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THE USE OF ULTRASONIC TO INCREASE THE EFFICIENCY OF OIL EXTRACTION FOR MICROALGAE INDIGENOUS ISOLATES FROM POND GRESIK, EAST JAVA

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ABSTRACT
The prospect of microalgae biomass as a biodiesel is as a renewable energy. The microalgae biomass is aimed to substitute the petroleum. The identification of microalgae was conducted on twice which was derived from Gresik ponds consist of 29 species. On the other hand, there were 10 species which were resulted from laboratory test of Biology, Science Faculty, UM. Among the isolated, there are 2 species that faster growth rate, Chlorella vulgaris and Spirogyra sp. Generally, extraction of oil from microalgae biomass requires a long time. The aims from the research are to examine three methods of oil extraction from the two species of microalgae, the experimental laboratory techniques. Biomass of microalgae dry powder was extracted using the solvent n- hexane by three methods, Soxhlet, maceration and ultrasonic waves. On the acquisition of microalgae oil the yield equivalent, compared with extraction time from the three methods. The smaller time of microalgae oil extraction is the more efficient extraction. The results showed: (1) the use of ultrasonic waves can improve the efficiency of microalgae oil extraction. (2) The yield of algae oil which is extracted using the method Soxhlet, maceration, and extraction with the aid of ultrasonic waves in a row is 1.58%, 1.03%, and 1.77% by the time it takes for 18 hours, 8 hours, and 2.33 hours.

Keywords: Microalgae, oil extraction, ultrasonic wave

1. INTRODUCTION

Energy supply for petroleum has still not fulfilled in Indonesia. The consequence of this situation is that government has to import petroleum which costs up to 10 billion US$. To solve this problem, government tries to use an alternative energy, such as liquefied gas, coal, and nuclear energy. However, those alternative energies spend a lot of money and need high technology equipment. Petroleum substitution, on the other hand, was also conducted. Using non-conventional energy for instance solar energy, wind, water, and biomass through a simple technology was developed. Abdullah (1990) stated that there is a possibility to run out of oil and gas. Therefore, we need a substitute energy especially renewable energy as well as choose the best method on technology, social and economic. To fulfill future energy, government still depends on petroleum and nuclear energy. There will be a number of nuclear reactors if the nuclear energy is the only alternative way. However, the negative impact of reactor leaking would be faced and would endanger for living things and environment.

Regarding to find the renewable energy, biomass is the hot issue recently. Biomass from microalgae has great prospect as renewable energy to fulfill an energy demand. Indonesia, actually, has water potential that can be used as a medium for microalgae growth. Microalgae are organism that have habitat in fresh water and sea. Microalgae capable to change light energy to be chemical energy that restored as organic compound (protein, saccharide and lipid). Maintance of lipid has varians number for different species, there is a species has high enough (net weigh 16-40%). If we cultivate this species, we will get energy subtitute for nabaty. Microalgae cultivation is one of efford to make available organic energy as going concern nabaty fuel supplies that ready to use for long time. Microalgae cultivation suitable to development Indonesian agriculture because a microalga is resource that can found around us. So that Indonesian has biomass energy prospect to developt but there is a single problem, how to prosses biomass energy that can be use in simple way dan for any people who needed. This problem need more concern through research that try to find out how to cultivation microalgae in efective way (as a new founding of renewable energy) and how to analyze cultivation as field energy for some purpose that give advantage to the society. As a solution is a national program to cultivate microalgae to involve source energy in the future. This program is conduct to get intensive research activity for founding renewable and appropriate energy. Depend on cost, there is only government can get subtitute energy in the future. And villagers hard to get alternative energy to supply their needed because they didn't have enough money to research and found alternative energy. There is a good prospect of microalgae variety in many swamps or fishpond on East Java. They are: *Chlorella, Ankistrodesmus, Chlorogonium, Chlamdomonas nasuta, Pando-rina, Pediastrum, Scenedesmus, Ulothrix, Saurastrum, Ochromonas, Mallomonas caudata, Mitzseia, Navicula, Phacus, Euglena, Agmene-llum, Anabaena, Spirulina, Tetradinium minus, Cystomidium iners*, and *Peridium* (Sitoresmi, et.al. 2011). *Chlorococcum, Pediastrum, Oedogonium,Chlorella, Spirogyra, Sphaerocytis, Palmella, Scenedesmus, Ulothrix, Euglena, Phacus, Diatom, Navicula,Pleurogaster,Meris-mopedia, Oscillatoria* (Fitriana, 2010). In Microbiology laboratory-Faculty of Mathematic and Natural Science, State University of Malang there are seven microalgae variety had been isolating, *Chlorella,*

