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Comparison of different lipid extraction procedures applied to three microalgal species

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Abstract

The increase in the world's energy demand has contributed to the emergence of new sustainable energy sources, such as microalgae, with their great potential to provide biofuels and other high value co-products for the food and health's markets. However, current biorefinery methodologies are either too complex to extract the targeted components such as high-value products, or require solvents with toxicity for humans and the environment. This work aims to evaluate different lipid extraction approaches applied to three microalgal species: *Chlorella zofingiensis*, *Phaeodactylum tricornutum*, and *Arthrospira platensis*, while employing less toxic and more economical solvents for the lipids extraction. Experimental results showed a promising outcome to tune current biorefinery methodologies, enhancing product yield as well as decreasing potential hazards.

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Keywords: Arthrospira platensis; Biodiesel; Biorefinery; *Chlorella zofingiensis*; Lipid extraction; Microalgae; *Phaeodactylum tricornutum*

1. Introduction

Processing of microalgae biomass for biodiesel production poses significant challenges [1] in particular for ensuring the sustainability of large-scale microalgae production systems [2]. For biodiesel production, a lipid extraction step is normally performed, defined as the process of disrupting/damaging the algal cell walls to extract the oil stored in the cells [3]. To accomplish lipid extraction, two techniques are normally applied: organic solvents and solvent-free approaches. Safety and environmental issues are the main concerns in applying organic solvents, as it is known that fugitive or open emissions can dominate the potential environmental impacts of a process [4]. Thus,

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the development of organic solvent-free approaches that are safe and environmentally friendly are of most interest. On the other hand, the process of lipid extraction from algal cells, using solvent-based approaches is costly, energy intensive, but fast. Most of the energy consumption is related to the process of solvents recovering after lipids extraction [5]. The extraction method used should be fast enough to avoid damaging the extracted lipids. Also, the choice of the solvent affects the extraction efficiency, as polar compounds are more soluble in polar solvents. Therefore, employment of co-solvents such as chloroform, methanol and n-hexane [6] can accelerate the procedure and improve the oil extraction efficiency [7], but the specific nature of the microalgal species also affects the lipids extraction efficiency.

2. Lipid extraction approaches from microalgae biomass

The methods used for microalgae lipids extraction are grouped as mechanical and chemical approaches [7]. Some of the mechanical extraction processes that do not necessarily require solvent assistance, include bead mills [8], expeller press procedure [9], microwave-assisted pyrolysis extraction as well as ultrasound-assisted extractions, pulsed electric field, and hydrothermal liquefaction [7]. The chemical approaches include: Soxhlet extraction, supercritical fluid extraction, and accelerated solvent extraction. The next sections briefly describe several extraction methods.

2.1. Mechanical methods for lipids extraction

The mechanical approaches are based on microalgal cell rupturing techniques to release lipid bodies stored in the algal cells. Although these methods are not fully developed, solvent-free extraction is seen to be a promising technique for the industrial production of primary extracted lipids [3]. Mechanical methods present an effective and reliable alternative, as they are independent on the type of microalgae. Besides, employing no-solvents, the hazard of contamination is decreased significantly [10]. However, sometimes the heat used to accelerate the process may damage the extracted lipids, and therefore, this requires to employ cooling facilities which results in increasing the final cost [10].

2.1.1. Expeller press

The most known mechanical method to extract lipids (e.g. from seeds), is mechanical pressure to compress out the oil from the disrupted cells. It is also the simplest and the oldest method invented to extract vegetal seed oil [10], which can be still applied to microalgal biomass for oil extraction [11]. In this approach high-mechanical pressure is first applied to disrupt cell walls, and then to squeeze out the oil from biomass. Although pressure can enhance the extraction, too much pressure leads to less lipid recovery, as it can heat up the biomass and finally decrease the quality of the extracted oil [9]. Studies reported by Niraj et al. [12] showed the efficiency of extracting around 75% of oil from dried microalgal biomass. However, as it was discussed, mechanical pressure demands large quantity of biomass [13] while it is a slow process, and costly due to the requirement of heating, and cooling facilities during the extraction [14]. One of the disadvantages of the mechanical pressure is the presence of color pigments along with oil. Therefore, it is required to remove them with additional methods, which increases the final costs [9].

