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The extraction of lipid from microalgae found in brackish water by terpenes

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Abstract

In this research**,** terpenes were used to evaluate the extraction of lipids from microalgae found in brackish water**.** *p*-Cymene, *α*-pinene, and *d*-limonene terpenes were used instead of petroleum-based solvents, which have a low boiling point and are toxic as well as highly flammable. The physical and thermal pretreatment method of grinding and then autoclaving at a temperature of 121°C and a pressure of 15 lb/in^2 was investigated to enhance the lipid extraction efficiency and excrete the intracellular lipid. The amount of lipid extracted by *p*-cymene was 29.23 ± 0.25 % (lipid wt./dry.wt. microalgae), whereas the amount of lipid extracted by n-hexane:methanol 3:1(v/v) was 13.5 ± 0.20 % (lipid wt./ dry.wt. microalgae). The quantity of fatty acid extracted from the microalgae was determined using GC-MS analysis. The results showed that the two main fatty acids were palmitic acid ($C_6H_{12}O_2$; 16:0) and oleic acid ($C_{18}H_{34}O_2$; C18:1). There was more palmitic acid (16:0) than oleic acid (C18:1) at 7.70% and 7.08%, respectively. This research found that using natural solvents to extract lipid from microalgae could be a feasible and sustainable alternative to petroleum solvents which are toxic, environmentally unfriendly and increase greenhouse gases causing climate change.

Keywords: *brackish water, extraction of lipid, green solvents, lipid extraction, microalgae, terpenes*

1. Introduction

Currently, climate change due to the emission of greenhouse gases from fossil fuels is the cause of many problems and a serious global issue. Many researchers have looked for a feasible and sustainable renewable energy source that will be safer for the environment. Microalgae are considered as a potential renewable energy source due to their carbon neutrality. However, their thick and rigid cell walls make it very difficult to extract lipids. There are two major types of microalgae lipid; polar and non-polar (Li et al., 2014). The most common lipid extraction solvents used in industry are n-hexane, methanol, and combinations of solvents such as Chloroform:methanol 2:1 (v/v) (Folch method), and Chloroform:methanol 1:2 (v/v) (Bligh and Dyer method) (Matyash, Liebisch, Kurzchalia, Shevchenko, & Schwudke, 2008). When used on a large scale, these volatile organic solvents derived from petroleum have many disadvantages, such as their low boiling point, high flammability, high toxicity, and environmental unfriendliness. To counter these disadvantages, researchers have investigated green solvents to extract lipids from microalgae (Madji et al., 2019). "Green and viable technologies" used to decrease hazardous waste disposal have focused on reducing toxic organic solvents and eliminating toxicological processes for environmental economics and sustainability (Ivankovic, Dronjić, Bevanda, & Talić, 2017).

Terpenes or terpenoids are outstanding natural aromatic compounds. They are simple lipids that are used in industrial applications, pharmaceuticals, and medicines (Schwab, Davidovich-Rikanati, & Lewinsohn, 2008). Terpenes are hydrocarbons with C_5H_8 isoprene units. Many terpenes are acyclic, bicyclic, or monocyclic (Tanzi, Vian, Ginies, Elmaataoui, & Chemat, 2012). They are non-polar molecules due to their hydrocarbons. Terpenes from citrus and other plants are essential oils. D-Limonene is the most common solvent present in citrus, which is low in cost and toxicity (Grazhdannikov et al., 2018). Madji et al. (2019) reported that the yield and crude microalgae lipid extracted by d-limonene were similar to those extracted by n-hexane. α-Pinene is a natural terpene hydrocarbon. p-Cymene, an important intermediate product in the chemical industry, is an aromatic hydrocarbon

(Cho et al., 2017). However, terpenes have a higher boiling point and require more energy for evaporation than petroleum-based solvents (Tanzi et al., 2012). Thus, using p-cymene, *α*-pinene, and *d*-limonene as terpenes to evaluate the extraction of lipids from microalgae found in brackish water instead of petroleum-based solvents is an interesting method of changing biomass to biofuel (Kumar, Rao, & Arumugam, 2015). In this study, the physical and thermal pretreatment method of grinding and then autoclaving at a temperature of 121°C and a pressure of 15 lb/in^2 was investigated to enhance the lipid extraction efficiency and excrete the intracellular lipid. Then, the microalgae lipid yields obtained from terpenes were compared with volatile organic petroleum solvents.

