM][$MeSO_4$]	*Nannochloropsis sp.*	Microwave-assisted extraction	40.9*	[46]
[EMIM]Cl	*Nannochloropsis gaditana*	Microwave-assisted extraction	13.9	[57]

Table 3 (cont'd). Green solvent employed for microalgae lipid extraction

Extraction Solvent	Microalgae	Cell Disruption Techniques	Lipid Extracted (%)	Ref
Deep eutectic solvent				
Ch-Aa: methanol-H_2SO_4 (60:40, v/v)	*Chlorella sp.*	Autoclave	13.91	[51]
Ch-Ca: DMC	*Phaeodactylum tricornutum*	Microwave-assisted extraction	11.00	[52]
ChCl: oxalic acid (1:2 v/v)	*Phaeodactylum tricornutum*	Microwave-assisted extraction	12.5%	[52]
ChCl: oxalic acid (1:2 v/v)	*Chlorella* sp.	-	12.5%	[53]
TMG: menthol (3:1, M/M)	*Nannochloropsis* sp.	Ultrasonic	172.3 mg/g	[54]
Supercritical CO_2	*Phaeodactylum tricornutum*	-	0.3	[52]
Switchable solvent				
TEPDA	*Nannochloropsis oceanica*	-	22.67*	[68]

Ch-Aa : Choline chloride-acetic acid; Ch-Ca: choline chloride- carboxylic acids; DMC : dimethyl carbonate; 2-MeTHF: 2-Methyltetrahydrofuran; [EMIM]Cl: 1-ethyl-3-methyl imidazolium chloride; [EMIM][MeSO4]: 1-ethyl-3-methylimmidazolium methyl sulphate; Isoamyl alcohol: 3-methyl-1-butanol; TEPDA: N, N, N', N'-tetraethyl-1,3-propanediamine. TMG: methnol: Tetramethylguanidine: methnol
*The lipid was extracted from wet microalgae biomass

Future perspectives for development of extraction solvents

Replacing conventionally used solvents in lipid extraction by green solvents is not an easy task. Although different lipid extraction methods using various green solvents have been proposed, no green solvent to date can successfully replace conventional solvents in industrial production as the toxicity, biodegradability and biocompatibility of some these green solvents remains questionable. Environmental assessments and toxicity tests must be conducted prior to the scale-up of the extraction process. Some of the green solvents such as 2-methyltetrahydrofuran and ILs are not suitable for large-scale used due to high production cost [39, 42]. In this context, the synthesis process of green solvents should be designed so that it is sustainable and the price is reasonable for scale-up. Researchers should also focus on the techniques that could reduce energy, volume of green solvent required and time taken for lipid extraction. Studies have showed that green solvents combined with mechanical processes can increase the lipid yield which is a promising avenue in this research field [52, 62].

Many researchers tried to search for the best solvent that for the lipid extraction with the lowest environmental impact. Notwithstanding, green solvents are just one of the aspects of green chemistry, the involved reactions in extraction should also be sustainable and have low environment impact [63]. Current processes probably could be improved to minimize environmental and health impact. For instance, Mustapha and Isa [64] devised the commonly used chloroform-methanol mixture to low toxic solvent mixture by modifying the composition and volume of each solvent.

In addition to lipids, microalgae also contained bioactive compounds such as carotenoids. These bioactive compounds have high global market demand and they are expected to grow in the future [65]. Therefore, the transformation of the single product approach to an integrated metabolite production system may help to enhance the production of microalgae biodiesel.

As some of the extraction solvents are toxic and may not easy to biodegrade, hence 3Rs (reuse, recycle and reduce) concept probably could be another consideration to minimize the exposure of these solvents to environment. Recent studies have shown some ILs [66-67], DES and switchable solvents [68] have the recycle and reuse capability, with a slightly decrease on extraction efficiency after several time consecutive cycles.

