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Ultrasound Extraction Assisted (UEA) of Oil from Microalgae (*Nannochloropsis sp)*

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Abstract

It has been well known that some of microalgae comprise oil and this kind of oil can be used as biofuel; one of algae types that meet this condition is *Nannochlopsis sp.* There are many methods can be used to extract the oil from plants. This study aims to investigate the use of ultrasonic wave which was combined with chemical solvents to extract microalgae oil. *N-Hexane*, and E*thanol* solvents were used by considering time and temperature varies in order to get the optimum condition. The result showed that solvent extraction which was assisted by ultrasonic has reduced the period of extraction time. The *n-Hexane* solvent with smaller polarity difference showed better extraction level comparing to the other solvents. Optimum condition for temperature and time for each solvent is 35^{O}C and 8 minutes for n-Hexane and 70^{O}C and 52 minutes for ethanol.

KEYWORDS: Microalgae, Nannochloropsis sp, ultrasonic extraction assisted, optimum condition, oil algae.

1. Introduction

Efforts to get biofuel to reduce fossil fuel dependency have become the focus of many researchers in many countries. Seeds of many plants have been recognized as the source of biodiesel oil [1]. However, in seeking for biodiesel fuel source, food and non food issue has become one significant issue in green energy discourse. There are many of the biodiesel sources which are derived from the seeds of the plants, and at the same time people need these plants for food supply. Therefore, there is an urge need to find a source of green energy that will not be a problem for the world's food supply. The use of microalgae to replace fossil fuel has become one focus of attention as the use of this plant is beneficial since it has lesser or nearly no effect to the world's food supply. There are three important parts of this plant, i.e. carbohydrate, protein and lipid [2]. It is believed that microalgae are more advantageous comparing with other biofuel sources [3]. Some of their benefits are they comprise higher oil intensity and they can be harvested in a very short time [5]. Extraction is one method used to obtain/squeeze oil from plant. Some of the well known extraction models are press, solvent, osmosis pressure, supercritical and ultrasonication [5, 6]. Ultrasonic is chosen in this study because of two reasons, the first it has low operational temperature, and second it has a relatively short operational time [7]. It is known that high temperature will comprise more but low quality oil [8] and conventional extraction takes a longer time [9]. Hitherto, the use of ultrasonic to assist extraction has been done to some seeds such as seeds of tobacco, fennel, peganum, woad, rose hip, sunflower, soybean, and rape [10]. Microalgae flour of *nannochloropsis sp* is used in this study.
Cavities resulted by ultrasonic sound trigger crash/collision among particles in the body of the cell, which furthermore increase the heat, then this heat break the cell, and finally release the oil from the cell. This extraction process is faster comparing with the traditional methods, because the contact surface area between solid and liquid phase is much greater, due to particle disruption talking place [11]. Two kinds of solvent, i.e. ethanol [8, 9] and n-Hexane [10] are used to extract microalgae assisted by ultrasonic energy. The ultrasonication process was conducted at different temperatures: 24^{o}C, 40^{o}C and 60^{o}C [10]. The efficiency of ultrasonic extraction (UE) is similar or even better than solvent extraction, but UE reduces the extraction time drastically. The objective of this research is to identify the ultrasonic effect in the extraction process using different solvents, temperature and extraction time as the parameter.

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2. Material and Methods

2.1 Material

The algae flour used is *Nannochloropsis sp* and has been obtained from Indonesian supplier. This flour was previously dried in room temperature. N-Hexane (BP Grade; 69^{o}C, BM 86.2 g/mol), and ethanol (BP Grade; 79^{o}C, BM; 46.07 g/mol) have been used as the extraction solvents.

2.2 Ultrasonic Procedure

The extraction sample (10 gram of algae flour) was placed in a bottle (100 ml); The ratio of algae flour and solvent were 1:3, 1:5 and 1:10 g/ml. Time span used for sonication are 10, 20 and 30 minutes [9] using ultrasonic cleaning bath (JAC ultrasonic type 1505) which nominal power is 150 W/200W dimension 300x150x150 mm), frequency of 40 kHz and volume of 5.7 L. The oil extraction process was conducted at three different temperatures i.e. 23, 40 and 60^{o}C [10]. The extract liquid was separated manually. Then, filtrate was evaporated using rotary vacuum evaporator at 60 ^{o} C. The experiment was designed by Box-Behnken design of Industrial Statistics and Six Sigma of STATISTICA 6.1 to get the optimum value.

