Microwave assisted extraction of lipid from *Nannochloropsis gaditana* microalgae using [EMIM]Cl

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Abstract. Ionic liquids (ILs) have been recognized as superior solvent due to their exclusive properties, green and varied applications in chemical industries. ILs can be apply as solvent in extracting lipid from algal biomass. This study examined the application of microwave-assisted extraction (MAE) with ILs to extract total lipids from microalgae for biodiesel production. 1-ethyl-3-methyl imidazolium chloride ([EMIM]Cl) IL was utilized as the solvent and microwave irradiation applied for the pretreatment method. The experimental findings were compared with those from conventional method using Soxhlet extraction. For [EMIM]Cl plus microwave irradiation assisted extraction, it was revealed that the highest lipid yield was achieved 13.9% in the optimum condition at 3.3 w/v of solid loading, process duration of 5 min, temperature of 80 °C and microwave power of 800 W. Also, microwave irradiation with IL had improved the lipid extraction up to 3.1 times compared to the Soxhlet result. The findings from this study displays that the MAE with IL has the potential to be applied in extracting compounds from biomass.

Keywords: Microalgae; lipid extraction; ionic liquid; microwave assisted extraction; soxhlet.

1. Introduction:

Microalgae are recognized as a favorable platform for the extraction of lipid-based biofuels [1–3]. In comparison with other exist feedstocks such as plant oils, animal fats, etc., microalgae have prominent benefits like: they have potential to growing fast and transforming CO₂ into the considerable amounts of lipids [4,5]. Furthermore, microalgae have extremely high oil contents in comparison with those of terrestrial plant sources like palm, coconut, castor bean and sunflower seeds [6]. Some microalgae types capable to imbibe vital nutrients containing the carbon, nitrogen and phosphorus from exhaust gas and wastewater [7]. Moreover, many microalgae spices can grow and cultivated in fringe land by means of non-potable water. That is, microalgae do not compete for the land required for producing food and dominate the discord between food and fuel [8,9]. The improvement of energy effective, economically feasible, tolerable downstream processing and strategy making is critical for biodiesel production from these high lipid content microalgae. In fact, most microalgae kinds, lipids are situated inside the cell wall, which must be interrupted to permit extraction [10,11]. The toughness of microalgae walls and their organization into a complex multi-layered construction make interruption an energy-intensive process [12]. The most traditional techniques for the extraction of lipid from microalgae include chemical organic solvent extraction at atmospheric pressure, like chloroform/methanol extraction [13]; soxhlet extraction using hexane [13]; extraction with dimethyl ether [14]; subcritical organic solvent extraction, like ethanol [15], and supercritical CO₂ [16]; and ultrasonic-assisted extraction (UAE) [17]. Nevertheless, solvent extraction consume a large amount of organic solvents, which are usually inflammable, poisonous and can cause address the health, security, and environmental impacts. Environmental concerns have inspired scientists for augmentation and optimization of environment friendly extraction methods [18]. The necessity for an unconventional methodology lacking of these issues, which are also energy effective, ecofriendly and moderately low-cost has led scientists to emphasis on green solvents like ionic liquids (ILs). ILs have outstanding characteristics for usage in the treatment of cellulosic biomass [19]: tunable physicochemical properties, low vapor pressure, thermal stability over a varied range of temperature [20]. ILs also can increase the efficiency of lipids extraction from microalgae biomass own to their high solubility for lignocellulosic materials [21–23] which are the key components of cell wall. Though, most algal biomass have a thick cell wall which is a major
obstacle for the extraction stage. To prevail this concern, microwave assisted extraction (MAE) as mechanical cell disruption procedure is performed to develop the solvent entrance to the cell content. Besides, microwave, the alternating present signals with frequencies threshold between 0.3 and 300 GHz, converts electromagnetic energy into heat with the polarity of compounds [24]. Furthermore, MAE has become a fast, safe and economical technique for the production of biodiesel from biomass[25]. In the present study, the effect of MAE with [EMIM][Cl] as IL was examined in order to extract lipid from dry microalgae biomass. Consequently, the influence of IL loading on the extraction of total lipid was performed a comparison with soxhlet as the conventional method.

2. Material and methods:
The freeze dried biomass (*Nannochloropsis gaditana*) was bought and transported from Longevity Superfoods (Utah, USA) with batch no: 32490. The biomass was 100% freeze dried by the manufacturer and the powdered biomass was wrapped in the package until further experiment is being performed. Hexane, chloroform (>99.8%), and methanol were used in this study bought from R&M Chemicals and of analytical grade. The ionic liquid 1-ethyl-3 methyl imidazolium chloride ([EMIM][Cl]) (P94.5%) was purchased from Merk, Germany and utilized without any additional purification. The microwave reactor (Samsung, ME711K) was from Malaysia.

2.1 Lipid extraction by Soxhlet extraction method:
The Soxhlet extraction method was performed utilizing 5 gr of dry microalgae and hexane was used as a solvent. The solvent was heated and the extraction was accomplished for 7 hours. After the extraction, the hexane was vaporized and the lipid achieved was gravimetrically evaluated.