Chlorocoocum, Scenedesmus,Oedogonium(Divisio Chlorophyta), Pleurogaster, Navicula (Divisio Crhysophyta), and *Oscillatoria* (Divisio Cyanophyta) (Suarsini, 2011). Microalgae had been cultured on mixed medium, so need more accurate and more time to isolate each genus of microalgae. A problem in isolating is we didn't use specific medium culture for each genus. So in the first step of research we need to know specific medium for each algae. Some genus microalgae have successes in culture (although we still use mixed medium) as a stock in Microbiology laboratory- Faculty of Mathematic and Natural Science, State University of Malang.

Great potential must be harvest full optimal to fullfill society energy needed. So we must cultivate microalgae in efford to fulfill energy supplies and there is a potential of freshwater microalgae in some lake on East Java. So renewable can fulfill the energy needed for a number of years. To find out microalgae culture potential, research need to conduct to have oil extraction of *Chlorella vulgaris* and *Spirogyra sp*. And, to know characteristic of algae oil which is extracted using the method soxhlet, maceration, and extraction with the aid of ultrasonic waves. Isolating and selecting of freshwater microalgae, to find out characteristic of algae oil we must have propagation plan for product freshwater microalgae biomass that more inovative, effective, and efficient

2. METHOD

This research had experimental laboratory method that conduct to know ultrasonic wave to increase efficiency of algae oil extraction from *Chlorella vulgaris* and *Spirogyra sp* and to know characteristic of algae oil. Algae oil is characterized to determine its physical properties (density and refractive index) and chemical properties (acid number and iodine number). Chemical character analyzed with FBI- A01-03 methods.

This research have three steps, (1) make microalgae powder, (2) oil extraction from microalgae powder with several methods, there are Soxhlet, maceration and ultrasonic wave, (3) figured out characteristic of microalgae oil. Independent variable in this research is methods of oil extraction. Control variables are microalgae variety, *Chlorella vulgaris* and *Spirogyra sp*.

Dependent variable are yield of the microalgae oil and running-time of the three extraction methods.This research had been working for 3 years which include group research of lecturers and students as source of funding from D I P A 2009. There are 3 lecturers with a group of studies related to research projects on microalgae as renewable energy, and include some students to finish their thesis. We have continuous this program, they are include: isolation and selection of superior local microalgae; selection of medium culture; propagation in laboratory; planing and assemble small bioreactor for propagation in village; test and installation optimally; planning dan socialisation to whole villager; evaluation and complete research.

3. RESULTS

There are research result of extraction oil from *Chlorella vulgaris* and *Spirogyra sp*. shown in Table 1.

Table 1 Comparison Yield of Oil *Spirogyra sp*. through Ultrasonic, Soxhlet and Mace-ration Methods (Alfiati, 2010)

	Extraction Methods	Time	yield (%)
1.	Ultrasonic	10 minute	0,175
		30 minute	0,260
		50 minute	0,335
		70 minute	0,355
		90 minute	0,460
		110minute	0,505
		130minute	0,555
2.	Soxhlet	5400minute	0,360
		5760minute	0,465
3.	Maceration	3420minute	0,200
		3600minute	0,339

Note:
( ) = equivalent oil yield.

As shown in Table 1, equivalent yield of oil (± 0, 35 %) from three extraction methods had less time in ultrasonic wave. This research had same result of Lely (2010) that had yield of oil Chlorella vulgaris through soxhlet, maceration and ultrasonic methods are 1,5815%; 1,033%; and 1,777% with takes time 2,33 hours, 8 hours, dan 18 hours. Based on time, if we calculating ratio, ultrasonic is more efficiency 7614% than soxhlet and ratio ultrasonic is more efficiency 5043% than maceration Results between both researches shown that methods of ultrasonic extraction is greatly accelerate extraction processes. Because using an ultrasonic reactor. Ultrasonic waves are used to create cavitation bubbles in a solvent material. When these bubbles collapse near the cell walls from the algae, the resulting shock waves and liquid jets cause those cells walls to break and release their contents into a solvent.