3. Result and discussion

3.1. Extraction process by Hexane solvent.

3.1.1 Effect of temperature and n-Hexane volume on oil yield

Figure 1 shows the effect of the volumes of n- hexane and temperature on the oil yield. The increasing of temperature followed by the increasing of solvent volume has enhance the oil yield. It shows that the rise of temperature and the quantity of solvent volume create higher possibility for interaction of microalgae cell and solvent; moreover higher temperature causes cavity process to run well and likewise increases the number of broken microalgae cell and leads to the rise of extracted oil. For example, while the obtained oil of 50ml n-Hexane and of 23^{o}C was 0.4 gram, the resulted oil of 100 ml n-Hexane and of 40^{o}C was 0.55 gram. The cavity waves resulted from ultrasonication has intensified the moving of microalgae particle cells and then generates heat that will break the microalgae cells and finally cause them release the oil [12]. The addition of environment temperature in the extraction process speed up the fracture of microalgae cell, therefore there will be more oil that can be collected. N- Hexane with smaller polarity is easy to absorb the oil from the microalgae cell fractures. The higher the solvent volume use, the better ability to take and tie the oil, thus the more oil will be released.

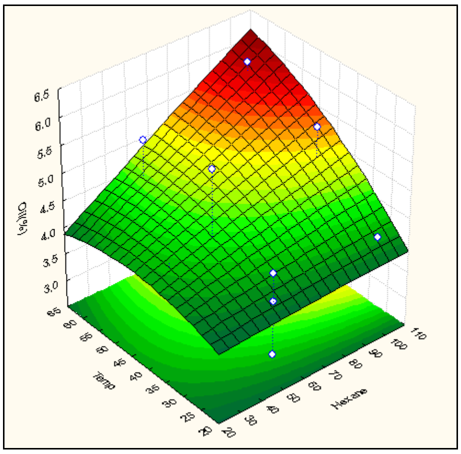

Fig.1. The effect of temperature and solvent volume to the oil yield

3.1.2 Effect of sonication time and n-Hexane volume on oil yield

The time has influenced to the oil yield as shown in figure 2. At the same volume (50 ml) and temperature of 23^{o}C, the oil obtained was 0.4 gram for 10 minutes extraction time and 0.45 gram for 30 minutes extraction time. Eventhough the oil yield rose but it was not significant. This result is similar with the previous research in which the use of ultrasound wave tends to accelerate the extraction time [7]. It reveals that given significant different time, the oil yield was relatively similar. The process of microalgae cell fracture has nothing to do with the time length of ultrasonic wave drop to the extracted material. The oil yield was influenced by the ability of the working process to break the microalgae cells; the better the fracture process, the more oil will produce; conversely if the fracture process is worse the oil yield will be less significant. In ultrasonic extraction case, the ultrasonic wave is able to break the cell wall faster up to particular time; therefore the extension of extraction time span to increase the oil result is not significant [12].

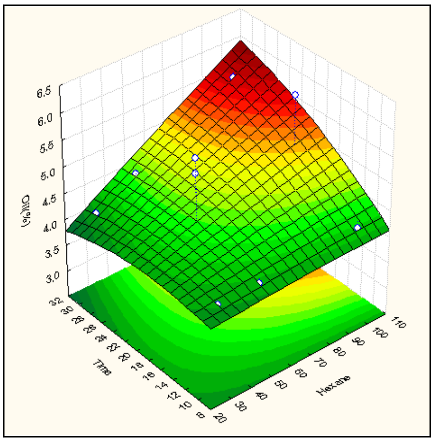

Fig.2. The time span effect to the oil yield in n-Hexane solvent

3.1.3. The effect of temperature and time on oil yield

Fig. 3 shows the effect of temperature and time extraction to the algae oil yield. The picture shows us that the effect of temperature and time by n-Hexane solvent is not significant in ultrasonic extraction. Table 1 shows the combination of time and temperature gives greater *p*-value (0.0865497) comparing to the other combinations, the high *p*- value indicates that the effect of these two factors is not significant to the oil yield. In the case of time extraction, the extraction will run faster up to 13 minutes and will be stable at 30 minutes [11]; time addition after 30 minutes has no effect to the oil yield. Similarly, given the higher the temperature the quantity of the oil yield will rise, and then will be stable; the rise of temperature up to specific time limit will disrupt the extraction process because of the evaporation of the solvent and this will influence to the quality of the oil [8]. In table 1, the combination between solvent and extraction time are two influential factors of the oil yield. N-hexane solvent influences more to the yield increase comparing with temperature and time, this was shown from the data that the solvent ratio 1:10 produced more oil comparing to 1:3 and 1:5. The optimum time and temperature were shown in table 2, i.e. at 34.67 ^{o} C and 7.9 minutes.