2.1.2. Ultrasound waves

As the thickness of cell wall increases in microalgal biomass, cell breakdown becomes more difficult. In this regard, to avoid generation of heat during crushing, ultrasound wave can assist, simulating high-pressure and low-pressure cycles. These cyclic waves penetrate into the biomass, propagate between cells and generate high-pressure, low pressure cyclic waves in which crush adjacent cells, without producing heat. This process results in the disruption of the cell structure to release the stored lipid. Studies have shown that the frequency of 20 kHz is adequate to be used during the extraction procedure [15].

2.1.3. Microwave

Microwaves are used passively to heat up the polar components of microalgal biomass and improve the efficiency of the lipids extraction. The employed waves are mostly between 0.3 to 300 GHz of frequency, and they can affect polar compounds, such as water molecules. Therefore, this method is a suitable approach to extract lipid from wet biomass. The aforesaid method uses less chemical solvents comparing to chemical extraction approaches. Iqbal and Theegala [5] reported improved lipid extraction yield, for biodiesel production, compared to other traditional toxic solvents used in chemical approaches.

2.2. Chemical methods for lipid extraction

In theory, an ideal solvent to be used in lipid extraction needs to easily solve lipids from other co-products while it should be separated with the lowest possible energy during the extraction phase to obtain pure lipids [7]. Both polar and non-polar solvents can be used during extraction, based on the type of lipids to be extracted. Non-polar solvents that are most frequently employed are hexane, benzene, toluene, diethyl ether, chloroform and polar solvents can be listed as methanol, acetone, ethyl acetate, and ethanol [16]. The combination of chloroform and methanol (with the ratio of 1 to 2 v/v) is the most widely used organic solvent to obtain lipid from algal biomass, resulting in less extraction time and high yield.

2.2.1. Folch approach

This method is one of the oldest initiatives for lipids extraction, which formed the basis for the development of further extraction procedures with improvements [10]. Folch et al. [17] used a mixture of chloroform/methanol 2/1 by volume, for extracting lipids from algal biomass. Briefly explained, the biomass is mixed and dilated with a saline solution. Then, the resulting mixture is maintained to be separated into two layers. Lipids, which are settled as the upper phase, are then separated and can be extracted. Rapid and easy processing of large number of samples is the major advantage of this method. However, it is less sensitive when compared with other latest procedures [10].

2.2.2. Bligh and Dyer approach

The Bligh and Dyer method is one of the widely practiced methods for lipid extraction, mostly used for total lipid extraction and quantification [18]. It is very similar to the Folch method, but mainly differs in solvent/solvent and solvent/tissue ratios [10], while lipid extraction and partitioning are performed simultaneously. The main solvents being used are methanol and chloroform, and water is used as co-solvent.

This procedure is initiated by extracting lipids from biomass suspension containing 1/2 (v/v) chloroform/methanol. The lipids from the chloroform phase are then extracted through different approaches proposed to enhance the extraction, which are not all, described here. Originally, this method proposed to use water as the co-solvent to enhance the separation; however, several investigations have suggested to use other co-solvents, instead, as explained in the next section.

2.2.3. Other co-solvent approaches

There have been investigations to evaluate the modifications of the Bligh and Dyer method [18]. The most common proposal to modify the Bligh and Dyer method is the addition of 1 M NaCl instead of water, to prevent binding of acidic lipids to denatured lipids. Alternatively, Hajra [19] proposed to employ phosphoric acid with the concentration of 0.2 M to improve lipid recovery with a shorter separation time, and Jensen [20] also suggested to use HCl for the same purpose.