Ultrasonication can enhance basic enzymatic extraction. The combination "sonoenzymatic treatment" accelerates extraction and increases yields (Hielscher, 2011). Then, Soxhlet extraction is method of chemical solvent extraction is. In this method, oils from the algae are extracted through repeated washing, or percolation, with an organic solvent such as hexane, under reflux in special glassware. The value of this technique is that the solvent is reused for each cycle. One more methods is maceration, process of sample submerged with organic soluble in the room temperature. The rule is the process with oil isolation because cell wall and cell

membrane of microalgae submerged will be broken as consequence differences inside and outside pressure. Secunder metabolism in sitoplasma will mixed with organic soluble. Selection of soluble for maceration process will increase effectively, according to their solubility natural compound. The compound extraction can b e a perfect process after we control time in submerged liquid. So, from three methods are shown that ultrasonic is the best to increases yields of algae oil. As shown that yields in Spirogyra sp 0.55% less than C. vulgaris 1.777%. According to result of extraction methods of both researchers, we'll choose ultrasonic wave extraction methods to get superior microalgae quickly because this methods more efficient than maceration and Soxhlet methods. The characterization of oil Spirogyra sp is their density 0,934 g/mL, refractive index 1,474, acid number 70, 7256 mg KOH/g, Iod number 3, 4153 gI_2/100g oil and saponification number 493,98 mg KOH/g (Alfiati, 2010). Otherwise, characteristic of C Vulgaris h a v e mass 1,535, acid number 34,1539 mg KOH/g, Iod number 149,394 gI_2/100g, and saponification number 83,668 mg KOH/g (Lely, 2010). Characterization oil result from both of microalgae shown there is a difference in quantity and quality. In physic, qualities of lipid are according to density and refraction index, in other wise chemical quality according to acid number, iod number and saponification number. Density is comparison between weight and their volume. Density depend on atomic weight or moleculer weight of compound that acording to their molecule density. More high their molecule density, more high density and their viscosity. Oil refraction index conduct to compare lenght of carbon chain to double bond total in lipid acid structure. Ketaren (1986) explain that refraction index can be used to predict number of double bond. More lenght carbon chain and their total double bond take effect more high their refraction index. Accor-ding to their physic haracteristic, microalgae oil of C. vulgaris has better quality than Spirogyra sp. Acid number in miligram KOH had to neutralize 1 gram of oil. This test conduct to know free acid lipid level in a spesific oil or lipid. If there is a low acid number, so their oil quality is high.

According to two researchers result shown that acid number of Spirogyra sp. higher than C. vulgaris, so we can had descript that C. vulgaris oil quality better than Spirogyra sp. because not easy to rancidity. Iod number shows weight of iod (I2) in gram that had bound with 100 gram of oil or lipid. The number of iod reserve show number double bond or their unsaturated oil. Glycerida with high unsaturated oil will get bond with large number of Iod. One moll of reserved iod equivalent with one moll of double bond. If we know iod number, so we will know their level of unsaturated oil or lipid (Parlan and Wahjudi, 2005). Based on iod number from both researchs shown that iod number of Spirogyra sp less than C. vulgaris number. So that lipid C. vulgaris have more double bond than in Spirogyra sp. Saponification number is the number of base needed to shed the oil. The higher number of saponification showed the longer of the C- chain oil. For the purposes of the longer C-chain of biodiesel will be difficult to burn. The results Lely (2011) research; based on the number of C. vulgaris lipid saponification, it can be selected as appropriate for the purposes of biodiesel. C. vulgaris is one of individual species of green algae differ considerably in their ecological preferences, ranging from broad spectrum organisms to species with very restricted habitats. Spirogyra occurs in a wide range of habitats, where it is typically attached to stable substratum (as periphyton) but also occurs as free floating mats (Lembi et al., 1988 in Barsanti, L. and Gualtieri, P., 2006) – derived either by detachment of from periphyton (vegetative propagation) or from benthic zygotes (sexual derivation). Spirogyra in streams from a wide range of biomes – including desert chaparral, temperate and tropical rainforests and tundra. Morphology of Spirogyra has cylindrical cells that are joined end to end to form an unbranched filament.

