THE EXTRACTION OF OIL FROM *Nannochloropsis* sp. MICROALGAE USING ULTRASONIC AND FERMENTATION AS CELL DISRUPTION

EKSTRAKSI MINYAK MIKROALGA *Nannochloropsis* sp. MENGGUNAKAN ULTRASONIK DAN FERMENTASI SEBAGAI METODE PEMECAHAN SEL

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ABSTRAK

Alga dapat menjadi sumber bahan baku biofuel yang menjanjikan, tetapi data mengenai kadar lemak alga berbeda-beda karena metode ekstraksi yang digunakan sangat bervariasi dan jumlah lipid tersebut sering tidak membedakan diantara jenis lipidnya. Salah satu jenis alga yang dapat digunakan adalah *Nannochloropsis* sp. Dua metode pemecahan sel yang berbeda, yaitu ultrasonik dan fermentasi yang diikuti oleh ekstraksi maserasi menggunakan campuran ethanol/heksana (1:1,v/v) sebagai pelarut ekstraksi diteliti untuk mengetahui efektivitas metode tersebut dalam mengekstrak lipid mikroalga *Nannochloropsis* sp. Parameter yang divariasikan pada proses ultrasonik adalah waktu kontak dan amplitudo. Pada proses fermentasi, parameter yang divariasikan adalah perlakuan hidrolisis, yaitu *Dillute Acid Pretreatment* (DAP), hidrolisis enzimatik, dan DAP dengan hidrolisis enzimatik. Hasil penelitian menunjukkan bahwa proses ultrasonik diikuti oleh ekstraksi maserasi adalah metode yang paling efektif untuk ekstraksi lipid *Nannochloropsis sp*. Kondisi yang paling ideal terdapat pada amplitudo 40 % dan waktu kontak 5 menit dengan kadar lemak 63,59 % berat kering.

Kata kunci: *dillute acid*, ekstraksi lipid, fermentasi, *Nannochloropsis* sp., ultrasonik

ABSTRACT

Algae are a promising source of biofuel but claims about their lipid content can be ambiguous because extraction methods vary and lipid quantitation often does not distinguish between particular lipid classes. One of algae types that meet this condition is Nannochloropsis sp. Two different cell disruption methods,i.e ultrasonic and fermentation followed by maceration extraction using a mixture of ethanol/hexane (1:1, v/v) as a solvent extraction were studied for their effectiveness in extraction of algae lipids from Nannochloropsis sp. Contact time and amplitude were varied in ultrasonic process. Hydrolysis treatment was varied in fermentation process, i.e Dillute Acid Pretreatment (DAP) hydrolysis, enzymatic hydrolysis and DAP with enzymatic hydrolysis. The result showed that ultrasonic process followed by maceration extraction was more effective for Nannochloropsis sp. lipid extraction. The most ideal treatment was at amplitude 40% and contact time 5 minutes with lipid content of 63.59% of dry weight.

Keywords: *dillute acid, fermentation, lipid extraction, Nannochloropsis sp., ultrasonic*

I. INTRODUCTION

Microalgae are microscopic plants that grow in water, can produce high oil content in a range of 20-50% dry mass of biomass and are capable of doubling their biomass in a period of 3.5 h (Chisti 2007). *Nannochloropsis* sp. is a unicellular, non motile, of about 2-5 µm cell size, golden green algae belonging to the class *Eustigmatophyceae* (El

Nabris 2012, Rodolfi et al. 2003). This microalgae is one of the most interesting phytoplankton in the field of marine biotechnology because it represents a valuable source of various natural products which have several aplication. Tropical marine microalgae are very interesting subjects of investigation as they produce various types of natural oils (triacyglycerols, TAGs; and fatty acids, fatty acids either in saturated, unsaturated, or polyunsaturated) as feedstocks for biodiesel (fatty acid methyl ester, FAME), Chloropyll, and carotenoid compounds. Lipids have been recovered from microalgae via a multitude of extraction methods described in the literature. Because of the nature of microalgae, regular extraction methods (used for example for food) may not be applicable. First of all, microalgae are single cells, which surrounded by an individual cell wall. Furthermore, they often contain ‘‘unusual’’ lipid classes and fatty acids differing from those in higher animal and plant organisms (Eline et al. 2011). For these reasons, it is necessary to have a profound look at methods for extraction of lipids from microalgae. Microalgae lipid extraction usually follows two steps: cell disruption and solvent extraction. Because of large variations in algae cell shape, size, cell wall structure and characteristic of algae lipids, various lipid extraction methods work differently on various algae species (Shen et al. 2009).