2.2 Lipid extraction by Microwave assisted extraction (MAE) with [EMIM][Cl]:
Domestic microwave assisted extraction (Samsung, ME711K) with temperature controller system was utilized. The system used 800 W of energy at a frequency of 2.45 GHz. The instrument was pre-calibrated according to manufacturer's conditions. The 0.5 gr of dry microalgae was mixed with distilled water appropriately (3.3 % wt). Samples were extracted at 80 °C, for 5 min. After microwave heating, methanol and chloroform are added to the sample and after phase separation using centrifuge (4000 rpm, 10 min), lipids are recovered in the chloroform phase. Next, chloroform phase washed using the mixture of Hexane: H2O to collect the residual IL in this layer. In the last, lipid is obtained after vaporized the chloroform. Dried residual lipid was measured and yields were determined with equation (1):

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\text{Lipid yield (%) = } \frac{\text{lipid weight (gr)}}{\text{Microalgae weight (gr)}} \times 100
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3. Result and discussion:
Three different methods comprising utilizing a different ratio of MeOH:CHCl3 to screening the best ratio and put it fix during the lipid extraction procedure from *Nannochloropsis gaditana* microalgae and the results are displayed in Figure 1.
Figure 1. Screening of lipid extraction efficiency through different approaches using MAE with MeOH:CHCl₃ (1:1; 1:2; 2:1) at 80°C, 5 min, and 800 Watt as comparison with traditional Soxhlet extraction method (reflux heating). *MeOH: methanol; CHCl₃: chloroform

The usage of MeOH:CHCl₃ as solvents (recover lipid step) in MAE yielded approximately 10.51%, 11.22%, and 9.85% respectively for the ratio of 1:1; 1:2; 2:1 which are comparable to that of the Soxhlet extraction method (4.472%). The outcome demonstrates that the lipid extracted by MAE using MeOH:CHCl₃ as solvents with the different ratio are more promising than the traditional soxhlet extraction method which uses hexane and normal heating during the lipid extraction procedure. The highest lipid extraction efficiency belonged to MeOH:CHCl₃ (1:2) with 11.22% yield. Studies accompanied by [26,27] also recommended that a mixture of chloroform and methanol in the ratio of 2:1 (v/v) could extract lipids more thoroughly from animal, plant or bacterial tissues than other simple solvent combinations. So, the MeOH: CHCl₃ with the ratio of (1:2) was fixed during the lipid extraction in other steps.

The results of H₂O volume influence on the extraction of lipid from microalgae in a MAE process is revealed in Figure 2.

Figure 2. Effect of H₂O volume on lipid extraction yield (%) from Nannochloropsis gaditana microalgae, at 80°C, 5 min, 800 Watt, and MeOH:CHCl₃ (1:2).
It can be detected that increasing the H$_2$O volume increases the extraction yield up to 11.22% (0.0561 gr total lipid weight) at 15 ml H$_2$O. A head from that point, the yield dropped to 5.76% (0.0288 gr total lipid weight) at 50 ml of H$_2$O. Shortly, high amount of water has negative effect on the lipid extraction from microalgae Nannochloropsis gaditana. Therefore, no extra water was added into the extraction system in the following extraction experiments and 15 ml H$_2$O was fix during the lipid extraction. Also, in a study performed by [28], the effect of water content on three different strain of microalgae (C. sorokiniana, N. salina and G. sulphuraria ) was assessed. It was found that in extraction of lipid from microalgae, the H$_2$O content can unfavorably affect the lipid extraction yield.

Figure 3 shows the results of evaluation the influence of IL loading (gr) on yield of lipid extraction (%).

Figure 3. Effect of IL loading (gr) on the yield of lipid extraction (%) from Nannochloropsis gaditana, at 80 $^\circ$C, 5 min, 800 Watt, MeOH:CHCl$_3$ (1:2), and 15 ml H$_2$O.

Seven different values of IL loading was considered for this part of experiments. According to the results, the presence of [EMIM][Cl] as IL has positive effect on the lipid extraction from microalgae. The result shown that the extraction yield was constantly aggregate until the maximum yield of 13.9% which gained after 2 gr IL from the onset of extraction. Though, the yield was observed to drop as it passed 2 gr IL until it reached 12.51% at 3 gr IL. In a study conducted by [29] the effect of microwave assisted extraction with ILs with different anion types (Cl, Ac and Te:N) and cations (AMIM, BMIM, and HMIM) on the extraction of essential oil was performed. Different IL concentration from (0.10 to 0.60 mol/l) and operation conditions 60 $^\circ$C, 5 min on the extraction of essential oil was examined. The outcome shown that the high efficiency of essential oil (9.61%) was achieved when the concentration was 0.50 mol/l with cleveger apparatus. Additional enhancing the concentration of IL did not demonstrate any development in the extraction efficiency.

4. Conclusion:

In this study, microwave-assisted extraction with IL has been used for the extraction of total lipids from dry microalgae Nannochloropsis gaditana. Lipid has been extracted using three different methods and comparison with the conventional and innovative techniques has been carried out. The characteristics H$_2$O volume and IL loading on lipid extraction from Nannochloropsis gaditana were analyzed. As a result, using MeOH: CHCl$_3$, with the ratio of (1:2, v:v) gain the most favorable result for the extraction of lipid from microalgae. Also, the high amount of H$_2$O had a negative effect on the lipid extraction and the effect of IL on lipid extraction was investigated. 2 gr of [EMIM][Cl] as green solvent extracted more lipid from Nannochloropsis gaditana.
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