The cell walls are firm and have a thin film of mucilage on the outside, giving them a slimy feel. Chloroplasts have a helical shape and there can be up to 15 per cell. Numerous pyrenoids are present. The nucleus, often visible in live material, is in the centre of the cell. Cells may be between 10 and 160 µm in diameter and up to 590 µm long. (Bellinger, E.G and Sigee, D.C., 2010). Filaments fragment easily at the cross walls, each fragment growing into a new filament. Sexual reproduction in Spirogyra involves conjugation between cells of different filaments and results in the production of a resistant zygote. It is widely distributed in shallow ponds and ditches here it can form dense green masses. Can grow in large enough amounts in shallow open water treatment filters to cause blockage. C. vulgaris cells are spherical to subspherical with a single parietal chloroplast which nearly fills the cell. A single pyrenoid is present. Cells 2–10 µm in diameter common in nutrient-rich waters but easily overlooked because of their small size. Its small size can also mean that it can pass through traditional water treatment sand filters giving rise to colour problems in the treated water (Bellinger, E.G and Sigee, D.C., 2010). This cell surface consists of a simple or modified plasma membrane. The unit membrane is a lipid bilayer, 7–8 nm thick, rich of integral and peripheral proteins. Several domains exist in the membrane, each distinguished by its own molecular structure. Some domains have characteristic carbohydrate coat enveloping the unit membrane.

The carbohydrate side chains of the membrane glycolipids and glycoproteins form the carbohydrate coat. Difference in thickness of plasma membrane may reflect differences in the distribution of phospholipids, glycolipids, and glycoproteins (Barsanti and Gualtieri, 2006). Based on characteristic of microalgae oil, we select C. vulgaris, because it have more prospective oil

characteristic as biofuel. The selection of this species depends to acid number, more low more high quality of oil/lipid. Oil from microalgae extraction is collect from oil in cell level. The biomass of microalgae can be estimated by phospholipid fatty acid (PFLA) analysis. In the nature, we can found biomass as biofilm. In celluler level, lipid has important function of cell membran, line up in globulers, other part of the cell (Belinger and sigee, 2010). Lipid acid production depends on growth of microalgae, although both of them depend to medium composition and environment (such as carbon resource). Lipid production consists in bordered growth. In linear growth, stress cell had production much lipid. On the other hand, it has negative effect to DHA production. Highest quality of lipid occur if glucose used as carbon resource and if cell content dan lipid of cell in the low level (Barsanti and Gualtieri, 2006). This is same that nutrient limitation, especially N and Si, is well recognised to influence lipid content. For green algae, nitrogen deprivation was reported to increase lipid content. This conclusion supported by data of content lipid C. vulgaris that cultured in nutrient medium replete = 25% dw, but medium N deficiency = 42% dw (Griffiths and Harrison, 2009).

Biomass and lipid productivities of C. vulgaris under different growth conditions had investigated. While autotrophic growth did provide higher cellular lipid content (38%), the lipid productivity was much lower compared with those from heterotrophic growth with acetate, glucose, or glycerol (Liang et al., 2009). The yield of microalgae oil in both researches showing its lower level than earlier research. This condition can be explained by theory that culturing on laboratory had been doing in optimal. The condition had effect to microalgae that no stress, so this research only had little lipid. According to both research experiences, to selection of microalgae culture medium need to set up environment that can make microalgae in the stress growth, so we can get much more of lipid production. Based on research result that had cross check with the theory, we had prediction for next experiment, used laboratory propagation of C. vulgaris we will plan and assemble small bioreactor for propagation in rural. The expectation of this experiment can be solid model of cultivate microalgae so farmer society will have alternative way to increase their economic level in the future. Farmer society will get more benefit because algae can produce up to 300 times more oil per acre than conventional crops, such as rapeseed, palms, soybeans, or jatropha. The duration of the cell cycle, from daughter cell, through growth and division, to the next daughter cells, varies considerably between algal species. Under conditions of optimum temperature, nutrient supply, and light, duration depends primarily on cell size (Sigee, D. C. 2005). As algae have a harvesting cycle of 1–10 days, it permits several harvests in a very short time frame, a differing strategy to yearly crops (Chisti, 2007). Algae can grow 20 to 30 times faster than food crops. Otherwise, farmer society cans easily growth algae in land, because algae can be grown on land, for instance arid land, land with excessively saline soil and drought-stricken land.

4. CONCLUSION

According to analyse we figured some conclusion, they are: (1) The use of ultrasonic waves can enhance the yield of extraction microalgae oil in an efficient. (2) Species of microalgae that can be selected for propagation are C. vulgaris that the yield of algae oil which is extracted using the method of soxhlet, maceration, and extraction with the aid of ultrasonic waves in a row is 1.58%, 1.03%, and 1.77% by the time required for 18 hours, 8 hours, and 2.33 hours. (3) We recommend using nutrient-deficient culture media so as to increase the harvest of many lipid from cultured C. vulgaris.

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