Conclusion

Microalgae biodiesel is a potential candidate to substitute conventional fossil fuels. Selecting an appropriate extraction solvent for lipid extraction is extremely important to achieve efficient extraction of the desired lipid content that can be transformed into high quality biodiesels. Several types of extraction solvents such as conventional chlorinated solvents, bio-based solvents and ionic liquids have been discussed in this paper, each with their plus points and disadvantages. In addition, the future perspectives for development of extraction solvents were also intensively discussed. Overall, the environmental assessments of green extraction solvents, using integrated metabolite production and improvement of extraction processes based on green chemistry should be the main focus in future research and development of biofuels.

Acknowledgement

The authors would like to acknowledge Ministry of Higher Education Malaysia, MOHE: FRGS/1/2020/STG02/UNITEN/02/1 (60%) and UNITEN BOLD Research Grant 2020: RJO10517844/044 (40%) for the financial support.

References

1. Javed, F., Aslam, M, Rashid, N., Shamair, Z., Khan, A. L., Yasin, M., Fazal, T., Hafeez, A., Rehman, F., Rehman, M. S. U., Khan, Z., Iqbal, J. and Bazmi, A. A. (2019). Microalgae-based biofuels, resource recovery and wastewater treatment: A pathway towards sustainable biorefinery. *Fuel*, 255: 115826.
2. Catone, C. M., Ripa, M., Geremia, E. and Ulgiati, S. (2021). Bio-products from algae-based biorefinery on wastewater: A review. *Journal of Environmental Management*, 293:112792.
3. Sun, S., Zhao, W., Mao, X., Li, Y., Wu, T. and Chen, C. (2018). High-value biomass from microalgae production platforms: Strategies and progress based on carbon metabolism and energy conversion. *Biotechnology for Biofuels*, 11: 1-23.
4. Lin, T. S. and Wu, J. Y. (2015). Effect of carbon sources on growth and lipid accumulation of newly isolated microalgae cultured under mixotrophic condition. *Bioresource Technology*, 184: 100-107.
5. Calixto C. D., Santana, J. K. S., Lira, E. B., Sassi, P. G. B., Rosenhaim, R., Sassi C. F. C, Conceição, M. M. and Sassi, R. (2016). Biochemical compositions and fatty acid profiles in four species of microalgae cultivated on household sewage and agro-industrial residues. *Bioresource Technology*, 221: 438-446.
6. Ridley, C. J. A., Parkera, B. M., Norman, L., Schlarb-Ridley, B., Dennis, R., Jamieson, A. E., Clark, D., Skill, S. C., Smith, A. G. and Davey, M. P. (2018). Growth of microalgae using nitrate-rich brine wash from the water industry *Algal Research*, 33: 91-98.
7. Nagarajan, D., Kusmayadi, A., Yen, H. W., Di Dong, C., Lee, D. J. and Chang, J. S. (2019). Current advances in biological swine wastewater treatment using microalgae-based processes. *Bioresource Technology*, 289: 121718.
8. Balasubramanian, R. K., Doan, T. T. Y and Obbard, J. P. (2013). Factors affecting cellular lipid extraction from marine microalgae. *Chemical Engineering Journal*, 215–216: 929-936.