Biodiesel production from microalgae consists of the following steps including species selection, cultivation, harvest, and cell disruption. Cell disruption is particularly an important step as cell walls are generally thick and consist of multiple layers. Since cell wall and membrane present in algae are formidable barriers to permeation by extraction solvents, cells have to be disrupted prior to extraction, which enhances oil recovery. To make it more economically attractive, a feasible cell disruption method should be established to ensure a low operating cost, high product recovery, and high quality of the recovered lipids (Surendhiran and Vijay 20140). Cell disruption is performed to release intracellular products into the culture broth making them available for further separation processes, most notably chromatography or solvent extraction (Sander and Murthy 2012). Several methods for algae cell disruption have been evaluated including ultrasonication, bead beating, microwave (at 100°C), osmotic shock (with NaCl) and autoclaving (at 121°C) with varied results. Sonication has the advantage of being able to disrupt the cells at relatively low temperatures when compared to microwave and autoclave (Jeon et al. 2013). Ultrasound is a very effective processing method in the generation and application of nano-size materials. In general, ultrasonic cavitation in liquids may cause fast and complete degassing: initiate various chemical reactions by generating free chemical ions (radical); accelerate chemical reactions by facilitating the mixing of reactants; enhance polymerization and depolymerization reactions by temporarily dispersing aggregates or by permanently breaking chemical bonds in polymeric chains; increase emulsification rates; improve diffusion rates; produce highly concentrated emulsions or uniform dispersions of micron-size or nano-size materials; assist the extraction of subtances such as enzymes from animal, plant, yeast, or bacterial cells; remove viruses from infected tissue; and finally, erode and break down susceptible particles, including micro-organism (Tomas 2005).

The increment of oil from *Nannochloropsis* sp. microalgae was studied in this experiment through extraction process and there was several pretreatments before extraction process, i.e ultrasonic and fermentation which are function as processes for cell disruption. The aim of this study was to obtain oil from *Nannochloropsis* sp. microalgae using ultrasonic and fermentation as processes for cell disruption.

II. METHODOLOGY

A. Microalgae

The species of microalgae used in the extraction using ultrasonic and fermentation as processes for cell disruption was *Nannochloropsis* sp. obtanined from BBPBL (Balai Besar Pengembangan Budidaya Laut) Lampung.

B. Selection Process of Extraction Methods and Solvent Extraction

In selection process of extraction methods, several extraction methods were used, there are maceration, soxlet, soxlet followed by maceration and osmotic shock. Soxlet extraction is method of chemical solvent extraction. In this method, lipid from the algae are extracted through repeated washing, or

percolation, with an organic solvent under reflux in special glassware. Then, maceration is process of sample submerged with organic soluble in the room temperature (Suarsini & Subandi 2012). One more method is osmotic shock, a sudden reduction in osmotic pressure. After getting the best extraction methods, then the solvent type will be varied for the purpose of finding the best solvent extraction for cell disruption process.

C. Cell Disruption

1. Ultrasonic

500 g slurry of microalgae mixed with 500 mL aquades and then sonicated with varying amplitude. At each amplitude variation, contact time of ultrasonic process also varied. The contact time varied are 5, 10 and 15 minutes, while the amplitude varied are 40%, 50% and 60%. After the process, slurry was dried and then extracted by maceration using a mixture of ethanol/hexane (1:1, v/v) as a solvent extraction. The ultrasonic equipment that have been used is Qsonica Sonicator Q1375 with 20KHz frequency.

2. Hydrolysis and Fermentation Process

Hydrolysis treatment in this study are Dillute Acid Pretreatment (DAP) hydrolysis and enzymatic hydrolysis. Hydrolysis treatment was varied in fermentation process, i.e DAP hydrolysis, enzymatic hydrolysis and DAP with enzymatic hydrolysis. DAP performed using H_2SO_4 1% by 1,5% dry weight of microalgae and heated in autoclave at 130°C for 30 minutes. Enzymatic hydrolysis performed using enzyme mixture of Celluclast® 1.5L, Novozyme 188, Viscozyme® L, and Xylanase (Sigma, Cat. No. X2753) at pH ±6.

DAP was conducted using 1 Liter aquadest with 250 gram of microalgae slurry. Then, 10 mL sulphuric acid was added to slurry and followed by neutralization with NaOH 10 M until pH reached ±6. The process was carried out in an autoclave at 130°C for 30 minutes. The mixture was then cooled to room temperature. In the fermentation process, *C. beijerinckii* was used as bacterial culture. The fermentation were conducted in anaerobic chamber at 37°C for 72 hours.