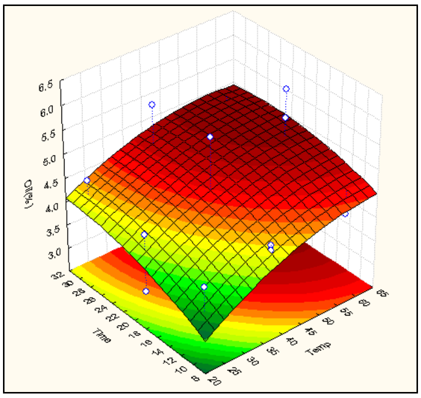

Fig.3. The effect of time and temperature to the oil yield

Table. 1. Effect estimate of parameter using n-hexane solvent

Factor	Effect	Std. Err	T(5)	p	-95.% cnf.Limt	+95.% Cnf.Limt	Coeff	Std Err. Coeff	-95.% Cnf.Limt	+95.% Cnf.Limt
Mean/Interc	**4.481444**	**0.276864**	**16.18645**	**0.000016**	**3.76974**	**5.193146**	**4.481444**	**0.276864**	**3.76974**	**5.193146**
(1)Hexane (L)	0.896913	0.817582	1.09703	0.322629	-1.20475	2.998573	0.448456	0.408791	-0.60237	1.499287
Hexane (Q)	0.013449	0.625592	0.02150	0.983680	-1.59469	1.621583	0.006724	0.312796	-0.79734	0.810792
(2)Temp (L)	1.024316	0.601140	1.70396	0.149114	-0.52096	2.569596	0.512158	0.300570	-0.26048	1.284198
Temp (Q)	0.203711	0.491064	0.41484	0.695460	-1.05861	1.466032	0.101856	0.245532	-0.52930	0.733016
(3)Time (L)	0.633579	0.622888	1.01716	0.355741	-0.96761	2.234766	0.316790	0.311444	-0.48380	1.117382
Time(Q)	0.152330	0.473608	0.32164	0.760738	-1.06512	1.369778	0.076165	0.236804	-0.53256	0.684889
1L by 2L	0.690429	0.964795	0.71562	0.506242	-1.78965	3.170513	0.345125	0.482397	-0.89483	1.585257
1L by 3L	0.651813	0.808349	0.80635	0.456664	-1.42611	2.729741	0.325907	0.404175	-0.71306	1.364870
2L by 3L	-0.150343	0.843267	-0.17829	0.865497	-2.31803	2.017344	-0.075172	0.421634	-1.15902	1.008672

Table.2. Critical values of variables using n-hexane

Factor	Observed Minimum	Critical Value	Observed Maximum
Hexane	30.00000	-11.4596	100.00000
Temp	23.00000	34.6740	60.00000
Time	10.00000	7.9395	30.00000

3.2. Extraction process by Ethanol solvent.

3.2.1. Effect of temperature and ethanol volume on oil yield

The effect of ethanol solvent and temperature in the extraction process of algae oil was shown in Fig 4. Similar with the use of n-Hexane solvent, ethanol solvent also contributes to enhance the oil yield, although less significant comparing to n-Hexane solvent. Increasing temperature level causes the of oil yield drastically as shown in the figure. Temperature contributes the acceleration of microalgae cell fracture, although ethanol has less power to tie the oil which was shown from the oil yield in the figure. It is because ethanol has bigger polarity comparing to the n–Hexane which further influences its ability to tie the oil released from the fractured cell [8], although quantitatively the obtained oil is greater, in this case comparing to n-Hexane.

