

Comparison of Cell Disruption and Lipid Extraction Methods for Improving Lipid Content of *Schizochytrium* sp. S31

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Abstract

Downstream processing steps required to obtain lipid from microalgal biomass once large scale production process is completed. Different types of cell disruption and extraction methods have been used in the literature to recover microalgal lipids. According to our results, ultrasonication with hexane method increased the total lipid yield significantly with clear appearance. Sonication resulted in a 1.4-fold increase in lipid yield when compared with solvent alone. Soxhlet extraction and incubation method were also compared. Because of the soxhlet type of lipid extraction apparatus is not suitable for the extraction of thermolabile biological products, incubation method which is also known as cold extraction was used for lipid extraction. However, these traditional lipid extraction methods use large amounts of solvents that are mostly toxic. Supercritical extraction has also been employed since it does not use toxic solvent. Based on contour plot analysis, 30.2% lipid yield was obtained under optimum extraction conditions.

Keywords: Cell distribution, lipid extraction, *Schizochytrium* sp. S31

INTRODUCTION

Cell disruption is a key step in influencing lipid extraction yields [1,2,3]. Therefore, applying an appropriate cell disruption method is essential for lipid extraction. The most efficient cell lysis method has not been determined yet for microalgae due to cell wall variations among the species [4]. Sonication (ultrasound), high-pressure homogenizers, grinding, enzymatic reactions (protease, alcalase), chemical hydrolysis (NaCl, CTAB) are most known cell disruption methods applied for microalgae. The cell wall properties of the microalgae play a critical role in the extraction of lipid [5, 6]. The ideal cell disruption method should lyse the cell wall to maximize the lipid yield and can be used in large scale. It should also not cause any contamination to product and hinder further steps of the process [7].

Neutral lipids are generally known as storage lipids such as TAG and extracted with non-polar solvents such as hexane, chloroform, benzene, diethyl ether. Neutral lipids interact with their long hydrophobic fatty acid chains with Van der Waals attraction. Therefore, non-polar lipids in cytoplasm come together and form globules [8,9]. Polar lipids are generally associated with cellular membrane and dissolve in polar solvents such as ethanol or methanol. Either organic solvent (hexane) or supercritical fluid is used to extract microalgal lipid. Extraction solvent extracts the cellular and membrane lipids out of the matrices during lipid extraction. Extraction temperature and time are the critical factors for lipid extraction. Either soxhlet or incubation method is used when organic solvent is applied as extraction solvent. Soxhlet lipid extraction was originally designed for continuous extraction of analytes from a solid into an organic solvent. Different types of solvents and extraction methods

have been used in the literature to recover microalgal lipids. Hexane, methanol, ethanol, isopropanol are the typical solvents used for lipid extraction.

MATERIALS and METHODS

Different physical and chemical cell lysis methods were examined for cell lysis to increase the total lipid content. Both physically lysis methods such as high frequency sound waves (sonication), french press and chemical lysis methods such as CTAB and SDS were performed and compared. Different lipid extraction methods such as soxhlet, incubation and supercritical fluid extraction method were also compared.

High frequency sound waves (sonication)

The sound waves are delivered using an sonicator (Ultrasonic Homogenizer, Model 3000, 115V/60hz) with a vibrating probe. Sample were sonicated 50W for 15min in hexane with multiple short bursts (On/Off per 30sec). Biomass-hexane mixture is placed into ice while sonication process to prevent excessive heating.

French press homogenization

Cell biomass is suspended in Tris-EDTA solution. 3ml of the sample is placed into French press (French pressure cell press, ThermoSpectronic) and high pressure is applied by pressing the sample with piston. When the sample is forced to pass through a tiny hole in the press, most of the cells are lysed. Two runs at 10 MPa were conducted for each sample.

