



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

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

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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 mikroalga 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. Namun, 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

membicarakan pelarut organik konvensional dan pelarut hijau yang digunakan dalam pengekstrakan lipid mikroalga. 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.

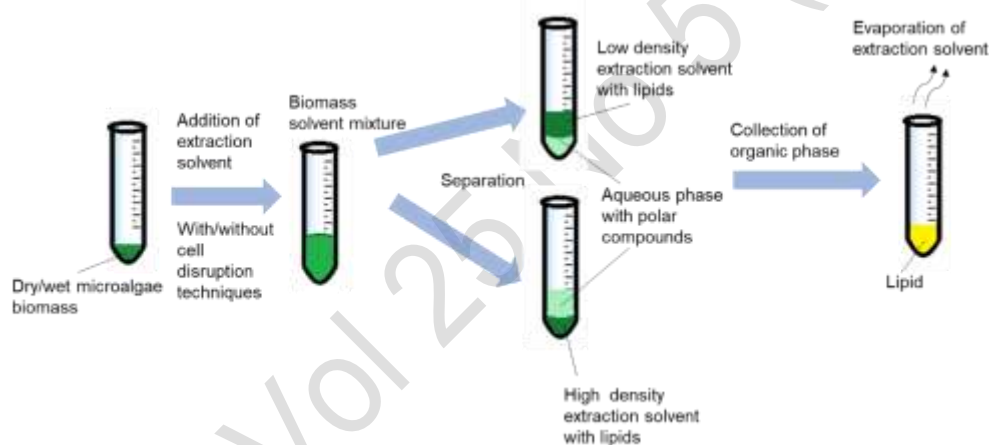


Figure 1. Common method for microalgae lipid extraction

Table 1. Microalgae lipid extraction efficiency of conventional solvents

Extraction Solvent (Volume/Volume)	Microalgae	Cell Disruption Techniques	Lipid Extracted (%)	Ref
Chl: Met (2:1)	<i>Oedogonium</i>	Soxhlet extraction	14	[32]
Chl: Met (2:1)	<i>Oedogonium</i>	Bligh and Dyer	11.5	[32]
Chl: Met (2:1)	<i>Chlorella vulgaris</i> UTEX 395	Accelerated solvent extraction	25.1	[33]
Chl: Met (2:1)	<i>Chlorella sorokiniana</i>	Accelerated solvent extraction	25.8	[33]
Chl: Met (2:1)	<i>Nannochloropsis gaditana</i>	Accelerated solvent extraction	29.8	[33]
Chl: Met (2:1)	<i>Chlorella vulgaris</i>	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)	<i>Chlorella vulgaris</i>	Chemical extraction	38.57	[31]
Chl: Eth (2:1)	<i>Chlorella sp.</i>	Soxhlet extraction	11.76	[21]
Chl: Met (2:1)	<i>Scenedesmus obliquus</i>	Microwave-assisted extraction	19.25	[34]
Chl: Eth (1:1)	<i>Scenedesmus obliquus</i>	Microwave-assisted extraction	10.08*	[34]
Chl: Met (2:1)	<i>Tetraselmis sp. KCTC12429BP</i>	Chemical extraction	5.51	[35]
Chl: Met: Water (5:10: 4)	<i>Acutodesmus obliquus</i>	Bligh and Dyer	7.41	[14]
DCM: Met (2:1)	<i>Scenedesmus obliquus</i>	Microwave-assisted extraction	19.00	[34]
Hex: Met (1:1)	<i>Tetraselmis sp. KCTC12429BP</i>	Chemical extraction	5.16	[35]
Hex: Met (1:2)	<i>Chlorella vulgaris</i>	Chemical extraction	25.17	[31]
Hex: Eth (1:2)	<i>Acutodesmus obliquus</i>	Soxhlet extraction	6.82	[14]
Hex: Eth (1:2)	<i>Acutodesmus obliquus</i>	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-methylimidazolium 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 (of extraction?), 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 CO₂ - 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)	<i>Chlorella pyrenoidosa</i>	Chemical extraction	99.6 mg/g	[39]
d-limonene	<i>Chlorella vulgaris</i>	Soxhlet method	1.29	[40]
α -pinene	<i>Chlorella vulgaris</i>	Soxhlet method	0.91	[40]
p-cymene	<i>Chlorella vulgaris</i>	Soxhlet method	1.52	[40]
Ethyl lactate	<i>Nannochloropsis sp.</i>	Soxhlet method	31.1	[41]
2-MeTHF	<i>Nannochloropsis sp.</i>	Soxhlet method	19.1	[41]
Ionic liquid				
Butyrolactam hexanoate	<i>Chlorella sp.</i>	Chemical extraction	70.90 mg/g*	[45]
Butyrolactam hexanoate	<i>Chlorococcum sp.</i>	Chemical extraction	19.86 mg/g*	[45]
[EMIM][MeSO ₄]	<i>Nannochloropsis sp.</i>	Microwave-assisted extraction	40.9*	[46]
[EMIM]Cl	<i>Nannochloropsis gaditana</i>	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 ₂ SO ₄ (60:40, v/v)	<i>Chlorella sp.</i>	Autoclave	13.91	[51]
Ch-Ca: DMC	<i>Phaeodactylum tricornutum</i>	Microwave-assisted extraction	11.00	[52]
ChCl: oxalic acid (1:2 v/v)	<i>Phaeodactylum tricornutum</i>	Microwave-assisted extraction	12.5%	[52]
ChCl: oxalic acid (1:2 v/v)	<i>Chlorella sp.</i>	-	12.5%	[53]
TMG: menthol (3:1, M/M)	<i>Nannochloropsis sp.</i>	Ultrasonic	172.3 mg/g	[54]
Supercritical CO ₂	<i>Phaeodactylum tricornutum</i>	-	0.3	[52]
Switchable solvent				
TEPDA	<i>Nannochloropsis oceanica</i>	-	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-methylimidazolium methyl sulphate; Isoamyl alcohol: 3-methyl-1-butanol; TEPDA: N, N, N', N'-tetraethyl-1,3-propanediamine. TMG: methanol: Tetramethylguanidine; methanol

*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.

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