9. Kuan, D., Du, W., Dai, L., Ma, G. and Liu, D. (2016). Effect of solvent on the extraction of microalgae lipid for biodiesel production. *Chemical Research in Chinese Universities* 32: 625-629.
10. Pohndorf, R. S., Camara, Á. S., Larrosa, A. P. Q., Pinheiro, C. P., Strieder, M. M. and Pinto, L. A. A. (2016). Production of lipids from microalgae *Spirulina* sp.: Influence of drying, cell disruption and extraction methods. *Biomass and Bioenergy,* 93: 25-32.
11. Shin, H.Y., Shim, S. H., Ryu, Y. J. Yang, J. H., Lim, S .M. and Lee, C. G. (2018). Lipid extraction from *Tetraselmis* sp. microalgae for biodiesel production using hexane-based solvent mixtures. *Biotechnology and Bioprocess Engineering*, 23: 16-22.
12. Vieira, B. B., Soares, J., Amorim, M. L., Bittencourt, P. V. Q., Superbi, R. C., Oliveira, E. B., Coimbra, J. S. R. and Martins, M. A. (2021). Optimized extraction of neutral carbohydrates, crude lipids and photosynthetic pigments from the wet biomass of the microalga *Scenedesmus obliquus* BR003. *Separation and Purification Technolog,y* 269:118711.
13. Silitonga, A. S. Masjuki, H. H., Ong, H. C., Mahlia, T. M. I and Kusumo, F. (2017). Optimization of extraction of lipid from *Isochrysis galbana* microalgae species for biodiesel synthesis. *Energy Sources, Part A: Recovery*, *Utilization and Environmental Effects*, 39: 1167-1175.
14. Escorsim, A. M., Rocha, G., Vargas, J. V. C., Mariano, A. B., Ramos, L. P., Corazza, M. L. and Cordeiro, C. S. (2018) Extraction of *Acutodesmus obliquus* lipids using a mixture of ethanol and hexane as solvent. *Biomass and Bioenergy,* 108: 470-478.
15. Singh, R., Kumar, A. and Sharma, Y. C. (2020). Evaluation of various lipid extraction techniques for microalgae and their effect on biochemical components. *Waste and Biomass Valorization,* 11: 2603-2612.
16. Bernaerts, T. M. M., Verstreken, H., Dejonghe, C., Gheysen, L., Foubert, I., Grauwet, T. and Loey, A. M. V. (2019). Cell disruption of *Nannochloropsis* sp. improves in vitro bioaccessibility of carotenoids and ω3-LC-PUFA. *Journal of Functional Foods*, 65: 103770.
17. González-González, L. M., Astals, S., Pratt, S., Jensen, P. D. and Schenk, P. M. (2019). Impact of osmotic shock pre-treatment on microalgae lipid extraction and subsequent methane production. *Bioresource Technology Reports*, 7: 100214.
18. Yao, L., Gerde, J. S., Lee, S., Wang, T. and Harrata, K. A. (2015). Microalgae lipid characterization. *Journal of Agricultural and Food Chemistry*, 63(6): 1773-1783.
19. Penga, Z., Feng, L., Wang, X. and Miao, X. (2019). Adaptation of *Synechococcus* sp. PCC 7942 to phosphate starvation by glycolipid accumulation and membrane lipid remodeling. *Biochimica et Biophysica Acta - Molecular and Cell Biology of Lipids*, 1864: 158522.
20. Cooney, M., Young, G. and Nagle, N. (2009). Extraction of bio-oils from microalgae. *Separation & Purification Reviews*, 38: 291-325
21. Ramluckan, K., Moodley, K. G. and Bux, F. (2014). An evaluation of the efficacy of using selected solvents for the extraction of lipids from algal biomass by the Soxhlet extraction method. *Fuel*, 114: 103-108.
22. Anthony, R and Stuart, B. 2015. Solvent extraction and characterization of neutral lipids in *Oocystis* sp. *Frontiers in Energy Research*, 3: 1-5.
23. Byreddy, A. R., Gupta, A., Barrow, C. J. and Puri, M. (2015). Comparison of cell disruption methods for improving lipid extraction from *Thraustochytrid* strains. *Marine Drugs*, 13: 5111-5127.
24. Supaporn, P. and Yeom, H. S. (2016). Optimization of a two-step biodiesel production process comprised of lipid extraction from blended sewage sludge and subsequent lipid transesterification. *Biotechnology and Bioprocess Engineering*, 21: 551-560.

