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Comparison of cell disruption methods for improving lipid extraction from *Porphyridium cruentum*

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Abstract

Cell disruption and extraction processes are very important downstream processing steps in microalgae-based in industrial bioprocesses. Each type of microalgae has a varied cell wall structure, so it is necessary to optimize an effective and economical cell disruption method to maximize the extraction process. This study aimed to evaluate several cell disruption methods, including: osmotic shock method (NaCl 20% w/v), acid method (HCl 3M), and microwave (450W, 5 min) for lipid extraction for *Porphyridium cruentum* biomass. Lipid extraction was carried out using n-hexane as solvent. Among several tested methods, the highest yield of lipid extraction was obtained by using acid method (8.15 ± 0.12% w/w), while the microwave method only produced lipids of 2.79 ± 0.37% w/w, and the lowest yield was obtained by using osmotic shock (1.07 ± 0.44 % w/w). The acid method is a very simple method for disruption of *P. cruentum* cells, because it does not involve the use of expensive equipment. Therefore, this method is considered efficient and economical for use in the extraction process of lipid or other biomolecule compounds.

Keywords: cells disruption method*;* extraction*;* lipids*; Porphyridium cruentum*

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Introduction

Microalgae are promising microscopic plants in the industrial sector because of their potential to produce several metabolites, such as lipids, carbohydrates, bioactive compounds, pigments, and enzymes (Foo, et al., 2015). Compared with higher plants, microalgae have several advantages, namely have high growth speed and productivity, can be cultivated in various environmental conditions, do not compete with fertile agricultural land, and have high oil productivity (20 – 50% w/w biomass dry) (Singh & Dhar, 2011). Therefore, due to their several advantages, microalgae have much interest in lipid production. Biodiesel has been known as an environmentally friendly fuel that produces safe gas emissions. Microalgae are included in autotrophic organisms which can carry out photosynthesize, the unicellular structure of microalgae can convert solar energy into chemical energy so that it is very suitable to be used as an alternative to produce biodiesel (Chisti, 2007). It is estimated that microalgae are more effective in producing 200 times more oil than other oil-producing plants such as palm oil at their best conditions. Extraction of oil from microalgae is a decisive step in increasing the yield of vegetable oil, so an effort is needed to maximize the oil extraction process (Purwanti, 2014). The method commonly used to extract microalgae oil is to use a non-polar solvent such as *n*-hexane (Magota et al., 2012).

To maximize the oil extraction, the microalgae cell walls has to be breakdown to come out the oil from the cells and allow it to contact with the solvent. Cell disruption and extraction processes are very important downstream processing steps in microalgae-based industrial bioprocesses. Each microalgae has a varied cell wall structure, so it is necessary to optimize an effective and economical cell breakdown method for maximizing the extraction process. This study aimed to evaluate several cell disruption methods, including: osmotic shock method (NaCl 20% w/v), acid method (HCl 3M), and microwave (450W, 5 min) to increase lipid extraction from *Porphyridium cruentum* biomass.

Methods

Porphyridium cruentum was obtained from the Biochemistry Laboratory of the Bandung Institute of Technology, Bandung, Indonesia, and cultivated in 1 liter transparent glass bottle filled with sterile seawater using Walne medium (Anderson, 2004). The culture conditions were pH 8, salinity 25 ppt, room temperature, light intensity 3,000 lux, photoperiodism light:dark = 24:0. The initial cell density used was 1×10^{-6} cells/ml. When the culture reached stationary phase

at day 10, the biomass was harvested by using a flocculation technique with NaOH at pH 10, and filtration to get thick algae paste. Then the algal paste was rinsed with distilled water to remove residual salts, and then freeze-dried.

Each of 0.4 g of dry algal biomass was broken down using three methods, namely: 1) the acid method using 3M HCl and heated at 80^{o}C for 1 hour; 2) osmotic shock method using 20% w/v NaCl and vortexed for 1 minute, then incubated for 48 hours; and 3) microwave at 450W for 5 min. Cell slurries from the three treatments were subjected to oil extraction. In brief, the biomass suspension was mixed with *n*-hexane with a ratio of biomass : solvent = 1 g : 10 mL, vortexed for 5 min, and centrifuged. The supernatant was transferred to preweighed flasks. Remaining biomass was re-extracted 3 times with the same solvent, and the supernatant was collected in the same flasks. Thereafter, the flasks were then placed in a hot air oven for complete evaporation of the solvent and were weighed again. The total lipid was calculated after obtaining the differences of final and initial flask weights. The lipid content was defined as dry weight ratio of extracted lipids to biomass. The oil extraction yield (%, w/w) was determined by following formula:

$$\text{Oil extraction yield } (\%) = \frac{W_o}{W_b} x100 \qquad (1)$$

where, W_o is weight of extracted oil (g), and W_b is weight of algal biomass (g).