Another modification, which will be studied in this research was proposed by Matyash et al. [21], using methyl-tert-butyl ether (MTBE) as a solvent for the extraction of lipids for better extraction of all type of stored lipids. The improvement is achieved since the lipids are settled as a low-density upper phase, which is easy to extract completely. Briefly explained, a mixture of harvested microalgae sample, 1.5 ml of methanol and 5 ml of MTBE are prepared and vortexed well and then incubated for 1 h at room temperature in shaker. To separate the phases, 1.25 ml of water is added to the mixture. Settling for 10 min at room temperature is enough to allow the separation. Centrifugation in proceeded at 1000 g for 10 min to collect the upper phase. To have a more complete recovery of all lipids of the lower phase, it has been proposed to mix 2 ml of MTBE/methanol/water (10/3/2.5, v/v/v). The excess solvent for both recovered phases can be drained using vacuum drier [21].

Lee et al. [22] evaluated the performance of five different solvents (chloroform/methanol, hexane/ isopropanol, dichloromethane/methanol, dichloromethane/ethanol, and acetone/dichloromethane), reporting a higher lipid yield, of 28.6%, for the chloroform/methanol (2/1, v/v).

3. Materials and methods

Employment of toxic solvents such as hexane and chloroform has a major drawback, which can result in severe health and environmental hazards [23]. This normally occurs when the solvent extraction techniques are cheaper and easier than mechanical approaches, either for research or commercial applications, and thus, the widespread application of toxic solvents and the longer execution times can increase their harmful consequences. Therefore, it is worth to study alternative methods that depend less on bio-hazardous solvents, or employ them as little as possible. In this research, it was applied the method proposed by Matyash et al. [21] and compared it with a modified Bligh and Dyer method [24]. Therefore, this research study aims to investigate two chemical extraction approaches for lipids extraction from three microalgal species: *Chlorella zofingiensis* (SAG-211.14) and *Arthrospira platensis* (SAG-85.79), both obtained from the Experimental Phycology and Culture Collection of Algae at the University of Goettingen, Germany), and *Phaeodactylum tricornutum* Bohlin (originating from Cañar Blanco, La Serena, IV Region, Chile).

The *Chlorella zofingiensis* microalgal species was cultured individually in sterile Bold's Basal Medium (BBM) [25]. The cultures consisting of two 5 L flasks, were monitored during 60 days of growing. The harvest was taken into place on the day 61. Harvested microalgal biomass was freeze-dried for 4 days.

The microalga *Arthrospira platensis* was cultured in sterile Zarrouk medium [26], in 5 L flasks, for 20 days. The harvest was taken into place on the day 21. Harvested microalgal biomass was freeze-dried for 3 days.

Phaeodactylum tricornutum was previously cultured [24] in 20 L plastic carboys containing sterile Walne medium [27] and silicate, prepared from natural seawater for approximately 14 days [28,29]. Harvested microalgal biomass was freeze-dried for 3 days.

To extract lipids from the dried biomass, a modified Bligh and Dyer method was applied, as described in [24], using an amount of 100 mg of lyophilized biomass and a proportion of chloroform, methanol and distilled water of 1:2:0.8 (v/v), respectively, in the first extraction. For the second round of Bligh and Dyer extraction it was used a ratio of 2:2:1.8 (v/v) of chloroform, methanol and distilled water respectively. Additionally, a Matyash based lipid extraction was performed as a standard protocol, by using 100 mg of lyophilized biomass. For both methods, the lipid layer was carefully recovered after solvent extraction, and transferred into another pre-weighted glass tube, using a glass Pasteur pipette. After solvent evaporated at room temperature, the extracted lipids were gravimetrically weighted in order to estimate the total lipid content. The samples were extracted in triplicates. Chloroform, methyl tert-butyl ether (MTBE) and methanol of analytical grade (Merck) were used in this study.

4. Results and discussion

In order to investigate the lipid content and accuracy of the extraction during the measurements, the samples were analyzed in triplicate for each extraction method. The results in Table 1 show the percentage of the extracted lipids recovered.