25. Xia, A., Sun, C. Fu, Q., Liao, Q., Huang, Y., Zhu, X. and Li. Q. (2020). Biofuel production from wet microalgae biomass: Comparison of physicochemical properties and extraction performance. *Energy*, 212: 118581.
26. Escorsim, A. M., Rocha, G., Vargas, J. V. C., Mariano, A. B., Ramosa, L. P., Corazzac, M. L. and Cordeiro, C. S. (2018). Extraction of *Acutodesmus obliquus* lipids using a mixture of ethanol and hexane as solvent. *Biomass and Bioenergy*, 108: 470-478.
27. Dvoretsky, D., Dvoretsky, S. Temnov, M., Akulinin, E. and Peshkova, E. (2016). Enhanced lipid extraction from microalgae *chlorella vulgaris* biomass: Experiments, modelling, optimization. *Chemical Engineering Transactions*, 49: 175-180.
28. Ryckebosch, E, Bruneel, C., Termote-Verhalle, R., Muylaert, K. and Foubert, I. (2014). Influence of extraction solvent system on extractability of lipid components from different microalgae species. *Algal Research*, 3: 46-43.
29. Mahmood, W. M. A. W, Theodoropoulos, C. and Gonzalez-Miquel, M. (2017). Enhanced microalgal lipid extraction using bio-based solvents for sustainable biofuel production. *Green Chemistry*, 19: 5723-5733.
30. Hidalgo, P., Ciudad, G. and Navia, R. (2016). Evaluation of different solvent mixtures in esterifiable lipids extraction from microalgae *Botryococcus braunii* for biodiesel production. *Bioresource Technology*, 201: 360-364.
31. Moradi-kheibari, N., Ahmadzadeh, H. and Hosseini, M. (2017). Use of solvent mixtures for total lipid extraction of *Chlorella vulgaris* and gas chromatography FAME analysis. *Bioprocess and Biosystems Engineering*, 40: 1363-1373.
32. Ramola, B., Kumar, V., Nanda, M., Mishra, Y., Tyagi, T., Gupta, G. and Sharma, N. (2019). Evaluation, comparison of different solvent extraction, cell disruption methods and hydrothermal liquefaction of *Oedogonium* macroalgae for biofuel production. *Biotechnology Reports*, 22: e00340.
33. Tang, Y., Zhang, Y., Rosenberg, J. N., Sharif, N., Betenbaugh, M. J. and Wang, F. (2016). Efficient lipid extraction and quantification of fatty acids from algal biomass using accelerated solvent extraction (ASE). *RSC Advances*, 6: 29127-29134.
34. Ansari, F. A., Gupta, S. K., Shriwastav, A., Guldhe, A., Rawat, I. and Bux, F. (2017). Evaluation of various solvent systems for lipid extraction from wet microalgal biomass and its effects on primary metabolites of lipid-extracted biomass. *Environmental Science and Pollution Research*, 24: 15299-15307.
35. Shin, H. Y, Shim, S. H., Ryu, Y. J., Yang, J. H., Lim, S. M. and Lee, C. G. (2018). Lipid extraction from *Tetraselmis* sp. microalgae for biodiesel production using hexane-based solvent mixtures. *Biotechnology and Bioprocess Engineering*, 23: 16-22.
36. Kapoore, R. V., Butler, T. O., Pandhal, J. and Vaidyanathan, S. (2018). Microwave-assisted extraction for microalgae: from biofuels to biorefinery. *Biology*, 7: 18.
37. Khoo, K. S. Chew, K. W., Yew, G. Y., Leong, W. H., Chai, Y. H., Show, P. L. and Chen, W. H. (2020). Recent advances in downstream processing of microalgae lipid recovery for biofuel production. *Bioresource Technology*, 304: 122996.
38. Schuur, S., Brouwer, T., Smink, D. and Sprakel, L. M. J. (2019). Green solvents for sustainable separation processes. *Current Opinion in Green and Sustainable Chemistry*, 18: 57-65.
39. Jesus, S. S., Ferreira, G. F., Fregolente, L. V. and Filho, R. M. (2018). Laboratory extraction of microalgal lipids using sugarcane bagasse derived green solvents. *Algal Research*, 292: 292-300.
40. Tanzi, C. D., Vian, M. A., Ginies, C., Elmaataoui, M. and Chemat, F. (2012). Terpenes as green solvents for extraction of oil from microalgae. *Molecules*, 17: 8196-8205.
41. Mahmood, W. M. A. W, Theodoropoulos, C. and Gonzalez-Miquel, M. (2017). Enhanced microalgal lipid extraction using bio-based solvents for sustainable biofuel production. *Green Chemistry*, 19: 5723-5733.