2. Materials and methods

2.1 Microalgae cultivation and harvesting

Microalgae were collected from the following sources of brackish water in Thailand: accommodation next to the sea in Bang Poo, Bang Poo mangrove forest, and the watersides next to Asogaram and Srichan temples. Modified Watanabe's medium was used to cultivate the inoculum. A spectrofluorometer was used to determine the lipid content and a gravimetric analysis was performed to compare then select suitable microalgae. The microalgae from Bang Poo mangrove forest had the highest lipid content (Labua, Inprasit, & Ngamcharoen, 2015). After scale-up and cultivation of microalgae from Bang Poo mangrove forest, the biomass was flocculated with 1.2 g/l $Al_2(SO_4)_3{\cdot}18H_2O$ and then harvested by membrane filtration. Subsequently, the wet microalgae were dried in a hot air oven at 110°C until a constant weight was obtained.

2.2 Materials and equipments

p-Cymene, *α*-pinene, *d*-limonene, n-hexane, chloroform, and methanol used for the extraction were supplied by Sigma-Aldrich and others. The Soxhlet apparatus, extraction equipment, and glassware were supplied by the chemistry laboratory.

2.3 Lipid extraction

The solvents for total lipid extraction in the microalgae were performed based on n-hexane and combinations of n-hexane:methanol 3:1 (v/v), chloroform:methanol 2:1 (v/v) (Folch method), and chloroform:methanol 1:2 (v/v) (Bligh and Dyer method) (Kumar et al., 2015) and then compared to *p*-cymene, *α*-pinene, and *d*-limonene as terpene solvents. The Soxhlet apparatus was used for lipid extraction.

2.4 Fatty acid analysis

GC-MS, which was used to analyze the fatty acid composition, consisted of an Agilent Technologies GC model 7890 A, an Agilent 5975C Mass Spectral Detector (MSD), and a mega-5 MS column (30 m x 0.25 mm). The flow rate of carrier gas (He) was 1 ml/min. The ionization source and injection temperatures were 230°C and 250°C, respectively. The initial oven temperature was 90°C, held for 2 min, then increased to 300°C at a rate of 4°C/min and held for 15 min. The Electron Impact (EI) mode was 70 eV with a mass spectra scan during 40-900 a.m.u. All samples were injected in volume 1 µl. The quantification of fatty acid was identified by a mass spectral database.

3. Results and discussion

3.1 Microalgae yields

The microalgae from Bang Poo mangrove forest were cultivated outdoors. Atmospheric instability is a weather condition associated with temperature and light. The microalgae culture were aerated throughout cultivation. After cultivation for 15 days, the microalgae were flocculated with 1.2 g/L $Al_2(SO_4)_3{\cdot}18H_2O$, filtered through a membrane filter, then dried in a hot air oven at 110°C until a constant weight was obtained. The microalgae contained a total yield of 13.30 g dried biomass/L culture. This result concurs with Halim, Danquah, & Webley (2012), who found that the biomass concentrations of microalgae were within the range 10-450 g dried biomass/L.

Figure 1 Dried microalgae

3.2 Lipid extraction yields

Before studying the lipid extraction yields, dry microalgae were ground into a fine powder by mortar and pestle. Dry microalgae (3 g) added with water (100 ml) were autoclaved at a temperature of 121°C and a pressure of 15 lb/in^2 for 7 min to disrupt the cell membrane and excrete the intracellular lipids. Suspensions of microalgae were filtered by membrane filtration and then placed in a cellulose thimble. 150 ml of solvents used for the extraction were added and mixed in each combination in a Soxhlet apparatus. The extraction time was 8 h. Subsequently, the solvents were separated and evaporated under a fume hood. The extracted lipid yields were calculated, as shown in Table 1.