CTAB method

50 ml of freshly prepared extraction buffer [100 mM Tris-Cl, pH 8.0, 25 mM EDTA, 1.5 M NaCl, 2.5% CTAB, 0.2% β-mercaptoethanol (v/v) and 1% Polyvinylpyrrolidone,

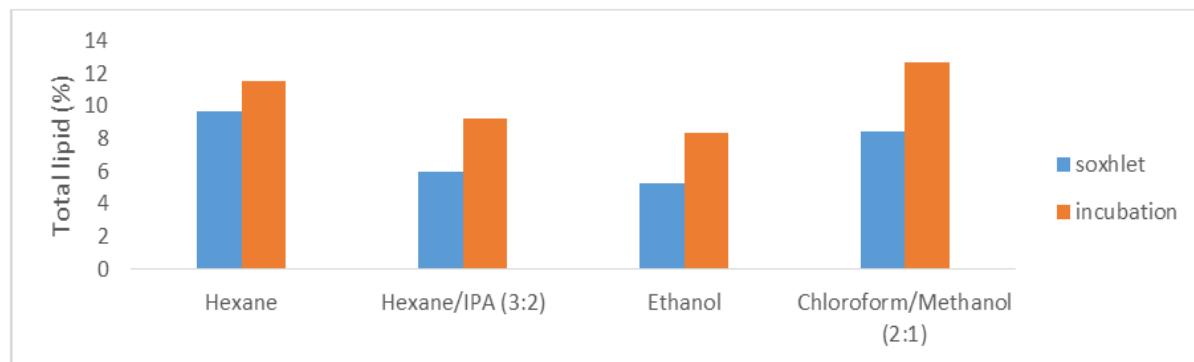


Figure 1: Comparison of soxhlet and incubation methods with different solvents

MW 40,000 (PVP) (w/v)] were added to 5g of cell pellet and mixed by inversion. The mixture was incubated at 60°C in a shaking water bath (100 rpm) for 30 min.

SDS method

50 ml of lysis buffer [10 mM Tris-Cl, pH 8.0, 10 mM EDTA, 2% SDS and 100µg/ml proteinase K were added to cell pellet and mixed. The mixture was incubated at 65°C in a shaking water bath (100 rpm) for overnight

Alcalase method

Alcalase® CLEA ≥5 U/g (Sigma) were used for enzymatic cell lysis protocol. It added at 1% (w/w of fresh algae) level to biomass-buffer complex. The flasks were incubated at 37°C shaker bath for 3 hours.

Solvent based lipid extraction and solvent free supercritical fluid lipid extraction method were performed for lipid extraction from *Schizochytrium* sp. S31. Hexane, hexane-IPA, Ethanol, methanol-chloroform solvents were examined with both soxhlet and incubation method for lipid extraction.

Soxhlet extraction

Soxhlet lipid extraction was designed for continuous extraction of lipids from a seed and/or cell into an organic solvent. Solvent containing flask is heated to evaporate hexane. Vapor rise in the larger outside tube and then diffuse into sample containing thimble. Extracted lipid return to the solvent containing flask. This circulation continues for generally 6 hours. Soxhlet apparatus was set and 5g dried biomass subjected to cartridge. Hexane is filled into the solvent vessel. Lipid extraction was performed at 110-130 °C for 6 hours.

Incubation method

5g dried biomass was dissolved in 100ml solvent containing erlenmeyer and incubated at 25°C, 225rpm for 6 hours.

Supercritical fluid lipid extraction

Supercritical liquid extraction (SFE) generally uses carbondioxide as a solvent at high pressure to extract lipid and/or nutraceutical products with higher selectivity in shorter extraction times. 5 g of freeze dried microalgae biomass was packed into a stainless steel extraction vessel. The system consisted of an extractor with an internal volume of 24 ml. CAMO Software AS. (Version 10.3, Norway) was used to perform the experimental design and statistical analysis.

RESULTS and DISCUSSIONS

Physical, chemical and biological lysis methods were examined and compared with regard to % lipid content. Comparative results of different cell lysis and lipid extraction methods were shown in Table 1.