42. Yoo, C. G., Pu, Y. and Ragauskas, A. J. (2017). Ionic liquids: Promising green solvents for lignocellulosic biomass utilization. *Current Opinion in Green and Sustainable Chemistry*, 5: 5-11.
43. Pan, J., Muppaneni, T., Sun, Y., Reddy, H. K., Fu, J., Lu, X. and Deng, S. (2016). Microwave-assisted extraction of lipids from microalgae using an ionic liquid solvent [BMIM][HSO_4]. *Fuel*, 178: 49-55.
44. Valerie C. A. and Rehmann, O. R. (2016). Ionic liquids for the fractionation of microalgae biomass. *Current Opinion in Green and Sustainable Chemistry*, 2: 22-27.
45. Shankara, M., Chhotaray, P. K., Agrawal, A., Gardasd, R. L., Tamilarasan, K. and Rajesh, M. (2017). Protic ionic liquid-assisted cell disruption and lipid extraction from fresh water *Chlorella* and *Chlorococcum* microalgae. *Algal Research*, 25: 228-236.
46. Wahidin, S., Idris, A., Yusof, N. M., Kamis, N. H. H. and Shaleh, S. R. M. (2018). Optimization of the ionic liquid-microwave assisted one-step biodiesel production process from wet microalgal biomass. *Energy Conversion and Management*, 171: 1397–1404.
47. Jordana, A. and Gathergood, N. (2015). Biodegradation of ionic liquids – a critical review. *Chemical Society Reviews*, 44: 8200-8237.
48. Sydow, M., Owsianiak, M., Framski, G., Woźniak-Karczewska, G., Piotrowska-Cyplik, A., Ławniczak, Ł., Szulc, A., Zgoła-Grześkowiaka, A., Heipiepere, H. J. and Chrzanowski, Ł. (2018). Biodiversity of soil bacteria exposed to sub-lethal concentrations of phosphonium-based ionic liquids: Effects of toxicity and biodegradation. *Ecotoxicology and Environmental Safety*, 147: 157-164.
49. Biczak, R., Pawłowska, B., Telesiński, A. and Kapuśniak, J. (2017). Role of cation structure in the phytotoxicity of ionic liquids: growth inhibition and oxidative stress in spring barley and common radish. *Environmental Science and Pollution Research*, 24: 18444-18457.
50. Chen, C. and Mu, T. C. (2021). Revisiting greenness of ionic liquids and deep eutectic solvents. *Green Chemical Engineering*, *2*: 174-186.
51. Chen. J., Wang, Q., Liu, M. and Zhang, L. (2017). The effect of deep eutectic solvent on the pharmacokinetics of salvianolic acid B in rats and its acute toxicity test. *Journal of Chromatography B*, 1063: 60-66.
52. Pan, Y., Alam, A., Wang, Z. Huang, D., Hu, K., Chen, H. and Yuan, Z. (2017). One-step production of biodiesel from wet and unbroken microalgae biomass using deep eutectic solvent. *Bioresource Technology*, 238: 157-163.
53. Tommasi, E., Cravotto, G., Galletti, P., Grillo, G., Mazzotti, M., Sacchetti, G., Samorì, C., Tabasso, S., Tacchini, M. and Tagliavini, E. (2017). Enhanced and selective lipid extraction from microalgae *P. tricornutum* by dimethyl carbonate and supercritical CO_2 using deep eutectic solvents and microwaves as pre-treatment. *ACS Sustainable Chemistry and Engineering*, 5: 8316-8322.
54. Lu, W., Alam, M. A., Pan, Y., Wu, J., Wang, Z. and Yuan, Z. (2016). A new approach of microalgal biomass pretreatment using deep eutectic solvents for enhanced lipid recovery for biodiesel production. *Bioresource Technology*, 218: 123-128.
55. Cai, C., Chen, X., Li, D. and Tan, Z. (2021). Three-phase partitioning based on CO_2-responsive deep eutectic solvents for the green and sustainable extraction of lipid from *Nannochloropsis* sp. *Separation and Purification Technology*, 279: 119685.
56. Obluchinskaya, E. D., Pozharitskaya, O. N., Zakharova, L.V., Daurtseva, A.V., Flisyuk, E.V. and Shikov, A.N. (2021). Efficacy of natural deep eutectic solvents for extraction of hydrophilic and lipophilic compounds from *Fucus vesiculosus. Molecules*, 26: 4198.