Table 1 Extracted lipid yields

Solvents	Yields (%) (lipid wt./dried algae wt.)
n-Hexane	3.00 ± 0.25
n-Hexane:methanol 3:1 (v/v)	13.50 ± 0.20
Chloroform:methanol 2:1 (v/v)	18.00 ± 0.35
Chloroform:methanol 1:2 (v/v)	25.00 ± 0.15
d-Limonene	21.01 ± 0.35
α-Pinene:*p*-cymene 1:29 (v/v)	25.81 ± 0.30
p-Cymene	29.23 ± 0.25

A comparison of microalgae lipid yields showed that n-hexane, a combination of hexane:methanol and a combination of chloroform:methanol, gave lower lipid yields than the terpenes. A combination of hexane:methanol 3:1 (v/v) gave higher lipid yields than hexane due to methanol being more polar than single hexane, which is a non-polar organic solvent. Chloroform is non-polar and has a higher toxicity than methanol. A combination of chloroform:methanol could extract both polar and non-polar microalgae lipids. Higher proportions of methanol such as chloroform:methanol 1:2 (v/v) could extract higher lipid yields than chloroform:methanol 2:1 (v/v), respectively. This result indicates that lipid yields are dependent on the polarity of solvents. Petroleum solvents with higher polarity could extract higher lipid yields, which corresponds to Li *et al*. (2014).

Terpenes are low toxicity non-polar molecules due to their hydrocarbons. However, they have more polarity than volatile organic petroleum solvents such as chloroform and hexane. *p*-Cymene could extract higher lipid yields than *d*-limonene and *α*-pinene due to its higher solubility and Hansen solubility parameter (δ), it was better dissolved in solutions than *d*-limonene and *α*-pinene. Moreover, the higher boiling point of *p*-cymene during extraction could decrease viscosity and increase the dissolving ability to extract microalgae lipid. The lipid yields extracted by *p*-cymene, *α*-pinene:*p*-cymene 1:29 (v/v) and *d*-limonene were 29.23 ± 0.25%, 25.81 ± 0.30%, and 21.01 ± 0.35%, respectively. This result agrees with Mamidipally and Liu (2004).

3.3 Fatty acid composition analysis

GC-MS was used to analyze the fatty acid composition. The quantity of microalgae common fatty acids extracted by the terpenes was calculated according to a mass spectral library database, as shown in Table 2 and Figure 2.

Table 2 Fatty acid composition of lipids

Peak No.	Retention Time	Corr % max.	Area %	Library/ID
1	5.271	100.00%	49.53%	p-Cymene
2	9.349	28.45%	14.09%	Ethanone
3	31.166	15.55%	7.70%	n-Hexadecanoic acid or Palmitic acid
4	35.280	14.29%	7.08%	Oleic acid