After 72 hours fermentation process, the mixtures were autoclaved at 121°C for 30 minutes to stop the fermentation process. The mixtures were then cooled to room temperature. The solid fraction separated from the liquid, then subjected to extracted by maceration method using a mixture of ethanol/hexane (1:1, v/v) as a solvent extraction.

D. Extraction Analytical Procedures

After disruption cell process, then the algae were dried under sun light. Dry algae then crushed using blender. The dried algae next extracted using maceration method. A total of ±20 gram dried algae put into erlenmeyer with volume of 250 mL and added of ethanol/hexane (1:1, v/v) mixture. The erlenmeyer that containing disrupted algae cells and solvent was shaken on a reciprocating shaker (150 rpm/min) for 24 hours. After that, the suspensions were transferred to 50-mL centrifuge tubes. After that, the tube was centrifuge at 4000 rpm for 15 minutes to remove the algae solids. The fluid fraction were carefully collected and vaporized by rotavaporation and then dried in an oven at 70°C for 24 hours. Lipids left in the flask without solvent were weighed to calculate lipid content.

III. RESULTS AND DISCUSSION

A. Selection Process of Extraction Methods

The process of selecting the solvent is divided into two steps. The first step is the selection of extraction methods and after obtain the best extraction method, then the second step is varied the type of solvent extraction. The type of microalgae used was *Scenedesmus* sp. Although the types of microalgae that have been used different from the cell disruption processes (ultrasonic and fermentation) which is using *Nannochloropsis* sp. microalgae, but the obtained data would have same tendency.

Table 1
Comparison of lipid content of *Scenedesmus* sp. with variation of extraction methods

Method	Solvent	Lipid content (% of dry weight)
Maceration	Hexane and Ethanol (1:1,v/v)	6.36
Soxlet	Hexane	4.48
Soxlet followed by maceration	Hexane - Hexane/Ethanol (1:1, v/v)	4.98
Osmotic shock	HCl 5 M	2.23

1. Variation of Extraction Methods

Table 1 shows the lipid content of *Scenedesmus* sp. in several extraction methods, there are maceration, soxlet, soxlet followed by maceration and osmotic shock. The highest lipid content was occured in maceration process with value of 6.36% of dry weight. Its probably caused by diffusion process in maceration is better than the others processes.

2. Variation of Solvent Extraction

Extraction process that has been used is the maceration method using a mixture of hexane and ethanol as a solvent extraction by ratio of 1:1. The mixture of hexane and ethanol obtained from the best results in solvent extraction variation (see Table 2). From this table, the extraction solvent was varied, i.e mixture of hexane and ethanol (1:1, v/v), hexane and ether (1:1, v/v) and hexane only. The results of the research showed in Table-2. It can be seen that the solvent extraction using a mixture of hexane and ethanol by ratio of 1:1 produce the highest of microalgae lipid content with value of 5.710% of dry weight. Based on these results, then the mixture of hexane and ethanol as a solvent extraction used in this research.

Table 2
Comparison of lipid content of *Scenedesmus* sp. with solvent extraction variation

Method	Solvent	Lipid content (% of dry weight)
Maceration	Hexane and Ether (1:1,v/v)	5.573
Maceration	Hexane and Ethanol (1:1,v/v)	5.710
Maceration	Hexane	5.380

B. Cell Disruption Using Ultrasonic

Ultrasonication is another liquid-shear method of disruption. Ultrasound, sound of frequency higher than 15-20 kHz which is inaudible to the human ear, it is known to cause both inactivation and, at higher acoustic power inputs, disruption of microbial cells in suspension (Chisti & Moo-Young 1986).

The effect of contact time and amplitude to the oil yield using ultrasonic as a cell disruption method are shown in Figure 1. The content of the highest lipid for a time contact 5 minutes occurs in amplitude 40% (63.59% of dry weight), time contact 10 minutes in amplitude 60% (1.66% of dry weight), and time contact 15 minutes in amplitude 50% (63.53% of dry weight). From these data, the highest lipid content occur in contact time 5 minutes with amplitude 40% with value 63.59% of dry weight. Its value is higher than the control (36.86% of dry weight). Contact time of 5 minutes with amplitude 40% resulting in rupture of the cell walls and intracellular materials release to the liquid. Based on Jeon et al. (2013), ultrasonication has been commonly used for cell lysis and homogenization, and could be an effective treatment for breaking up the rigid cell envelopes of microalgae. Based on Chisti (2007), that the content of *Nannochloropsis* sp. lipid is 31-68% of dry weight, therefore the lipid content from this research accordance with the result of Chisti's research. The process of ultrasonic, dry algae and crude alga oil after ultrasonic process showed in Figure 2.