57. Torregrosa-Crespo, J., Marset, X., Guillen, G., Ramón, D. J. and Martínez-Espinosa, R. M. (2020). New guidelines for testing ‘‘Deep eutectic solvents” toxicity and their effects on the environment and living beings. *Science of the Total Environment*, 704: 135382.
58. Hayyan, M., Hashim, M. A., Al-Saadi, M. A., Hayyan, A., AlNashefc, I. M. and Mirghani, M. E. S. (2013). Assessment of cytotoxicity and toxicity for phosphonium-based deep eutectic solvents. *Chemosphere*, 93: 455-459.
59. Du, Y., Schuur, B., and Brilman, D. W. F. (2017). Maximizing lipid yield in *Neochloris oleoabundans* algae extraction by stressing and using multiple extraction stages with n-ethylbutylamine as switchable solvent. *Industrial & Engineering Chemistry Research*, 56: 8073-8080.
60. Du, Y., Schuur, B., Kersten, S. R. A. and Brilman, D. W. F. (2015). Opportunities for switchable solvents for lipid extraction from wet algal biomass: An energy evaluation. *Algal Research*, 11: 271-283.
61. Bazel, Y., Rečlo, M. and Chubirka, Y. (2020). Switchable hydrophilicity solvents in analytical chemistry. Five years of achievements. *Microchemical Journal*, 157: 105115.
62. Motlagh, S. R., Harun, R., 1, Biak, D. R. A. and Hussain, S. A. (2020). Microwave assisted extraction of lipid from *Nannochloropsis gaditana* microalgae using [EMIM]Cl. *IOP Conf. Series: Materials Science and Engineering*, 778: 012164.
63. Poliakoff, M. and Licence, P. (2007). Green chemistry. *Nature*, 450: 810–812.
64. Mustapha, S. I. and Isa, Y. M. (2020). Utilization of quaternary solvent mixtures for extraction of lipids from Scenedesmus obliquus microalgae. *Chemical Engineering*, 7: 1788877.
65. Molino, A., Mehariya, S., Sanzo, G. D., Larocca, V., Martino, M., Leone, G. P., Marino, T., Simeone Chianese, S., Roberto Balducchi, R. and Musmarr, D. (2020). Recent developments in supercritical fluid extraction of bioactive compounds from microalgae: Role of key parameters, technological achievements and challenges. *Journal of CO2 Utilization*, 36: 196-209.
66. Malekghasemi, S., Kariminia, H. R., Plechkova, N, K. and Ward, V. C. A. (2021). Direct transesterification of wet microalgae to biodiesel using phosphonium carboxylate ionic liquid catalysts. *Biomass and Bioenergy*, 150: 106126.
67. Orr, V. C. A., Plechkova, N. V., Seddon, K. R. and Rehmann, L. (2016). Disruption and wet extraction of the microalgae *Chlorella vulgaris* using room-temperature ionic liquids. *ACS Sustainable Chemistry and Engineering*, 4: 591-600.
68. Jun Cheng, Guo, H., Qiu, Y., Zhang, Z., Mao, Y., Qian, L., Yang, W. and Park, J. Y. (2020). Switchable solvent N, N, N′,N′-tetraethyl-1, 3-propanediamine was dissociated into cationic surfactant to promote cell disruption and lipid extraction from wet microalgae for biodiesel production. *Bioresource Technology*, 312: 123607.