Results and Discussion

Each type of microalgae has different biochemical composition cell walls. Various kinds of cell disruption methods have been carried out to determine the most effective cell disruption method to increase lipid extraction from various types of microalgae (Breddy et al., 2015; Rakesh et al., 2015; Ramola et al., 2019). In this study, three different cell disruption methods were used to extract lipids from *P. cruentum* biomass, namely acid method (HCl 3M), osmotic shock (NaCl 20% w/v), and microwave (450 W, 5 min). The disrupted cell wall of *P. cruentum* will easily release its intracellular components. The amount of total lipid extracted from *P. cruentum* was considered as an indicator of the efficiency of the cell disruption method used. Cell disruption will affect the amount of lipid that will be extracted (Wang et al., 2015). The results showed that the extraction of lipids from microalgae biomass which was broken down by the acid method produced the highest lipids, reaching 8.15 ± 0.12% w/w, compared to the microwave method (2.79 ± 0.37% w/w).) and osmotic shock (1.07 ± 0.44% w/w), as shown in Figure 1. The results of statistical analysis using one-way ANOVA showed that the lipid yields of the three cell disruption methods were significantly different (P<0.05).

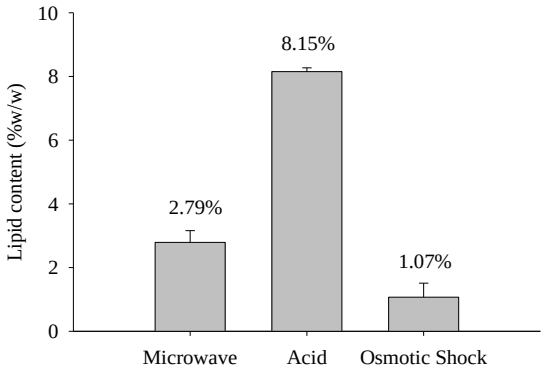

Figure 1. Efficiency of lipid extraction by using various cell disruption methods from *P. cruentum*.

Among the cell disruption methods in this study, the acid method using 3M HCl is the most efficient for *P. cruentum*, because the cell walls of *P. cruentum* contain very complex polysaccharides (Setyaningsih et al., 2013). The acidic conditions are able to completely hydrolyze constituent polysaccharides. Another advantage of this acid method is that it is easy, inexpensive and does not require special equipment and large amounts of energy. The difference in the process of separating lipids from the remaining extracted biomass can be seen from Figure 2.

The cell wall *of P. cruentum* is composed of sulfated polysaccharides in the form of a gel with acidic characteristics and lacks cellulose microfibril components (Gujar et al., 2019). This sulfated polysaccharide can be completely hydrolyzed by acid, so that there is no visible polysaccharide gel in the middle layer in the treatment with the acid method (Figure 2A). This causes the non-polar phase containing lipids and the polar phase containing non-lipid compounds to separate properly. Laurens et al. (2015) reported that the use of dilute acid can assist the extraction of lipids up to 97% w/w of the total use of n-hexane. This can make the cell walls of microalgae completely hydrolyzed.

In contrast to the microwave and osmotic shock methods, the polysaccharide gel is still visible in the middle because these methods are not able to completely hydrolyze the polysaccharides that make up the cell wall. It causes the polar and non-polar phases to not separate properly (Figures 2B and 2C) and makes it difficult to extract the lipids. Lee et al. (2010) suggested that the cell disruption method using a microwave oven gave the highest yield for microalgae *Botryococcus* sp. The different results from previous studies may occur due to the different types of microalgae used. Different types of microalgae cause different characteristics of one microalgae to another. The cell wall structure of microalgae has unique characteristics based on the growth phase of each microalgae species, resulting in differences in thickness, hardness and chemical composition.