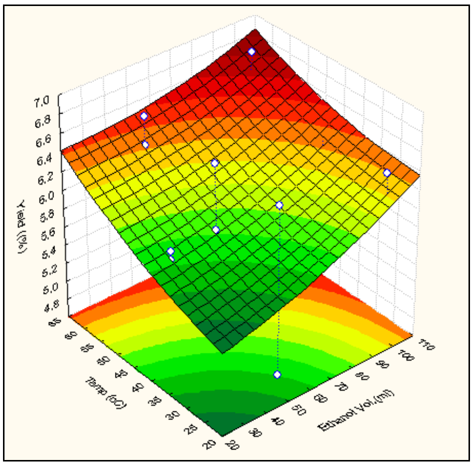

Fig. 4. Effect of temperature and ethanol volume on the oil yield

3.2.2. Effect of time and ethanol volume on the oil yield

Fig 5 shows the effect of time to the oil yield by ethanol solvent. Given the same volume (50 ml) and same temperature (60^{o}C) the oil yield change was 0.06 gram higher for 20 minutes comparing to 10 minutes, although there was a decrease at minutes 30. Similar with n-Hexane solvent, the addition of time will increase the oil yield but it was not very significant, this was indicated by the rise of the oil yield with less drastic time (Figure 5).

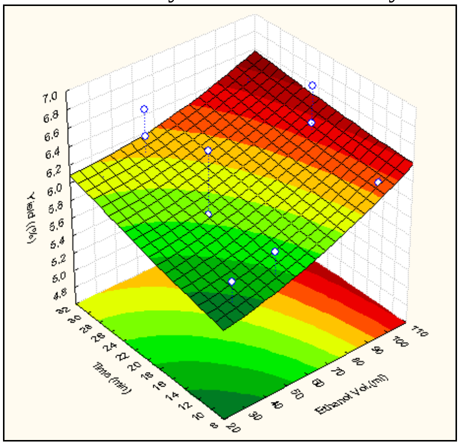

Fig.5. Effect of ethanol volume and time on the oil yield

3.3.3. Effect of temperature and time on oil yield

Figure 6 shows the effect of temperature and extraction time to the algae oil yield. This figure shows that the effect of temperature and time, it seem to be significant in ethanol solvent. From table 4 we can see that in ethanol solvent, temperature-time combination was significant to generate the oil comparing with the other combinations. This can be seen from *p* value which is relatively small i.e. 0.0646014. This data does not mean a contrary with the n-hexane solvent which shows that temperature ad time were not significant to the oil production. This data shows that ethanol solvent is not good to be used as the solvent in plant oil extraction [8]. The optimum time and temperature, which was shown in table 4, are at 51.60 minutes and 69.62^{O} C, respectively. This shows that comparing with n-hexane, the Ethanol solvent needs more time and higher temperature to get the optimum result.

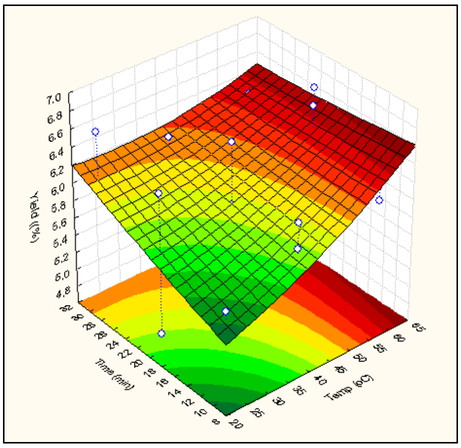

Fig. 6. Effect of temperature and time on the oil yield

Conclusion

The oil substance in microalgae *Nannochloropsis sp* is observable and this species is highly potential to be used as the raw material of biodiesel production. Ultrasonication process is one of method to used to extract the oil. The ultrasonic extraction was influenced by solvent types being used; the smaller polarity, the better yield. When the ethanol solvent was used, the number of oil yield was greater but the oil looked hazy/blurred as it contained a lot of impurities. N-Hexane solvent tied the oil more reactively compared to the ethanol. To achieve the maximum result, n-Hexane needs a low temperature of about 35^{O}C and short time of about 8 minutes compared to the ethanol for temperature of about 70^{O}C and time of about 52 minutes. Further investigation on others factor are needed to get the best ultrasonication process.