Table1: Comparison of different cell lysis methods and solvents for lipid extraction

	No lysis	Physical		Chemical		Biological	
		French Press	Ultrasonication	CTAB	SDS	Alcalase	
Hexane	24	32	34,5	12,3	13,4	11,4	
Hexane/IPA(3:2)	19,5	28	29	11,6	10,4	17,5	
Ethanol	17	24,4	26,5	9,4	15	11	
Chloroform/Methanol (2:1)	24,8	33,2	35	14,6	15,7	17,7	
SFE	30,2	-	-	-	-	-	

According to results, neither chemical nor biological method was effective for cell lysis as the low amount of lipid was extracted. The highest lipid extraction yields were obtained using sonication and french press cell disruption together with hexane extraction from *Schizochytrium* sp. S31 with 34,5% and 32% lipid yield, respectively. After optimization of pressure, temperature and time variables of SFE, 30.2% lipid yield was obtained.

Different cell disruption techniques show different efficiency and effects on lipid yield [10]. Ultrasonic pretreatment exhibited the best results as indicated by Chen and Oswald (1998) in previous studies, where lipid yield was improved by up to 33% [11]. Both sonication and high pressure homogenization (French press) could be useful methods for disruption of *Schizochytrium* sp. Solvent based extraction has been used in numerous studies; however, heat treatment can be detrimental to the sensitive bioactive components.

Different lipid extraction techniques were also compared to obtain higher amount of lipid. Soxhlet extraction and incubation method were compared for lipid extraction. Comparative results of soxhlet and incubation with different solvents were shown in Figure1.

The soxhlet type of lipid extraction apparatus is not suitable for the extraction of thermolabile biological

products, as the extracted matter is subjected to the boiling temperature. Therefore, incubation method which is also known as cold extraction was used for lipid extraction. Although chloroform/methanol extraction gave the best results for lipid extraction, appearance of the lipid was not clear suggesting that unwanted molecules were co-extracted. Therefore, hexane was used for the further analysis.

As a conclusion, improvement of downstream processes was critical step in this study. Each steps has to be performed to obtain high quality of lipid and reduce the cost of downstream processing [12, 13]. Alternative cell harvesting, drying, lysis and solvent based extraction methods including SFE of *Schizochytrium* sp. S31 microalgae were investigated and compared. Extraction of microbial lipid is mainly conducted with solvents such as hexane, coupled with mechanical disruption techniques [14]. According to the cell lysis and lipid extraction results, the highest lipid extraction yields were obtained using sonication and french press cell disruption together with hexane extraction from *Schizochytrium* sp. S31 with 34.5% and 32% lipid yield, respectively. Ultrasonication with hexane method increase the total lipid yield significantly with clear appearance. Sonication resulted in a 1.4-fold increase in lipid yield when compared with solvent alone. Araujo et al. (2013) showed that ultrasonic distribution of *C. vulgaris* resulted in the highest lipid extraction, suggesting a favorable potential for biodiesel production [1]. Another research with *C. Minutissima*, *Thalassiosira fluviatilis* and *Thalassiosira pseudonana* indicated that sonication-assisted method with n-hexane was efficient method for lipid extraction in these strains [15]. Solvent based soxhlet extraction has been used in numerous studies. However, heat treatment can be detrimental to the sensitive bioactive components. SFE is an alternative to liquid extraction using solvents such as hexane or chloroform. There will always be some residual solvent left in the extract and matrix, and there is always some level of environmental contamination from their use. In contrast, carbon dioxide is easy to remove simply by reducing the pressure, leaving almost no trace, and it does not require heat treatment. SFE extraction is a promising green technology that can potentially be used for food and nutraceutical application [16,17]. The effect of extraction temperature, pressure and time of SFE on the lipid yield and %DHA amount were investigated by using RSM. According to SFE results, pressure and temperature has significant effect ($p < 0.05$) on total lipid yield and DHA concentration. It is shown that at constant temperature, lipid yield and DHA concentration increases with higher pressure whereas decreases with higher temperature [18]. Based on contour plot analysis, optimum extraction conditions were found to be 425 bar pressure at 40.5°C for 97.5min. After optimizing SFE conditions, 30.2% lipid yield was obtained. Compare to traditional extraction, SFE has advantages to get solvent free value-added extracts.

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