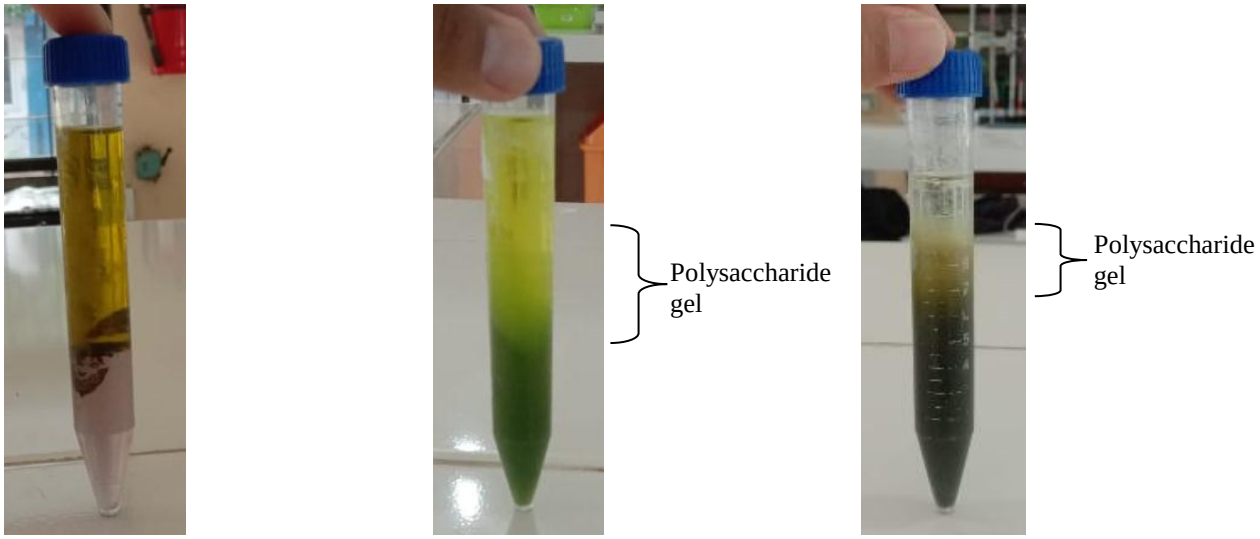

Figure 2. Differences in the separation of polar and non-polar phases from three cell disruption methods: (A) acid method, (B) microwave method, (C) osmotic shock method

Table 1
A comparison of cell disruption methods for total lipid extraction from different microalgae

No.	Cell disruption method used	Microalgae	The most efficient method	Lipid yield (% w/w)	References
1	Grinding, vortex with zirconia beads, osmotic shock, waterbath, sonikasi, shake mill with zirconia beads	*Schizochutrium* sp. S31	Osmotic shock	48.7	Byreddy et al. (2015)
		Thraustochytrium sp. AMCQS5-5	Osmotic shock	29.1	
2	Osmotic shock, microwave oven, autoklaf, pasteurisasi	*Chlorococcum* sp. MCC30	Microwave oven	24.12	Rakesh et al. (2015)
		Botryococcus sp. MCC32	Microwave oven	48.33	
		Chlorella sorokiniana MIC-G5	Osmotic shock	45.86	
		Botryococcus sp. MCC31	Osmotic shock	36.18	
3	Autoclave, bead beating, microwave, sonikasi, osmotic shock	*Botryococcus* sp.	Microwave oven	28.6	Lee et al. (2010)
		Chlorella vulgaris	Autoclave	11	
		Scenedesmus sp.	Microwave oven	11.5	
4	Microwave, HCl 3M, osmotic shock.	*Porphyridium cruentum*	HCl 3M	8.15	This study

Different results were also reported by Byreddy et al. (2015) that the highest lipid yield in freshwater microalgae *Schizochytrium* sp. and *Thraustochytrium* sp. obtained by disrupting cells using the osmotic shock method. However, in this study, the osmotic shock method actually obtained the lowest lipid yield. This was due to *P. cruentum* is a marine microalgae that grows under conditions of high osmotic pressure, so the osmotic pressure inside and outside the cell is almost the same and ultimately the cell cannot be broken. A comparison of several cell disruption methods for total lipid extraction from different microalgae can be seen in Table 1.

Table 2
Advantages and disadvantages of using the cell disruption methods

Cell disruption method	Advantages	Disadvantages
Microwave	• Cell disruption faster	• Use a lot of electrical energy
Osmotic shock	• Requires only salt water to breakdown cells • Requires longer time for cell disruption	• Cannot hydrolyze polysaccharides in *P. cruentum* cells, so they are not effective in lipid extraction
Acid	• Very suitable for use on *P. cruentum* • The cost used is cheaper	• The residual acid waster needs to be neutralized before being discharged into the environment

The efficiency of the cell disruption method can be seen based on the advantages and disadvantages of using the method used, as shown in Table 2.

Conclusion

The acid method is the most effective cell disruption method for the extraction of lipids from *Porphyridium cruentum.* This method is also very simple because it does not involve the use of expensive special equipment and does not require high energy consumption, so it is economical to use in the process of extracting lipids or other biomolecular compounds from *P. cruentum*.

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