Acknowledgment

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Table 3. Effect estimate of parameters using ethanol solvent

factor	effect	Std. Err	T(5)	p	-95.% cnf.Limt	+95.% Cnf.Limt	Coeff	Std Err. Coeff	-95.% Cnf.Limt	+95.% Cnf.Limt
Mean/Interc	**6.155625**	**0.216681**	**28.40876**	**0.000001**	**5.59863**	**6.712620**	**6.155625**	**0.216681**	**5.59863**	**6.712620**
(1)Ethanol (L)	0.501503	0.639859	0.78377	0.468658	-1.14331	2.146314	0.250751	0.319930	-0.57165	1.073157
Ethanol (Q)	-0.053537	0.489603	-0.10935	0.917179	-1.31210	1.205028	-0.026769	0.244802	-0.65605	0.602514
(2)Temp (L)	0.565093	0.470467	1.20113	0.283489	-0.64428	1.774466	0.282546	0.235233	-0.32214	0.887233
Temp (Q)	-0.075344	0.384319	-0.19605	0.52292	-1.06327	0.912578	-0.037672	0.192159	-0.53163	0.456289
(3)Time (L)	0.266211	0.487488	0.54609	0.608478	-0.98692	1.519338	0.133106	0.243744	-0.49346	0.759669
Time(Q)	-0.018128	0.370657	-0.04891	0.962886	-0.97093	0.934676	-0.009064	0.185328	-0.48547	0.467338
1L by 2L	-0.138701	0.755072	-0.18369	0.861472	-2.07968	1.802273	-0.069351	0.377536	-1.03984	0.901136
1L by 3L	-0.096717	0.632634	-0.15288	0.884471	-1.72295	1.52952	-0.048358	0.316317	-0.86148	0.764760
2L by 3L	-0.322246	0.659961	-0.48828	0.646014	-2.01873	1.374242	-0.161121	0.329981	-1.00936	0.687121

Table 4. Critical values of variables using ethanol

Factor	**Observed Minimum**	**Critical Values**	**Observed Maximum**
Ethanol	30.00000	66.99770	100.0000
Temp (C)	23.00000	69.62172	60.00000
Time (min)	10.00000	51.60132	30.00000

References

[1] R. Prihandana, R. Hendroko. (2008), Energi Hijau, Penebar Swadaya Perss,

[2] D.A. Feinberg.(1984). Fuel Option from Micro algae with Representative Chemical Compositions, Solar Energy Research Institute, SERI/TR-231-2427, US Department of Energy,

[3] N. Sazdanoff. (2006). Modeling and Simulation of the Algae to Biodiesel Fuel Cycle, Thesis on Department of Mechanical Engineering, College of Engineering, The Ohio State University,

[4] J. Sheehan, T. Dunahay, J. Benemann, P. Roessler. (1998). A Look Back at the U.S. Department of Energy's Aquatic Species Program—Biodiesel from Algae, National Renewable Energy Laboratory, NREL/ TP-580-24190, US Department of Energy,

[5] www.oilgae.com , retrieved on July 7th, 2007.

[6] S. Shweta. (2005). Extraction of Oil from *Jatropha curc*as L. Seed Kernels by Combination of Ultrasonication and Aqueous Enzymatic Oil Extraction, Int. J. Bioresource Technology 96 121-123

[7] www.ultrasonic-system.com; hielscher ultrasonic's gmbh, warthestrasse 21, 14513 teltow, germany. Retrieved on July 6th, 2007

[8] M.Y. Liauw. (2008). Extraction of Neem Oil (Azadirachta indica A. Juss) using n-Hexane and Ethanol: Studies of Oil Quality, Kinetic and Thermodynamic, ARPN Journal of Engineering and Applied Sciences VOL. 3, NO. 3,

[9] Zheng Dong.(2004), Application of Ultrasound in Extraction of Chinese Medicine, Proceedings of the 2004 International Conference on Intelligent Mechatronics and Automation, Chengdu, China , August

[10] T. Ivana. (2007). Ultrasonic Extraction of Oil from Tobacco (Nicotiana tabacum. L.) Seeds, Int. J. Ultrasonics Sonochemistry 14 646–652.

[11] M. Palma, Ultrasound Assisted Extraction of Compounds from Foods, PACS Reference: 43.35.Vz.

[12] K.S. Suslick. (1989). The Chemical Effects of Ultrasound, Scientific American, February