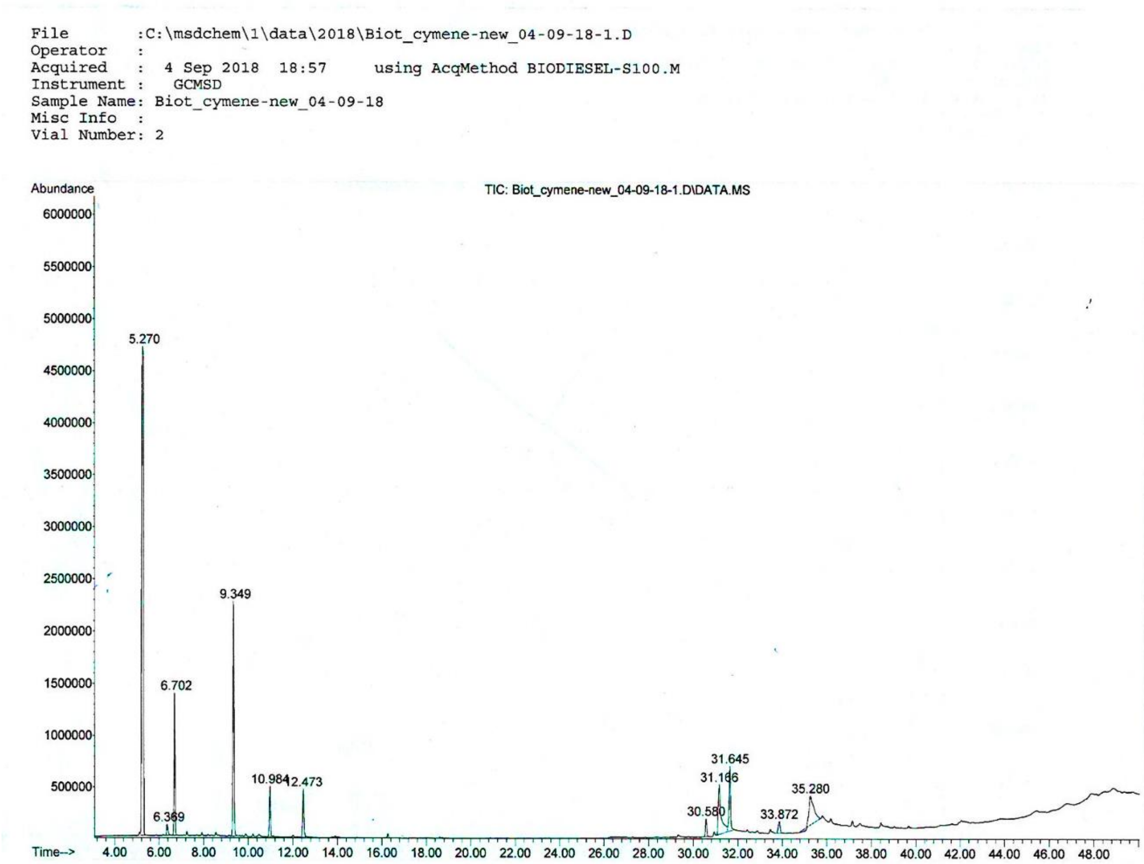

Figure 2 Fatty acid composition analysis

This study showed that the two main fatty acids were n-hexadecanoic acid or palmitic acid ($C_6H_{12}O_2$; 16:0) and oleic acid ($C_{18}H_{34}O_2$; C18:1), which concurs with Chen, Wang, & Qiu (2018), who reported that C16 and C18 were the most abundant fatty acids in microalgae. Nevertheless, the quantity of microalgae fatty acids depends on the species, cultivation, and environment. There was more Palmitic acid (16:0) than oleic acid (C18:1) at 7.70% and 7.08%, respectively. Interestingly, microalgae found in brackish water have the potential for biodiesel production and fuel properties according to their high oleic acid (Rashid, Anwar, Moser, & Knothe, 2008).

4. Conclusion

The principles of green or sustainable chemistry state that suitable substitutions for organic solvents must be safe for workers, sustainable, and environmentally friendly (Ivankovic et al., 2017). The disadvantage of using petroleum-based solvents for lipid extraction is that they are toxic and not environmentally friendly. Terpenes, which are natural solvents, were investigated to extract microalgae lipid found in brackish water. The results of this study demonstrated that more polar terpenes such as *p*-cymene, *α*-pinene, and *d*-limonene released more lipid than organic petroleum solvents. Moreover, terpenes are less hazardous than petroleum solvents. The microalgae found in brackish water contained high levels of oleic acid, making it suitable as a renewable energy source. This research found that using natural solvents to extract lipid from microalgae could be a feasible and sustainable alternative to petroleum solvents which are toxic, environmentally unfriendly and increase greenhouse gases causing climate change. However, the disadvantage of using terpenes is their high viscosity and density, and also the higher energy consumption related to solvent recovery by evaporation due to their higher boiling point and higher enthalpies of vaporization (Ivankovic. et al., 2017). Further research could investigate biological methods of breaking microalgae cell walls instead of mechanical methods such as grinding to find suitable, cost-effective methods of extracting microalgae lipid found in brackish water.

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