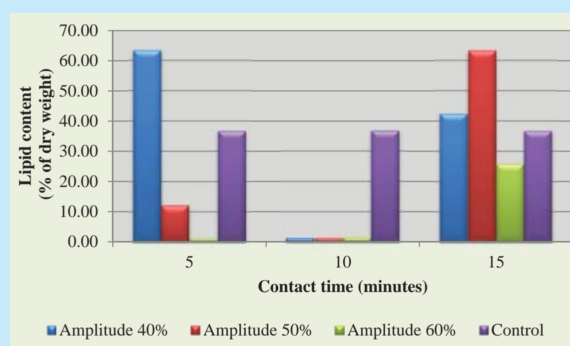

Figure 1
The effect of contact time and amplitude to the lipid content of *Nannochloropsis* sp. using ultrasonic as cell disruption method

Figure 2
Cell disruption using ultrasonic; (1) ultrasonic equipment; (2) dried algae and (3) crude alga oil

These results are initial research in cell disruption processes. The causes of lipid content in contact time

10 minutes less than 15 minutes cannot be identified yet. The influence of amplitude to lipid content also cannot be indetified too. Further investigation on influenced of amplitude and contact time to lipid content are needed to get the best cell disruption process condition using ultrasonic.

C. Cell Disruption Using Fermentation

The result shows (Figure 3) that fermentation with enzymatic hydrolysis process resulted the lowest lipid content than other treatments, which is 2% of dry weight. Its probably because there was no adding DAP treatment in cell disruption process. From this experiment, DAP treatment probably have a significant effect for microalgae cell disruption before fermentation process. Figure 3 shows that the fermentation with DAP treatment with the addition of enzymes and *C. beijerinckii* bacteria resulted in the highest lipid content, which is 11.52% of dry weight. Its probably caused when intact cells are disintegrated during cell disruption, intracellular lipids are liberated from the cellular structures and released into the surrounding medium. Furthermore, the acid hydrolysis and enzyme breaks down lignin which is the most powerful part of the cell wall, making the cell more easily extracted.

Surendhiran & Vijay (2014) was investigated the utility of various pretreatment (acid lysis, enzymatic, thermal, microwave, ultrasonic treatment and pretreatment with 40% NaCl solution) protocols to extract lipids by cell disruption. *Nannochloropsis oculata* microalgae was used for this research. It results that acid hydrolysis proved to be the appropriate method as cell disruption. Eventhough the *Nannochloropsis* microalgae type is different, but the result of this research approach to Surendhiran's result. With the hydrolysis to obtain glucose, it is expected that oil extraction microalgae for biodiesel will be high due to cell wall carbohydrates and lipids will be broken and easily to extracted. The mixtures of the liquid and dry microalgae after fermentation process showed in Figure 4.

IV. CONCLUSION

Extraction process is was influenced by initial cell disruption and solvent extraction Using ultrasonic as cell disruption method before extraction

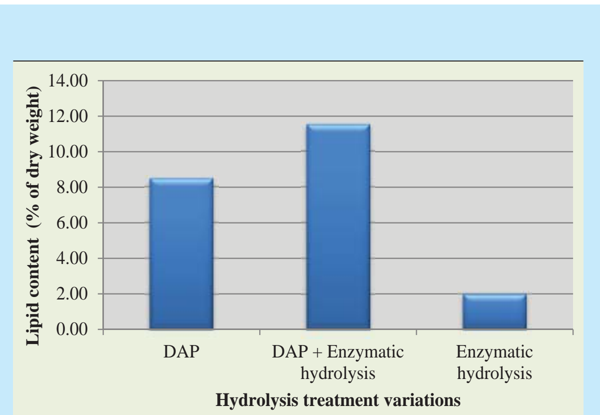

Figure 3
The effect of hydrolysis treatment followed by fermentation as cell disruption to the lipid content of *Nannochloropsis* sp.

Figure 4
(1,2) algae condition after fermentation process; (3) dried algae

process is one of the good method than fermentation process. It is clear from this work that cell disruption using ultrasonic under optimized conditions (amplitude 40%; contact time 5 minutes) can disrupt algae cells releasing high lipids (63.59% of dry weight) for biofuel production.

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