Table 1. Lipid yield obtained for different microalgae strains comparing two extraction methods.

Microalgae	Bligh & Dyer (%)	Matyash (%)
Chlorella zofingiensis	26.68 ± 1.96	34.06 ± 1.12
Phaeodactylum tricornutum	13.00 ± 1.57	13.59 ± 0.43
Arthrospira platensis	2.15 ± 3.89	3.89 ± 3.60

Furthermore, Fig. 1 depicts two steps during the lipid extraction procedure by Matyash approach. Fig. 1a shows the mixtures being incubated at room temperature using a shaker, after adding MTBE solvent. While Fig. 1b depicts how the lipids are extracted before evaporated the solvents.

Reportedly, Matyash et al. [21] approach has resulted in extracting more stored lipids in average, comparing to the modified Bligh and Dyer method [18]. It should be noted that since Matyash approach employs nontoxic solvent, it can prevent further hazard consequences during the extraction procedure. Further analysis of the recovered lipids must be performed to compare their composition. Besides, the lipid extracted via Matyash approach can be collected easier than the method proposed by Bligh and Dyer, since the extracted lipids consists of an additional layer on top of the mixture. This is in contrast with the Bligh and Dyer method in which the layer containing the lipids is settled as the bottom layer.

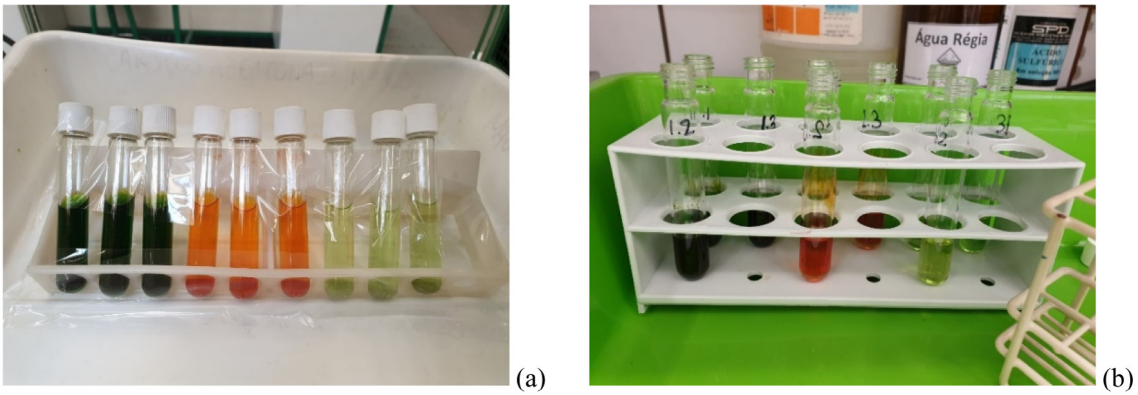

Fig. 1. Two consecutive stages of lipid extraction of three microalgal species (*Phaeodactylum tricornutum*, *Chlorella zofingiensis*, and *Arthrospira platensis*, respectively from left to right) via Matyash approach. Note that for each strain, three samples have been prepared. The samples are shown: (a) during the incubation in shaker, and (b) after collecting the top layer containing lipid and solvents (MTBE and methanol).

5. Conclusion

Lipids extraction from microalgal biomass can be accomplished using various mechanical or chemical approaches, which are more interesting since they are fast, easy and achievable in less time. However, application of toxic solvents increases the hazard for both the environment and the worker personnel health. In this research it was studied two chemical extraction methods, Bligh and Dyer and Matyash, being the former one of the most frequently used for lipids quantification in microalgae. Experimental results showed that the application of MTBE, instead of chloroform, could result in the increase of the extracted lipid, while it can decrease the chemical hazards by replacing chloroform with a nontoxic solvent. Briefly, experimental results obtained by the two approaches showed the potential of hazard-free solvents during the lipid extraction procedure.

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